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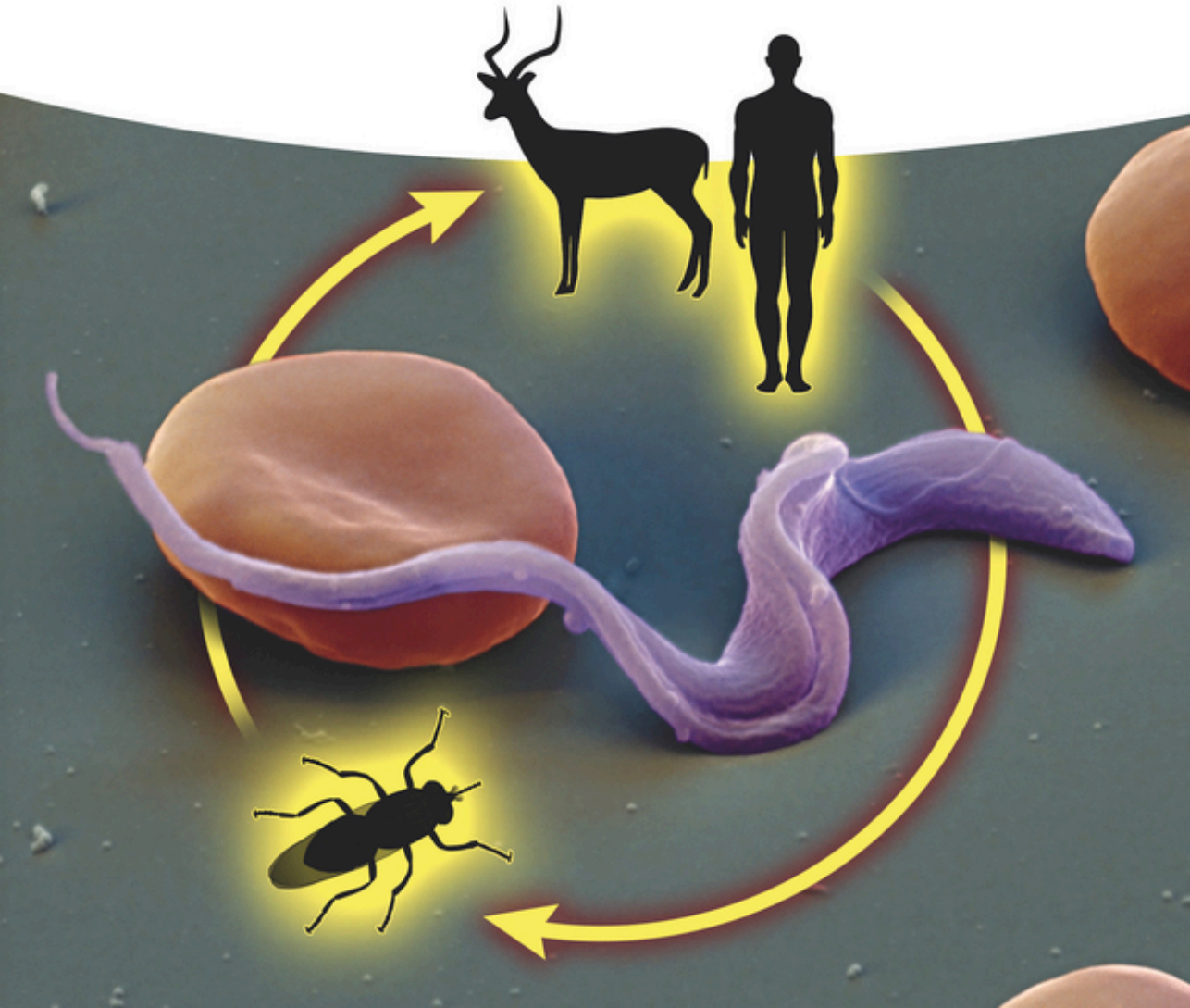

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The Biology of Parasites

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Contents

Preface

Parasitism is a specialized way of life, pursued by organisms that have evolved to thrive at the expense of a living host. Therefore, in a broad sense, all pathogens like viruses, bacteria, or eukaryotic infectious agents are parasites and thus share many common features. However, they also have important differences. For example, viruses and bacteria are genetically less complex and employ different strategies for exploiting a host, leading to different disease syndromes. As a consequence, different scientific fields have emerged, of which the discipline of parasitology is one that deals with eukaryotic pathogens, namely, protozoa, worms, and arthropods. Parasites, in this narrower sense, are a huge burden to mankind, with billions of infected people, mainly in tropical developing countries with relatively poor hygiene. Along with their medical and veterinary importance, parasites have a fascinating biology, which is the theme of this book. *The Biology of Parasites* is based on an earlier German book (Lucius & Loos-Frank (2008), Springer Verlag, Heidelberg), which has been extended and updated by the current team of authors.

The living host is a very particular niche; it is not a neutral place at all. Parasites are involved in a constant struggle with their hosts, who strive to rid themselves of the unwanted company, deploying all sorts of mechanisms against them. These range from defensive behavior to the effector molecules and cells of a complex immune system. In spite of such defenses, an extraordinary number of animals have adopted parasitism as a mode of life; some specialists believe that >50% of animal species are parasites or have at least a parasitic phase in their life cycle. It seems that the parasitic lifestyle is so rewarding that it has been worth the great effort parasites have made to develop most intriguing means of locating their hosts, survive within or on them, produce offspring, and ensure that the next generation reaches a new host. To exploit a host, parasites may change their morphology beyond all recognition: they may trick and cheat by disguising themselves or manipulate their host's cellular pathways or even their behavior. Because of these extraordinary, bizarre, or seemingly "otherworldly" abilities, parasites have always fascinated biologists and captured the attention of the general public.

The antagonistic relationship between pathogens and their hosts drives the evolution of both adversaries in a profound manner. This arms race has affected the evolution of some of the most important processes of life, for example, sexual reproduction and the immune system. It also shaped the genomes of both parties

to a degree that we have only recently discovered. Indeed, new molecular techniques developed in past few decades have opened an extraordinary range of perspectives on the interplay between eukaryotic parasites and their hosts. Genome projects have cast light on the peculiarities of parasite genomes. For example, we have learned that many protozoans and worms have undergone a reductive evolution in their genomes, especially with regard to those functions they have appropriated from their host, while other areas have been expanded, such as those needed for the manipulation of the host. This explosion in genomic knowledge has also provided us with the tools to discover and describe precisely parasite-specific metabolic pathways. It has also facilitated the dissection of molecular mechanisms used by parasites to detect host cues, invade host cells, or cope with immune effector mechanisms. This information has already allowed and will hopefully further allow us to design specific measures against parasites and their vectors, ranging from strategies to prevent infection, such as vaccines and pesticides, to drug development. However, it is not the sole goal of parasitologists to fight diseases, as worthy as that is, but to understand the intricacies of the parasitic lifestyle and to put them into a broader biological context. This greatly contributes to our wider understanding of key biological processes, such as evolution, ecology, and generation of biodiversity. Last but not least, the simple wonder and awe the extraordinary biology of parasites instills in us makes their study worthwhile in its own right.

This book is designed to provide advanced information to students of biology, medicine, or veterinary medicine and to interested lay persons. An introductory chapter on general parasitology, addressing crosscutting topics of parasitology, is followed by specific chapters on the biology of protozoan parasites, parasitic worms, and parasitic arthropods. The focus is on parasites of medical or veterinary importance, as these are best known from intensive research and are of the widest interest, although we also highlight parasites with interesting biological adaptations to emphasize those traits most typical of the parasitic lifestyle. To be concise, we discuss particular species as representatives of their taxon, while related parasites are briefly mentioned or treated in tabular form. Inevitably, the book cannot cover the entire field of parasitology. It does not give detailed treatment of the therapy or control of parasitic infections, parasite ecology, or evolutionary parasitology. Likewise, we have sparingly mentioned marine parasites or parasitoids and their interesting biologies. To cover cutting-edge topics, we have invited three renowned guest authors to contribute concise information from their field of research, namely, John Boothroyd (parasite–host interplay of *Toxoplasma*), Kai Matuschewski (vaccine development against the malaria parasite *Plasmodium*), and Nina Papavasiliou (new developments in trypanosome research).

We are thankful to many colleagues from different fields of parasitology and beyond for their helpful discussions. We thank specifically those who provided images of parasites or illustrative research data, in particular Oliver Meckes and Nicole Ottawa from *eye of science* for fascinating electron microscope pictures, Prof. Egbert Tannich for images of amoebae, and Dr. Heiko Bellmann for photos

of arthropods. We gratefully acknowledge the permission of the Departments of Parasitology of University of Hohenheim and of Humboldt University to utlize pictures from their archives. The life cycles and other drawings are based on the painstaking work of Flavia Wolf, Dr. J. Gelnar, and Hanna Zeckau, which is gratefully acknowledged. A heartfelt thank-you goes to Christine Nowotny for her most professional help with the organization of the manuscript and illustrations. This work would not have been possible without the continuous support of Dr. Gregor Cicchetti and Dr. Andreas Sendtko and their team from the publisher Wiley-VCH, which is gratefully acknowledged.

September 2016

Richard Lucius
Berlin

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General Aspects of Parasite Biology

Richard Lucius and Robert Poulin

The Biology of Parasites, First Edition. Richard Lucius, Brigitte Loos-Frank, Richard P. Lane, Robert Poulin, Craig W. Roberts, and Richard K. Grencis.

1.1 Introduction to Parasitology and Its Terminology

1.1.1 Parasites

Parasites are organisms which live in or on another organism, drawing sustenance from the host and causing it harm. These include animals, plants, fungi, bacteria, and viruses, which live as host-dependent guests. Parasitism is one of the most successful and widespread ways of life. Some authors estimate that more than 50% of all eukaryotic organisms are parasitic, or have at least one parasitic phase during their life cycle. There is no complete biodiversity inventory to verify this assumption; it does stand to reason, however, given the fact that parasites live in or on almost every multicellular animal, and many host species are infected with several parasite species specifically adapted to them. Some of the most important human parasites are listed in Table 1.1.

The term parasite originated in Ancient Greece. It is derived from the Greek word "parasitos" (Greek *pará* = on, at, beside; *sítos* = food). The name parasite was first used to describe the officials who participated in sacrificial meals on behalf of the general public and wined and dined at public expense. It was later applied to minions who ingratiated themselves with the rich, paying them compliments and practicing buffoonery to gain entry to banquets where they would snatch some food.

Table 1.1 Occurrence and distribution of the more common human parasites.

Parasite	Infected people (in millions)	Distribution
Giardia lamblia	>200	Worldwide
Trichomonas vaginalis	173	Worldwide
Entamoeba histolytica	500*	Worldwide in warm climates
Trypanosoma brucei	0.01	Sub-Saharan Africa ("Tsetse Belt")
Trypanosoma cruzi	7	Central and South America
Leishmania spp.	2	Near + Middle East, Asia, Africa, Central and South America
Toxoplasma gondii	1500	Worldwide
Plasmodium spp.	>200	Africa, Asia, Central and South America
Paragonimus sp.	20	Africa, Asia, South America
Schistosoma sp.	>200	Asia, Africa, South America
Hymenolepis nana	75	worldwide
Taenia saginata	77	Worldwide
Trichuris trichiura	902	Worldwide in warm climates
Strongyloides stercoralis	70	Worldwide
Enterobius vermicularis	200	Worldwide
Ascaris lumbricoides	1273	Worldwide
Ancylostoma duodenale and *Necator americanus*	900	Worldwide in warm climates
Onchocerca volvulus	17	Sub-Saharan Africa, Central and South America
Wuchereria bancrofti	107	Worldwide in the tropics

Source: Compiled from various authors.
*many of those asymptomtic or infected with the morphologically identical *Entamoeba dispar*.

The result was a character figure, a type of Harlequin, who had a fixed role to play in the Greek comedy of classical antiquity (Figure 1.1). Later, "parasitus" also became an integral part of social life in Roman antiquity. It also reappeared in European theater in pieces such as Friedrich Schiller's "Der Parasit." In the seventeenth century, botanists were already describing parasitic plants such as mistletoe as parasites; in his 1735 standard work "Systema naturae," Linnaeus first used the term "specie parasitica" for tapeworms in its modern biological sense.

The delimitation of the term "parasite" to organisms which profit from a **heterospecific** host is very important for the definition itself. Interactions between individuals of the same species are thus excluded, even if the benefits of such interactions are very often unequally distributed in the colonies of social insects and naked mole rats, for instance, or in human societies. As a result, the interaction between parents and their offspring does not fall under this category, although the direct or indirect manner in which the offspring feed from their parent organism can at times be reminiscent of parasitism.

Figure 1.1 Parasitos mask, a miniature of a theater mask of Greek comedy; terracotta, around 100 B.C. (From Myrine (Asia Minor); antiquities collection of the Berlin State Museums. Image: Courtesy of Thomas Schmid-Dankward.)

The principle of one side (the parasite) taking advantage of the other (the host) applies to viruses, all pathogenic microorganisms, and multicellular parasites alike. This is why we often find that no clear distinction is made between prokaryotic and eukaryotic parasites. With regard to *parasites,* we usually do not differentiate between viruses, bacteria, and fungi on the one hand and animal parasites on the other; we tend to see only the common parasitic lifestyle. Even molecules to which a function in the organism cannot be assigned are sometimes described as parasitic, such as prions, for example, the causative agent of spongiform encephalopathy, or apparently functionless "selfish" DNA plasmids that are present in the genome of many plants. Many biologists are of the opinion that only parasitic protozoa, parasitic worms (helminths), and parasitic arthropods are parasites in the strict sense of the term. Parasitology, as a field, is concerned only with those groups, while viruses, bacteria, fungi, and parasitic plants are dealt with by other disciplines. This restriction clearly hampers cooperation with other disciplines, something that seems antiquated in today's modern biology, where all of life's processes are traced back to DNA; it is gratifying that the boundaries have relaxed in recent years. However, eukaryotic parasites are distinguished from viruses and bacteria by their comparatively higher complexity, which implies slower reproduction and less genetic flexibility. These traits typically drive eukaryotic parasites to establish long-standing connections with their hosts, using strategies different from the "hit-and-run" strategies used by many viruses and bacteria. For these reasons, and for the sake of clarity and tradition, only parasites in the stricter sense of the term, that is, parasitic protozoa, helminths, and parasitic arthropods are dealt with in this book.

1.1.2 Types of Interactions Between Different Species

The coexistence of different species of organisms involves interactions among them that take many different forms in which the benefits and costs are often very unevenly distributed. Both partners benefit from mutualistic relationships, while in antagonistic relationships the advantage lies with only one side. However, a direct relationship between two species is seldom completely neutral. Different types of interactions are not always easy to distinguish, such that transitions between them are often fluid and the differences subtle. The spectrum of the partnerships between different organisms can be best illustrated by the use of concrete examples.

1.1.2.1 Mutualistic Relationships

If different partners rely on their coexistence and are limited in their viability or even nonviable when separated, this close association is described as a **symbiosis** (Greek: *sym* = together, *bíos* = life). For example, Lichens – a combination of fungi and photosynthetically active algae – can only colonize completely new habitats in this combined form. Another example is the partnership of termites with cellulose-digesting protozoa, which live in the intestinal blind sacs of the hosts. The metabolites of the protozoa complement the hosts' rather unbalanced diet.

When the two partners benefit from coexistence without losing the ability to live independently, it is known as **mutualism**. A close mutualistic relationship exists between clown fish (anemone fish) and sea anemones: the fish can gain protection from predators by snuggling into the tentacles of the sea anemones without being attacked by the latter's poisonous stinging cells (Figure 1.2) and always returns to the anemone when danger threatens. The sea anemone benefits in turn

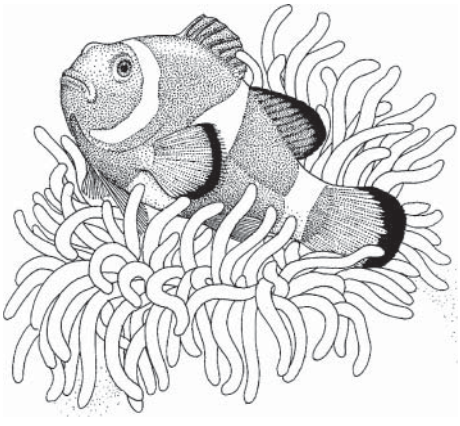


Figure 1.2 A clown fish in the tentacles of a sea anemone. The partners form a mutualistic symbiosis, but they can also survive independently. (Image: Richard Orr, courtesy of Random House Publishers, Munich.)

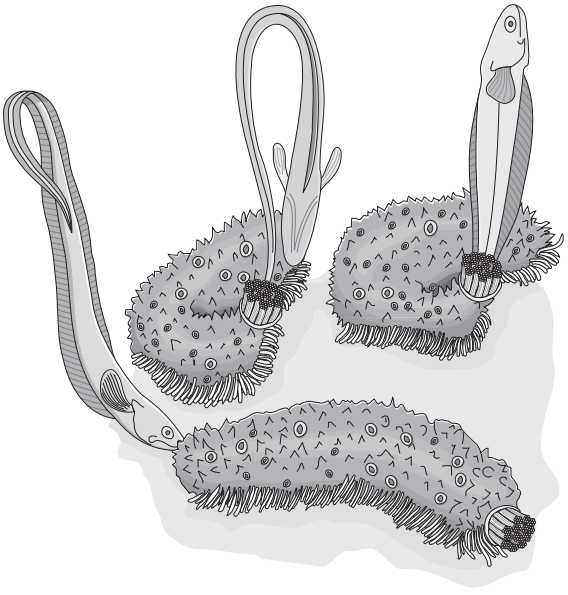


Figure 1.3 The pearlfish *Carapus* (syn. *Fierasfer*) *acus* lives in the water lungs of sea cucumbers. (Edited from Oche G. (1966) "The World of Parasites", Springer-Verlag Heidelberg.)

from the food remnants of the fish. Another example of a less intimate mutualistic association is the interaction between Cape buffalo and the cattle egret. While grazing, a buffalo flushes out insects, which are then snapped up by the egrets – and the danger-sensitive birds warn the buffalo by flying up when they spot big cats approaching.

Commensalism describes a feeding relationship, in which one partner benefits without providing any reciprocal benefits nor imposing any cost to the other. The commensal draws sustenance from the host's waste materials or from the components of the host's food, which are of no value to it. The flagellates that reside in the anal canals of arthropods provide an example, because these are areas of the digestive system where no more food absorption takes place.

However, there are symbiotic relationships in which a host is merely used as a living place. This involves organisms settling on the external surfaces of a different species (e.g., barnacles on crabs and shellfish), or even inside the host's body. One example of this is the pearlfish, a member of the cod family, which can grow to a length of approximately 20 cm. The fish lives in the water lungs of sea cucumbers into which it skillfully wriggles, pointed tail first (Figure 1.3). Pearlfish only leave their hosts to forage and reproduce.

1.1.2.2 Antagonistic Relationships

When a guest organism extracts nutrients from its host – and the host incurs a cost from the relationship – it is known as **parasitism**. Parasites can also cause damaging effects such as injury, inflammation, toxic metabolite, and other factors,

Figure 1.4 Coexistence of a typical parasite with its host. Tapeworms draw nourishment from their host and exploit the host in the long term – they are, however, only moderately pathogenic. (De bouche à Oreille by Claude Serre © Editions Glénat 2016)

and result in reduced evolutionary fitness of the host, even if the effects are only slight. Adult tapeworms may be regarded as typical examples here: they absorb nutrients from the digested food in the small intestine of the host, thereby harming the host, but do not attack its tissues. The host is thus weakened a little, but not killed, and the parasite lives of the interest without touching its capital. Claude Serre expresses all these qualities very aptly in his cartoon (Figure 1.4). Parasites are usually smaller and more numerous than their host, whereas predators are larger and less numerous than their prey. When one parasite settles on another, we call this **hyperparasitism**. *Nosema monorchis*, for example, a single-cell organism of the phylum Microspora, parasitizes the digenean *Monorchis parvus*, which is itself a parasite of fish.

We usually expect an intimate, physical relationship between parasite and host. Intimate contact like this exists in endoparasitism and (in many cases) ectoparasitism. There are also forms of parasitism in which the physical contact between the partners is less intimate, where the parasite does not exist as a pathogen, but exploits the host in other ways. The exploitation of interactions between members of social organisms is defined as **social parasitism**. In the case of social insects such as ants, the interactions of a host species are exploited by a parasitic species. The spectrum of social parasitism ranges from food theft to slavery and the targeted assassination of the queen, which is then replaced by the queen of a parasitic species. One specific form of social parasitism is the exploitation of a different species to rear one's own offspring, which is known as **brood parasitism**. A well-known representative of the brood parasites is the

Figure 1.5 Brood parasitism: A young cuckoo is fed by a warbler. (Image: Courtesy of Oldrich Mikulica.)

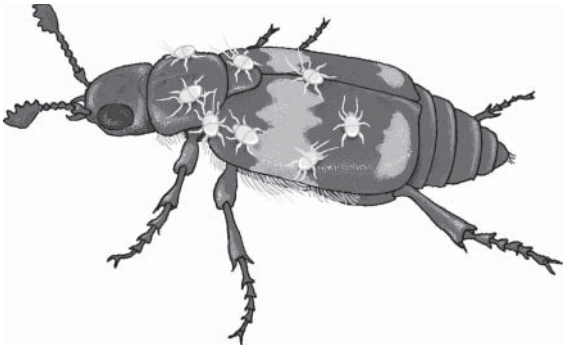


Figure 1.6 Phoresy: Mites latch on to a sexton beetle, "hitching a ride" to the nearest carrion. (Drawing from a photo by Frank Köhler.)

cuckoo (*Cuculus canorus*). The female of the species lays its eggs in the nests of smaller songbirds to have them raise its young ones (Figure 1.5). The cuckoo bee's behavior is very similar. Cuckoo bees account for 125 of a total of 547 species of bees in Germany – a fact that says much for the success of this form of parasitism. Not only food but also functions such as transportation can be exploited by parasites. For instance, some mites and certain nematodes latch on to insects for transportation. In this type of parasitism, **phoresy**, the carriers are referred to as transport hosts (Figure 1.6).

Parasitoidism occurs when the death of the host is almost inevitable following parasitic exploitation. One typical example of this involves ichneumon wasps, which lay their eggs on caterpillars. When the young wasps hatch, they feed on the host's tissues (Figure 1.7). The parasitic wasp larvae first devour the body fat, and then eat the muscle tissue and finally kill their hosts by consuming the neural

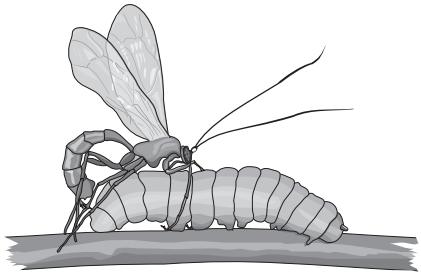


Figure 1.7 Parasitoidism: A parasitic wasp of the genus *Ichneumon* lays its eggs in a caterpillar. (From a photo in "The Animal Kingdom," courtesy of Marshall Cavendish Books Ltd.)

tissue. The larvae finally break out of the caterpillar and pupate. A parasitoid like this attacks the capital, rather than living of the interest. However, it does exploit the host for a relatively long period of time and only kills it when the parasitoid is finished with it.

The interaction between parasitoids and their host shows some similarities to **predator–prey relationships**, for example, between a lion and wildebeest (Figure 1.8). However, whereas the parasitoid only kills its host after eating most of it, the prey is immediately killed by the predator and then eaten. In addition, predators are typically larger than their prey, and consume more than one prey in their lifetime, two features that separate them from parasites.

Figure 1.8 Predator–prey relationship: A lion attacks a wildebeest. (Image: Ingo Gerlach, www.tierphoto.de.)

1.1.3 Different Forms of Parasitism

Organisms, which can live as parasites, but are not necessarily dependent on the parasitic mode of life represent **facultative parasites**. One example is the bloodsucking kissing bug, *Triatoma infestans.* It can also live as a predator by sucking out the hemolymph of smaller insects. **Obligate parasites** have no alternative other than their way of life. In the case of some organisms, only one sex lives parasitically. In mosquitoes, for instance, only the females need a meal of blood to produce eggs; the male feeds on the sap of plants. **Permanent parasites** are parasitic in all stages of their development, while **temporary parasites** spend only certain phases of their life in a host.

An **ectoparasite** attaches to the skin or other external surfaces (e.g., the gills) of its host, where it subsists on hair or feathers, feeds on skin, or sucks blood or tissue fluid substance. Included among ectoparasites are numerous **temporary parasites** (sometimes called micropredators), which only seek out their hosts to feed (e.g., bloodsucking mosquitoes), and many **permanent parasites** that remain in constant contact with their hosts (e.g., lice, or the monogeneans parasitic on fish). Parasites that live inside their hosts are known as **endoparasites**. The worms living in the gut lumen of vertebrates illustrate the simplest form of endoparasitism. The difference between rotting substances in the outside world and the contents of the digestive tract is not particularly significant and it is relatively common to find organisms that have adapted to endoparasitism of this type. One example of these residents of the gut lumen is the nematode *Strongyloides stercoralis*, the life cycle of which illustrates that it has the option of either the free-living or the parasitic modes of life. Other endoparasites either live in organs (such as the great liver fluke *Fasciola hepatica*), live freely in the blood of their hosts (such as *Trypanosoma brucei*), or inhabit body tissue (such as the filarial nematode *Onchocerca volvulus*). **Intracellular parasites** induce very pronounced changes in the host cell: using these highly specific mechanisms, these parasites (e.g., *Leishmania* and *Plasmodium*) invade host cells, reorganize them to fit their own needs, and exploit this extreme ecological niche due to a multitude of adaptations.

As an adaptation to their modes of life, many parasites have evolved complex life cycles, which include switching between multiple hosts and sexual and asexual reproduction (Figure 1.9). In the simplest forms of parasitism, only one host is exploited; these parasites as referred to as **monoxenous** (Greek *mónos* = single, *xénos* = foreign). In this case, transmission from one host to the next takes place among members of the same host species, and is referred to as **direct transmission**. By contrast, **indirect transmission** occurs when the parasite switches between two or more host species. These parasites are known as **heteroxenous** (*hetero* = differing); their complex cycles require two or three, sometimes even four, host species, depending on the parasite. By switching hosts from one stage of their life cycle to the next, heteroxenous parasites achieve greater overall fitness, or transmission efficiency, than they would by utilizing a single host per generation. For example, the use of bloodsucking mosquitoes

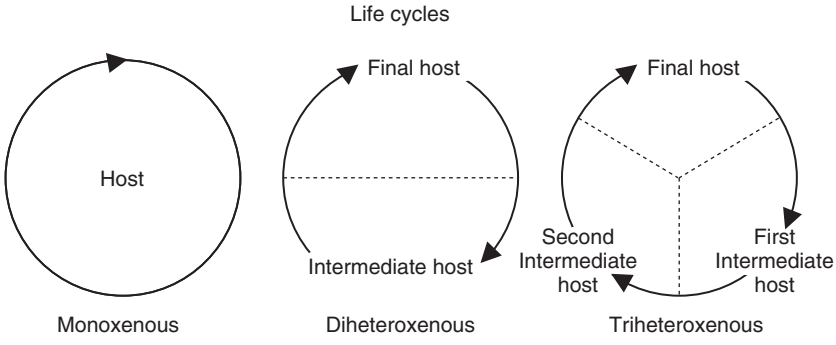


Figure 1.9 Life cycles of parasites. *Left*: Monoxenous cycle with one host, for example, *Ascaris lumbricoides*. *Center*: Heteroxenous cycle with final and intermediate hosts, for example, *Trypanosoma brucei*. Right: Heteroxenous cycle with final host and two intermediate hosts, for example, *Dicrocoelium dendriticum*.

as **vectors**, that is, carriers of the parasite between vertebrate hosts, results in a much higher level of efficiency in the transmission of malaria than has been measured in highly contagious viral diseases transmitted through direct transmission. The evolution of complex life cycles from what originally were simple ones has occurred in several unrelated parasite lineages, ranging from microorganisms to multicellular parasites, through the stepwise addition of a new host whenever this was favored by natural selection.

Modes of reproduction vary greatly among parasites, and many parasite species can switch from one reproduction mode to another during their life cycle. When a parasite alternates between sexual and asexual reproduction, this is known as **metagenesis**. The Apicomplexa, alternating between schizogony (asexual), gamogony (sexual), and sporogony (asexual), is a good example of metagenesis. Switching from sexual to asexual reproduction (i.e., parthenogenesis = virgin birth) is also seen in some intestinal nematodes, such as *Strongyloides stercoralis*, for instance; its life cycle illustrates a change between generations of parthenogenetically reproducing parasitic females and free-living sexually reproducing worms. Among sexually reproducing parasites, hermaphroditism is a common strategy, in which individual parasites possess both male and female reproductive organs. This is the case among platyhelminths such as tapeworms and flukes (except the blood flukes, or schistosomes). Hermaphrodites have the great advantage of being capable of reproduction even if they cannot find another member of their species in a host, by self-fertilization.

1.1.4 Parasites and Hosts

In heteroxenous life cycles a distinction is first made between the final host and intermediate host. Sexual reproduction takes place in the **final (definitive) host**. Some confusion in terminology can occasionally arise with this definition;

for plasmodia, for example, the more important host from an anthropocentric viewpoint is the human being. However, fertilization takes place in the mosquito, and hence the insect must be regarded as the definitive host. Another part of the life cycle of parasites takes place in the **intermediate host**, where significant developmental processes or asexual reproduction occur. This is the distinguishing factor between intermediate hosts and pure transmission agents (vectors), which transmit pathogens mechanically (e.g., through the stylets of bloodsucking insects). Several intermediate hosts may be exploited in succession during a life cycle, and these are known as first or second intermediate hosts. In some life cycles, a host individual plays the roles of the final and intermediate hosts simultaneously, as observed in the case of the nematode *Trichinella spiralis*. The trichina reproduces sexually in the gut of its host (final host function), and then forms resting stages in a completely different compartment, the muscle cell (intermediate host function). In many cases, transmission from intermediate to definitive hosts occurs by predation of the former by the latter. The larval stages of some parasites can also be transmitted from smaller to larger intermediate hosts through the food chain, without any significant morphological changes occurring. Such hosts, known as **paratenic hosts**, accumulate the larvae, and their insertion in the life cycle facilitates transmission of these larvae to the definitive host.

At each stage of their life cycle, many parasites are optimally adapted to a particular host species, which may represent their main host in fat, that is, the one they have coevolved with for a long time. On this host, growth and reproduction are optimal and the parasite enjoys a long life cycle. By contrast, living conditions are worse for the parasite in alternative host in fat that nonetheless allow the survival of the parasite, with the result that these hosts play a less significant role in the perpetuation of the life cycle. These alternative hosts, however, may serve as **reservoir hosts** in fat and be of major epidemiological importance – when control measures have been used on the main host, for example, chemotherapy on farm animals, the parasite cycle in wild reservoir hosts cannot be eradicated and a reinfection may take place via these hosts. By contrast, parasite development stages can sometimes occur in a **wrong host** or dead-end host, where no further transmission can take place (e.g., *Toxoplasma gondii* in humans).

One indispensable basis for the establishment of a host–parasite relationship is the **susceptibility** of the host. Susceptibility is essentially determined by the behavioral, physiological, and morphological characteristics of the host, and also by the host's innate and adaptive immune responses. Within a population, therefore, the host genotype often determines the individual degree of susceptibility – certain hosts may thus be predisposed for infection. Acquired characteristics, such as physical condition or age, can also affect an individual host's susceptibility to parasites.

Host **resistance** to a parasitic infection can depend on the immune responses of the host. This becomes clear when a host only becomes susceptible when elements of the immune system are disabled. For example, *Aotus* monkeys – used as experimental animals in malaria vaccine research – can only be reliably infected

after splenectomy. However, resistance can also be defined by biochemical factors. *T. brucei,* for instance, is killed by a protein in human serum, which is associated with high-density lipoproteins. **Immunity** is the term used when a past infection leaves behind protective immune responses. In the case of parasitic infections, an existing infection often provides immunity to further infections. A **concomitant immunity** (premunition) like this permits already-established parasites to survive, but leads to the elimination of new infective stages trying to infect the host. This situation can cause parasite density to be downregulated to a tolerable level for the host. Hosts with defective immune systems are often more susceptible to parasites; these hosts may consequently be colonized by **opportunistic pathogens**, which are present only in low densities or not at all in immunocompetent individuals. Such opportunistic parasitic infections are common in AIDS patients – and in many cases, these infections are the direct cause of death. Examples of this are the frequent occurrence of *Toxoplasma gondii*, *Cryptosporidium parvum*, and *Leishmania* species in AIDS patients and other immunocompromised persons.

Parasites can specialize in varying degrees in the way they exploit their hosts. The degree of specialization is expressed in the **host specificity**, which combines the number of host species that can be used at any stage of the life cycle, and the relative prevalence and intensity of infection by the parasite on these hosts. For instance, parasites that can infect only one host species or infect a few host species but achieve high prevalence and intensity on only one of these species have a high degree of host specificity ("narrow host specificity"). Feather lice (Mallophaga, see Section 4.4.2) are one example of highly host specific parasites. They are not only adapted to a particular host species omit – they can only colonize certain parts of the host bird's body (Figure 1.10). Other highly host-specific parasites include omit the larval stages of digeneans (flukes) for their molluscan first intermediate host. By contrast, parasites with wide host specificity can colonize a wide range of hosts successfully, and often achieve high prevalence or intensity of infection on many of these hosts. For example, certain stages of *Trypanosoma cruzi* and *Toxoplasma gondii* exploit almost all mammals as hosts and invade almost all types of the hosts' nucleated cells. Relying on the **host range** combined with information on prevalence and intensity of infection as a measure of specialization can be, however, misleading. Let us consider two related parasite species, A and B, each using four host species and achieving almost equal prevalence and intensity in all their hosts. However, the hosts of parasite A belong to distantly related families, whereas those of parasite B all belong to the same genus. Therefore, we can easily argue that parasite B displays higher host specificity than A, since its hosts are restricted to a narrower phylogenetic spectrum. Host specificity is the outcome of colonization of new hosts and adaptation to these hosts over evolutionary time, and the more host-specific parasites are those that cannot make the large jump necessary to colonize animal species not closely related to their main host. In this context, several parasites have made the "jump" from wild or domestic animals to humans; diseases caused by parasites transmitted between vertebrates and humans under natural conditions are referred to as **zoonoses** (e.g.,

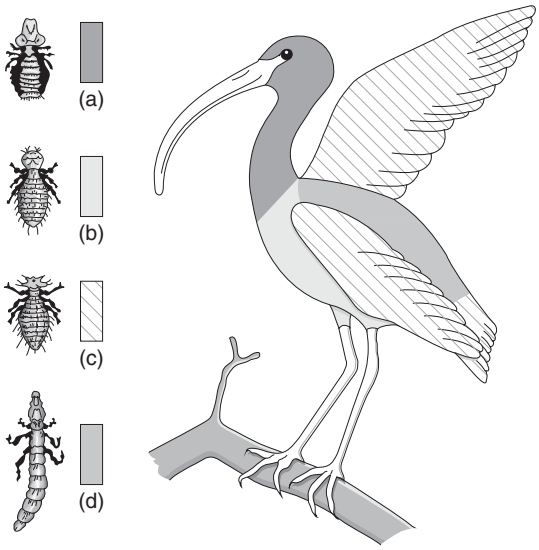


Figure 1.10 Distribution of various Mallophaga species on an Ibis (*Ibis falcinellus*), an example of high specificity for particular habitats on a host individual. (a) *Ibidoecus bisignatus*. (b) *Menopon plegradis*. (c) *Colpocephalum* and *Ferribia* species. (d) *Esthiopterum raphidium*. (From Dogiel, V.A. (1963) *Allgemeine Parasitologie (General Parasitology)*, VEB Gustav Fischer Verlag, Jena.)

T. spiralis, transmission between pigs and humans; and *Taenia saginata*, transmission between cattle and humans).

The establishment of parasites in a susceptible host results in an **infection**. In strict context, this term applies only if an increase in the number of parasites occurs by replication of the original parasite within the host, as in the case of protozoa. However, the term is now widely used in the case of helminths (worms) or arthropod parasites, too, where only one mature parasite develops from each infective larva. The term formerly used for these groups is "infestation." The period during which diagnostically relevant parasite stages appear, such as plasmodia in the blood, is known as **patency**. The period from infection to patency is called **prepatency** or the prepatent period, while the period until the onset of the first symptoms is known as the **incubation period**. For helminth parasites, prepatency corresponds to the period from initial infection of the host to the onset of egg production, when eggs start appearing in the feces or urine of the host; patency then corresponds to the adult life of the worm, from the onset of egg production to its death. In accordance with an international agreement, the infection and the resulting disease are known by the name of the parasite with the suffix -**osis**, for example, toxoplasmosis. However, for many diseases the suffix-iasis is in wide use.

The term used when an infection with the same pathogen occurs after a parasitosis has healed is **reinfection**. An infection contracted in addition to

an existing parasitosis and caused by the same species of parasite is known as **superinfection**. Simultaneous infections with multiple pathogen species are known as **mixed infections**. Both superinfections and mixed infections have consequences for host welfare, as the overall harmful effect on the host may depend on additive or synergistic effects between the different infections. If a host infects itself with stages that originate from its own infection, it is called **autoinfection**; an example of this is autoinfection with the pinworm *Enterobius vermicularis*.

The **harmful effect** which the host suffers from parasites may have different causes and manifestations, and is measured in different ways by different groups of researchers. As a measure of the impairment caused by parasites, evolutionary biologists use the reduction in the host's **genetic fitness** attributable to infection. This decrease in host fitness is referred to as the **virulence** of the parasite, and is quantified as the relative difference between the reproductive capacity of the infected host compared to what reproduction it could achieve without the infection. In the assessment of medical importance, the parameters **morbidity** (incidence of disease) and **mortality** (incidence of death) are used. A quantification is determined by calculating the ***disability-adjusted life years* (DALYs)**; this is a WHO index into which up to 140 individual parameters flow for the assessment of a disease. Finally, the harmful effect of parasitic infections in livestock is calculated by determining the loss of productivity (e.g., milk yield in cows and wool production in sheep) and the cost of infection control.

At a physiological level, parasitic infections usually have a pathogenic impact or effect; this is generally described as **pathogenicity** and the defined molecular factors that are important in this context are called **pathogenicity factors**. The amoebapore protein produced by *Entamoeba histolytica* is a pathogenicity factor, since it plays a crucial role during tissue invasion. The term used for the quantitative expression of pathogenicity is **virulence** (Latin *virulentus* = full of poison), which was originally a means of assessing a pathogen's degree of aggressiveness. Unfortunately, the same term is used by evolutionary biologists to refer to the parasite's effect on host genetic fitness. From a physiological perspective, the host is therefore not only harmed by food deprivation – the destruction of cells or tissues through the action of toxic metabolic products and immune responses that harm the host's own tissue (immunopathology) also cause damage. It used to be thought that phylogenetically ancient parasite–host relationships should be characterized by a relatively low pathogenicity, since parasites which only minimally harm their hosts may persist longer over the course of evolution. However, following both theoretical work and experimental studies with fast-evolving pathogens, it is now accepted that the pathogenicity (or virulence in its evolutionary sense) of parasites can increase over the course of evolution. Indeed, under a range of circumstances, natural selection can favor aggressive exploitation of the host, resulting in high parasite replication and transmission rates, at the expense of long-term host survival.

1.1.5 Modes of Transmission

Due to the different varieties of parasitic organisms, modes of transmission are very diverse. The simplest form of transmission is **by direct contact**, for example, through contact with the skin, such as in mites or lice. One special case of contact infection is **sexual transmission**, as in the case of infections caused by *Trichomonas vaginalis* and *Trypanosoma equiperdum*.

Many life cycles of parasites are based on **oral infection,** that is, the intake of infective stages via the mouth. Intake can occur via the food chain and foodstuff – a process known as **alimentary infection**, or in ecological terms, **trophic transmission**. Some of these cycles are based on a predator–prey relationship between the intermediate and definitive hosts (e.g., catching of mice by the final host, the fox, which simultaneously ingests metacestodes of *Echinococcus multilocularis*, the fox tapeworm). Transmission from an intermediate host to a plant-eating definitive host requires a slight adjustment; here, the parasite stages leave the intermediate host to encyst on food plants (*Fasciola hepatica*: encystment on wetland plants). Infection by **fecal-oral contamination** occurs when infective stages derived from feces (e.g., amoeba cysts, coccidia oocysts, and nematode eggs) are ingested orally; diverse media such as contaminated water, food, and air (airborne transmission) can be used as transmission media. Since transmission is usually left to chance in fecal-oral infections, large numbers of infective stages are typically produced by parasites using this route (e.g., several billion *Cryptosporidium parvum* oocysts per kilogram of cattle dung). Infections occurring through other body orifices such as the nose, ear, eye, rectum, genital apertures, and wounds are less common. One important decisive factor in whether or not infective stages are successful in transmission is their **persistence** in the external environment, that is, the infective stages' resistance to environmental influences such as extremes of temperature, desiccation, salinity, and the effects of chemical exposure.

Percutaneous infection or skin penetration plays an important role, particularly in helminth infections. In these cases, infective stages in the soil or water actively bore into the skin of the final or intermediate host (e.g., the cercariae of schistosomes and other digeneans, the infective larvae of the hookworm *Ancylostoma duodenale*).

One highly targeted and therefore extremely efficient mode of transmission occurs through the use of **vectors** (Latin *vectus* = carried) in the life cycle of many parasites, mostly those living in the blood of vertebrates. This may be purely mechanical, such as the transmission of *Trypanosoma equinum* via the lancets of tabanid flies. More commonly, however, the transmitting organisms have a function as hosts, for instance bloodsucking arthropods that ingest the parasites with their blood meal, with part of the parasite's development occurring inside the vector (e.g., malaria parasites inside the mosquito). Other bloodsucking animals can also serve as vectors of parasites, including leeches and vampire bats.

Many of the aforementioned transmission modes (direct contact, sexual transmission, fecal-oral contamination, transmission by vectors) enable transmission

from one host individual to any other within a population, which is known as **horizontal transmission**. By contrast, **vertical transmission** occurs when the infection is passed on to the offspring from the mother, that is, across generations. In **congenital infection**, parasites infect the offspring of a host in the womb or during birth. For example, some parasites migrate through the placenta to the fetus (e.g., *Toxoplasma gondii*).

The transmission pattern characterizing the spread of diseases and the quantification of all processes associated with transmission of parasites are studied in the science of **epidemiology** (Greek *epí* = via, *dē mos* = people). If a specific study involves animal diseases, the veterinary term **epizootiology** (Greek *zō on* = living creatures) is used. One commonly used measure of infection is its **prevalence**, that is, the proportion of individuals in a population that are infected at a particular point in time. The **incidence** of an infection refers to the number of new cases occurring over a period of time. The **intensity of infection** indicates the number of parasite stages per host. In recent years, the science of epidemiology has relied increasingly on mathematical models to predict the spread of diseases through a population, given some basic parameters such as host population density and parasite transmission efficiency. This growing theoretical framework provides us with the means to forecast the potential effects of climate change and other environmental factors on the future dynamics of diseases.

Further Reading

Goater, T.M., Goater, C.P., and Esch, G.W. (2014) *Parasitism: the Diversity and Ecology of Animal Parasites*, 2nd edn, Cambridge University Press, Cambridge.

Loker, E.S. and Hofkin, V. (2015) *Parasitology: A Conceptual Approach*, Garland Science.

Mehlhorn, H. (ed.) (2007) *Encyclopedia of Parasitology*, Springer-Verlag, Berlin, Heidelberg, New York.

Poulin, R. (2007) *Evolutionary Ecology of Parasites*, Princeton University Press, Princeton, NJ.

Poulin, R. (2011) The many roads to parasitism: a tale of convergence. *Adv. Parasitol.*, **74**, 1–40.

Test Questions

1. How is the term parasite defined?
2. Which major groups of eukaryotic parasites are generally recognized?
3. What is the difference between symbiosis and parasitism?
4. Give examples of temporary parasites and permanent parasites.
5. What is the difference between monoxenous and heteroxenous parasites?
6. Which phase of a life cycle takes place in the final (definitive) host?
7. What is a paratenic host?
8. Does a parasite with narrow host specificity infect many or a few species?
9. How do we refer to the phase during which parasite stages are detectable in the host, like in the blood, for instance?
10. What is a zoonosis?
11. What is the difference between horizontal and vertical transmission?

1.2 What Is Unique About Parasites?

1.2.1 A Very Peculiar Habitat: The Host

Parasites, in contrast to free-living organisms, occupy a very special ecological niche, namely a living host. On closer inspection, the host is, particularly in the case of endoparasites, an **extreme habitat**, which, in relation to its inhospitality, can doubtlessly be compared to a salt spring or deep-sea vent. The environment of the small intestine, for instance, in which tapeworms and other parasites live, is characterized, among other things, by oxygen deficiency, high concentration of active digestive enzymes, high osmolarity, and immune responses of the host. Yet, apparently compensating for these harsh conditions is the high abundance of nutrients. In order to be able to exploit such a habitat in the long run, morphological, physiological, and other adaptations are necessary. Such adaptations either existed as **preadaptations** before the ancestral switch from a free-living existence to a parasitic one, or they evolved rapidly after the transition to parasitism, through intense natural selection. Preadaptations would have originally evolved in free-living organisms in response to the demands of their particular habitat; they may have included morphological structures allowing attachment inside the host intestine, a tough cuticle to resist immune attack, or a metabolism capable of handling low oxygen availability. Further evolution after switching to a parasitic mode of life has lead to a **strong specialization** for parasitism, a universal feature of parasites. As specialists they exhibit a close relationship with their host. Therefore, the evolution of parasites proceeds jointly with that of their hosts, while the hosts, too, are reciprocally influenced by their parasites. This closely coupled evolution of two species is termed **coevolution**.

The distinctive feature of parasite–host coevolution is the fact that hosts respond to infection with parasites, for instance, by developing defense reactions. This is the reason why parasites not only have to adapt to inhospitable surroundings, but additionally have to evade the host's defense reactions if they are to survive and reproduce. Thus, an especially strong evolutionary pressure is exerted on parasites. As they are adapted very specifically to their hosts, they cannot easily evade this pressure by, for instance, infecting other host species with lesser defense reactions. Therefore, parasites are forced not only to adapt to the particular physical and physiological conditions associated with the host, but also to evade its defensive attacks that are flexible and change over the course of time (see Section 1.6). Hence, a typical feature of parasite–host interactions is the **antagonism** between the partners.

Parasites lower their hosts' genetic fitness and quality of life, acting as competitors for nutrients and exerting pathogenic effects due to lesions, inflammations, toxic metabolic substances, or merely by occupying space within the body cavity of the host. In contrast to most viral and bacterial pathogens exploiting their host only temporarily, the typical eukaryotic parasites **persist in their hosts for long**

periods of time. For example, intestinal nematodes often live several years in their hosts, hardly inducing any pathology as long as the worm burden is low. For such parasites, a **high prevalence** is typical, meaning a high percentage of infected individuals within a host population over a long time. By contrast, in flu epidemics, the susceptible individuals of a population become infected, produce viruses for a short time, and transmit these to their conspecifics, until the germs are eliminated by the immune system. As soon as protective immunity has developed in a sufficiently large number of individuals, the transmission practically ceases and the pathogen disappears from the population, only to perhaps reappear later, usually in an altered form.

The typical long-term strategy of parasitic worms, therefore, is in contrast to the hit-and-run strategy of many smaller pathogens. However, it should be mentioned that the persistence of parasitic worms in their host is not a singular feature, as there are also multicellular parasites relying on short-time exploitation, while some viruses and bacteria live for a long time in their hosts.

Still, why do typical eukaryotic parasites prefer long-term strategies? It is often claimed that protozoa and particularly helminths, due to their larger body size and complex genome, have long replication times and thus are less flexible genetically than viruses and bacteria. Although there are counterexamples, it is evident at first glance that organisms with a small genome and body size replicate more rapidly – and consequently can also change their genome more rapidly – than very large creatures (Figure 1.11). As a rough estimate, typical viruses usually possess several dozen genes, bacteria a few thousand, and eukaryotes around 10,000 or more (see also Table 1.2). While *Escherichia coli* has a generation time of 20 min, trypanosomes divide only every 6 h and some nematodes need more than 1 year to attain sexual maturity. Such a long development is not compatible with a hit-and-run strategy, but instead favors a long-lasting exploitation of the host.

A parasite's optimal level of virulence may be completely altered when the parasite encounters a novel host species. For instance, an infection with *T. brucei*, the causative agent of "Nagana," usually proceeds asymptomatically or with only few symptoms in wild ungulates, in which the parasite experienced a long coevolution. By contrast, the infection often leads to death in horses and donkeys to which the parasites are not adapted, as these host species have been relatively recently introduced into the endemic regions.

The **regulation of the parasite population density** prohibiting an overcrowding of the host is another requirement for the long-term exploitation of the host. It has been suggested that some unicellular parasites (e.g., plasmodiids and trypanosomes) self-regulate their population density to achieve an optimal utilization of the host, although the underlying mechanisms and molecules are not yet known. In many species of tapeworms, there exist density controlling mechanisms, which govern the number or size of the worms. These operate either by induction of host immunological responses or via molecules secreted by the tapeworms themselves, which act against their conspecifics. The outcome is that infections by few worms generally produce of large ones, whereas infections by many worms result mostly in very small individuals (**crowding effect**). Besides, it

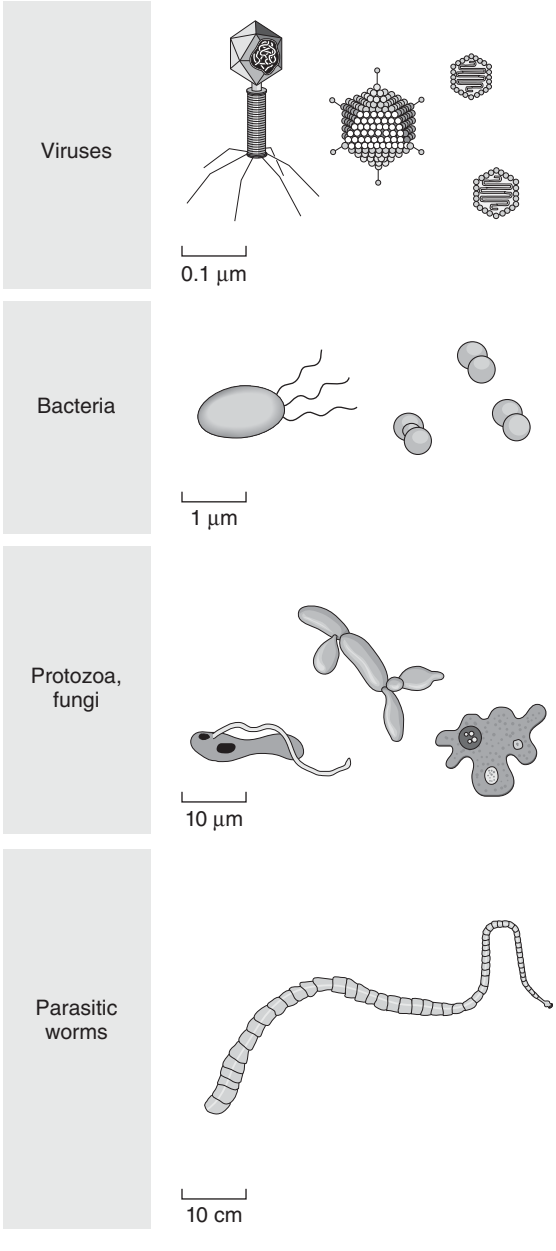


Figure 1.11 Dimensions of various parasitic organisms.

Table 1.2 Genome size and number of protein-encoding genes of some viruses, bacteria, and eukaryotes.

Organism	Number of protein coding genes	Genome size
Hantavirus	3	12.2 kb
Herpes simplex	74	152 kb
Smallpox	187	186 kb
Escherichia coli (K12)	4 377	4.6 Mb
Bacillus subtilis	4 221	4.2 Mb
Helicobacter pylori	1 589	1.6 Mb
Encephalitozoon cuniculi	1 997	2.9 Mb
Giardia lamblia	5 012	11.7 Mb
Entamoeba histolytica	9 938	24 Mb
Trypanosoma brucei	9 068	36 Mb
Leishmania major	8 311	32.8 Mb
Cryptosporidium parvum	3 807	9.1 Mb
Plasmodium falciparum	5 268	22.8 Mb
Babesia bovis	*3 671*	8.2 Mb
Theileria parva	4 035	8.3 Mb
Schistosoma mansoni	>11 809	363 Mb
Caenorhabditis elegans	21 733	100 Mb
Haemonchus contortus	23 610	320 Mb
Brugia malayi	~11 500	90 Mb
Anopheles gambiae	13 683	278 Mb
Mus musculus	24 174	2.8 Gb
Homo sapiens	~24 000	3.3 Gb

has been shown in many worm infections that established adult parasites induce immune reactions to repel superinfections without themselves being damaged. This premunition ("concomitant immunity") protects already-established parasites against conspecific competitors and simultaneously spares the host from overwhelming damage.

An infection of long duration requires the correct balance between exploitation of host resources on the one hand, and parasite growth and reproduction on the other. Highly pathogenic parasites that quickly kill their hosts have no chances to exploit them for prolonged periods of time, although they may achieve high replication rates during their brief use of the host. By contrast, parasites hardly impairing their host are more likely to prevail for a long time, but their less aggressive exploitation of host resources may only support modest replication rates. The evolution of parasite virulence toward its host may settle anywhere between these extremes, depending on the host–parasite association, as natural selection favors the combination which yields the highest overall transmission success.

Even if many parasites are amazingly well adapted to their hosts, infection results in a considerable burden for the host, as measured by reduced fitness (Section 1.3). Consequently, **hosts strive to eliminate their parasites**. They have evolved complex defense systems, for instance, innate immune responses or the

adaptive immune system, which, however, cannot provide a complete protection. Yet, a small number of parasites may not critically diminish host fitness. On the other hand, parasites are absolutely dependent on their hosts and have to be able to cope with their defence systems. Thus, the parasite–host relationship is an **asymmetric arms race**, in which the benefits of staying ahead appear greater for the parasites than for the host. As stated earlier, the necessity to adapt to their host has lead to such a pronounced specialization of parasites that life with another host species is not possible any more, let alone a return to a free-living existence (with very few exceptions). Hence, for most parasites, the way back is blocked and they depend on the host for better or worse.

1.2.2 Specific Morphological and Physiological Adaptations

Parasites have specialized very successfully and accomplished some remarkable feats of adaptation while doing so. However, these achievements cannot often be measured accurately against the current benchmarks, so they are seldom appreciated. Konrad Lorenz proposed that parasites do not need to acquire properties which have been developed by their hosts. They do not have to sing well, for example, or look attractive or be successful in capturing prey – they have more *subtle* skills which, upon closer inspection, are just as impressive as those of their hosts. For instance, a detailed look at the antigenic variation of trypanosomes or the induced behavioral changes of the intermediate host, which enable the transmission of the lancet fluke *Dicrocoelium dendriticum* to its definitive host, reveals that these parasites deserve the same level of interest as the evolutionary achievements of higher organisms. Parasites have often abandoned the features required by their free-living ancestors, because the host has taken over the relevant tasks for them. For instance, consider the loss of the intestinal tract of tapeworms and acanthocephalans; they can absorb their food from the host in solubilized form via their outer surface. This **loss of features** has often led to the assumption that parasites were simplified versions of their free-living relatives. Indeed, genome projects have actually shown that increasing specialization is often accompanied by a reduction of the genome size and the number of protein-coding genes, and hence well-adapted parasites transfer more tasks to the host. However, there are counterintuitive examples as the one of *Trichomonas vaginalis*, with one of the largest known parasite genomes, coding for 59 681 predicted protein genes. Given the complex mode of life of certain parasites, we may assume that a general tendency toward a reduction of genomic complexity is accompanied by the acquisition of new functions by the expansion of certain gene families. Any accurate statement about a possible genomic simplification of multicellular parasites will only become possible when sufficient genomes have been sequenced to enable us to compare the complexity of parasites and their free-living relatives with one other.

In the course of coevolution with their hosts, many parasites have lost structures that their free-living ancestors still required. The more intimate and prolonged is a

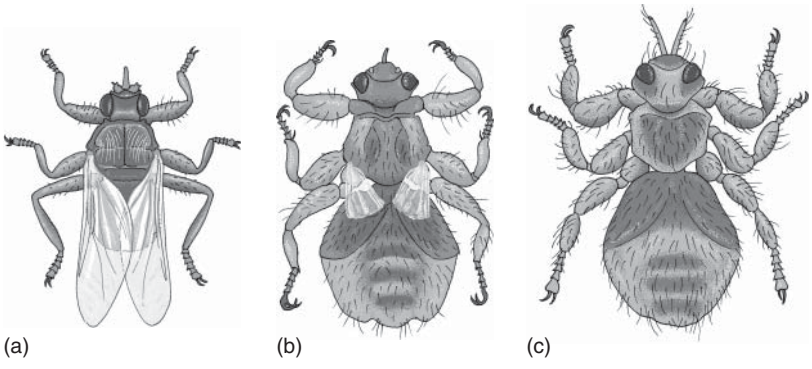


Figure 1.12 Louse flies with varying degrees of wing reduction. (a) *Lynchia maura* (continuously flight-capable). (b) *Lipoptena cervi*, sheds its wings when it has alighted on a host. (c) *Melophagus ovinus*, wingless. (Compiled from various authors.)

host–parasite association, the more pronounced are the changes. Among insects, temporary ectoparasites generally have all of their nonparasitic relatives' locomotor organs, while permanent ectoparasites tend to possess reduced wings and their legs have either been modified into clasping organs or lost completely. Louse flies are an example of this type of evolutionary change. While *Lynchia maura*, the pigeon louse fly, remains winged throughout its life, transferring among hosts at the adult stage, the deer ked or deer fly, *Lipoptena cervi,* sheds its wings immediately after finding its host. The sheep louse fly, *Melophagus ovinus* is stationary, completing its entire development on the host. It is transmitted by direct contact and therefore develops no wings at all (Figure 1.12). Similarly, the extremities of arthropods can be modified and are eventually largely lost with increasing intimacy of the host–parasite association. This can be seen in *Demodex folliculorum*, commonly referred to as the eyelash mite, whose grub-shaped body possesses extremely stubby limbs (see Section 4.2.4.1).

The best example of an extreme loss of morphological characteristics is the parasitic crustacean *Sacculina carcini* of the order Cirripedia (barnacles). They infest the European green crab, *Carcinus maenas*. The female parasite transforms itself into a network of roots, which pervades the entire body of the crab, absorbing nutrients which contribute to the development of the parasite's brood sac, the "externa." This sac-like organ replaces the female crab's egg case under its anal segments. The host keeps it clean and supplies it with fresh oxygenated water as if it were its own egg case (Figure 1.13). Male *Sacculina* larvae penetrate the young externa and change into extremely reduced dwarf males inside the female. In its adult stage, *Sacculina* therefore displays none of the characteristics of a free-living crustacean. This morphological degeneration has long been considered to be typical of parasites and as such has been given the name "sacculinization." However, when we consider the complex life cycle of *Sacculina* – a cycle which has been only fully understood relatively recently – we realize that this animal is not simplified, but highly specialized. The mode of life of *Sacculina* is an extreme form of

Figure 1.13 Beach crab with the externa of *Sacculina carcini*. The brood sac – the only externally visible part of this extremely modified barnacle – juts out under the anal segments of the crab. (Image: Courtesy of M. Grabert)

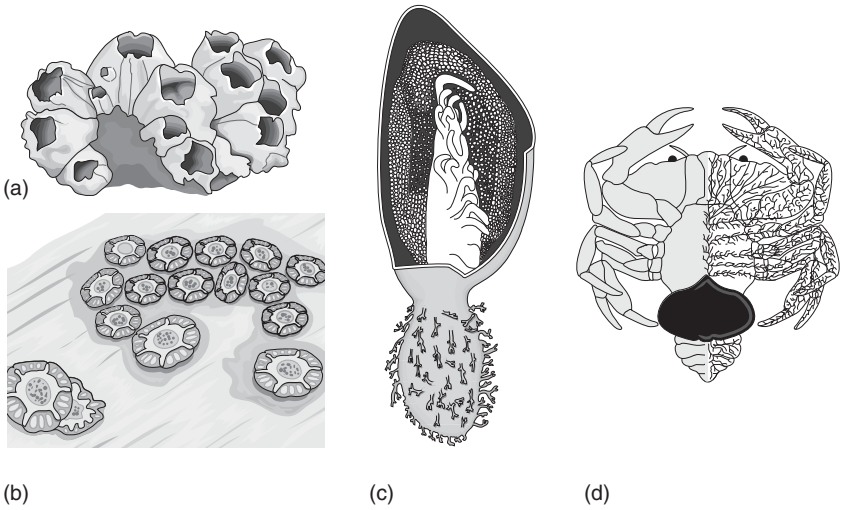


Figure 1.14 Transition to endoparasitism in barnacles. (a): Barnacle on a firm surface. (b) Whale barnacle on whale skin. (c) The shark parasite *Anelasma squalicola*, forming root-like extensions that extend into the host's skin and absorb nutrients. (d) The root system of *Sacculina carcini* runs throughout the entire body of the host; the externa is under the anal segments. (Compiled from various sources.)

a development which can also be found in other Cirripedia (Figure 1.14). Many of these barnacles are stuck to rocks and other objects and strain their food from water, such as the common barnacle *Chthamalus stellatus*. Related species have specialized in attaching to moving surfaces, where they live as external symbionts, such as the "crown" barnacles *Coronula diadema*, on the skin of whales. Other Cirripedia, for example *Anelasma squalicola*, which inhabits the skin of sharks, have evolved into genuine parasites, branching out into the host tissue to absorb food.

The highly specialized endoparasitic lifestyle of *Sacculina* probably developed in similar steps.

Reduction or total loss of organs, however, is not exclusive to parasites, but also occurs in free-living animals. Similar to the gradual loss of wings in ectoparasitic insects, the loss of legs in whales and snakes is an adaptation, not a retrogressive simplification. Even the gut can be reduced among free-living animals, as the example of the Pogonophora illustrates. These marine tube worms are related to the acorn worms. They live in deep-sea vents, where the main food source is chemotropic bacteria. Pogonophora have lost their gut – they absorb their food through a tentacle crown. In addition, the loss of sight organs in endoparasitic flatworms parallels the loss of eyes in fish and crustaceans inhabiting the complete darkness of underground rivers. Thus, basic structures have been reduced in a range of other free-living as well as parasitic organisms – natural selection indiscriminately favors the loss of any feature that has become useless ballast due to a new mode of life. Parasites are not less complex than free-living animals, but are complex in different ways.

Indeed, some structures that have evolved in parasites are not present in their free-living relatives, particularly structures serving for anchoring or attachment to the host. Across all parasite taxa, we find a wide array of impressive hook and anchor structures or adhesive disks and suckers with which parasites anchor themselves to the surface of their hosts, in mucous membranes or within cells (Figure 1.15). The entire body is often flattened to offer as little resistance as possible. Noteworthy in this respect is the formation of analogous structures in very different organisms. The common fish louse or carp louse, *Argulus*, an ectoparasitic crustacean and the single-celled intestinal parasite *Giardia lamblia*, for example, have a plate-shaped flattened body, which is held on the surface of the fish or the lining of the intestine by suction cups or a suction plate. A very different convergent development is found in the prehensile legs of true lice and the ectoparasitic crustaceans (Amphipoda) of whales, the "whale lice." These analogous structures also reflect the need for the parasite to anchor itself on the host animal. These are just some of the examples of clear morphological convergence among unrelated parasite lineages facing similar challenges.

Another striking feature of many helminth parasites is their **larger body size** than their free-living relatives. While soil-inhabiting nematodes rarely grow larger than a few millimeters in length, *Placentonema gigantissima*, a parasite of the placenta of the sperm whale, reaches a size of between 6 and 9 m. The nematodes *Dioctophyme renale* from the kidney of the dog and the Guinea worm, *Dracunculus medinensis* can grow up to a length of 1 m. In tapeworms, the broad fish tapeworm *Diphyllobothrium latum* (up to 20 m in length) is one of the record holders for length (Figure 1.16, see Section 3.1.2.5). Similarly, a digenean parasitic in the sunfish, *Mola mola*, reaches length of more than 10 m, dwarfing any of its free-living flatworm relatives. This evolutionary increase in body sizes is most apparent in endoparasitic helminths, but is also evident in copepods, where those taxa ectoparasitic on fish are orders of magnitude larger than their free-living relatives. These extraordinary body sizes can only come about if nutrients

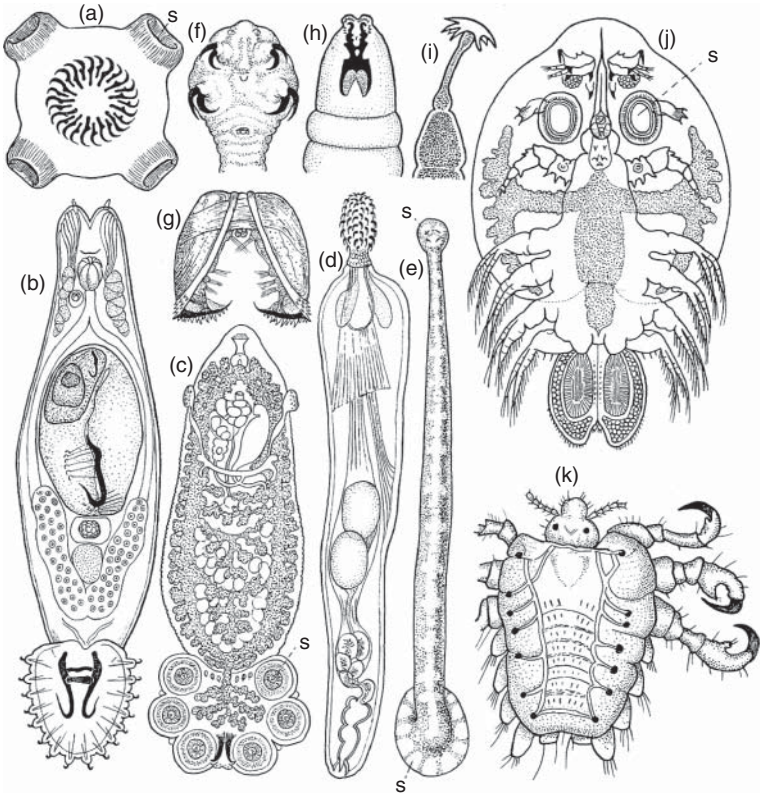


Figure 1.15 Adhesive and clasping organs in parasites. (a) Scolex of the pig tapeworm *Taenia solium*, front view. (b) Monogenean *Gyrodactylus elegans*. (c) Monogenean *Polystomum integerrimum*. (d) Acanthocephalan, or spiny-headed worm *Acanthorhynchus*. (e) Fish leech *Piscicola geometrica*. (f) Front end of the pentastomid, or tongue worm *Leiperia gracilis* as seen from the abdomen. (g) Larva of the pond mussel *Anodonta cygnea*. (h) Front end of the larva of a deer botfly (*Cephenomyia*). (i) Front end of a Gregarine (*Stylorhynchus*) from the intestine of a dragonfly larva. (j) *Argulus foliaceus*, a crustacean fish louse. (k) Crab louse (Insecta) of humans (*Pthirus pubis*). s: Sucker. (From Hesse-Doflein (1943) Tierbau und Tierleben, Verlag Gustav Fischer, Jena).)

are present in abundance, and they are seemingly the product of strong selection for increased gonad tissue and high reproductive output. Given the high mortality of eggs and larvae that fail to find a host, the reproductive strategy of many parasites is **the mass production of offspring**. Females of the nematode genus *Ascaris* (see Section 3.3.4.6) can each lay up to 200 000 eggs daily – around 70 million every year. This corresponds to 1700 times the female's body weight – in human terms, a woman weighing 60 kg would have to produce 102 tons of offspring per year – and that represents around 25 000 human babies of 4 kg each.

The beef tapeworm *Taenia saginata* produces up to 10 billion eggs during its 20 years of life, and a large body is necessary for this type of mass production.

Figure 1.16 Length of *Diphyllobothrium latum* compared to a medium-sized woman. (Image: Archive of the Department of Molecular Parasitology, Humboldt University, Berlin.)

Parasites that produce few offspring are usually smaller; one example of this is the intestinal nematode genus *Trichinella*. The female has a maximum length of 3 mm and only produces up to 2500 larvae. In fact, it is the size of reproductive organs in relation to body size that really matters, as shown by the nematode *Sphaerularia bombi* that lives in the body cavity of the bumblebee – the uterus grows outside of the female and becomes huge, while the rest of the body remains tiny. Since size is associated with the production of eggs, this also explains the fact that male worms are often smaller. A well-known example of dwarf males is seen in the nematode *Trichosomoides crassicauda*, which is found in the urinary bladder of the rat; the tiny male is rooted in the uterus of the female. Similarly, in parasitic copepods, the males are often minuscule compared with the females, being nothing more than mobile testes.

1.2.3 Flexible Strategies of Reproduction

Parasites that have found a suitable host are generally guaranteed an abundance of food for the rest of their life. Finding a mate, however, is not always so easy. Given the relative rarity of hosts and the difficulty involved in infecting them, it is common for a host individual to harbor only a single or very few parasites of a given species. A solitary life like this is basically unproblematic for unicellular parasites that can reproduce asexually. For multicellular parasites with sexual reproduction, finding a mate can be a major challenge, and evolution has favored different solutions to this problem in different taxa. For example, in some types of helminth or

arthropod parasite, once two sexual partners encounter each other, they remain together and thus avoid the struggle of finding another mate in the future. In schistosomes (blood flukes), the larger male worm fits the smaller female in a special groove along its ventral surface and keeps it lodged there, allowing the pair to mate continuously. The female may eventually be usurped by another single male, or leave its own, but the long-term pairing nevertheless serves to facilitate mating in conditions where potential mates do not encounter each other often. In some fish parasites, including copepods (family Chondracanthidae) as well as monogeneans (family Diplozoidae) and digeneans (family Didymozoidae), the two mates physically fuse together when they first meet, forming a permanent mating association. There are other adaptive solutions to the problem of rare mating encounters resulting from the uneven distribution of parasites among their hosts. Indeed, some parasites develop into hermaphrodite adults capable of self-fertilization (selfing), whereas others have adopted parthenogenesis. The latter mode of reproduction can also contribute to the rapid development of a population, since there is no investment in the production of males, which do not produce progeny.

Tapeworms are a good example of **hermaphroditism**. As protandrous hermaphrodites, the male sex organs develop first in the young proximal proglottids, while the female reproductive organs only become sexually mature in older, distal proglottids. This is why a single adult tapeworm can mate with itself. In a study of *Schistocephalus solidus,* a tapeworm of the order Diphyllobothriidea, the reproductive success of selfing was compared with that of cross-fertilization. The cestode larvae of *S. solidus* produced by selfing individuals were less successful in infecting a copepod intermediate host when compared with parasites produced as a result of cross-fertilization. With regard to infecting copepods, offspring produced from selfing achieved a lower prevalence and intensity of infection and a smaller body volume than that achieved by the progeny produced by cross-fertilization. This difference shows that the genetic quality of offspring produced by selfing is inferior – for a tapeworm isolated in a host without a partner, however, this type of reproduction can serve as a stopgap measure for producing offspring.

Various parasites can make their propagation more flexible through **parthenogenesis**, such as nematodes of the *Strongyloides* genus. These nematodes are an exception to the helminth norm – they are capable of the autoinfection of a host, which means that a self-perpetuating population can persist in the same host. The threadworm (pinworm) of the rat, *Strongyloides ratti*, inhabits the mucosa of the upper small intestine and, depending on current requirements, can change its mode of reproduction from producing parthenogenetic, parasitic females to producing dioecious individuals. At the beginning of the infections, parthenogenetic females are formed almost exclusively; this enables many offspring to be created when food availability is optimal and immune responses are low. The proportion of dioecious worms increases with the duration of the infection. The likely explanation for this is that increasing immune responses require greater genetic flexibility. This has been substantiated in experiments using immunosuppressive treatment with corticosteroids: In immunosuppressed rats, *S. ratti* produces more offspring,

in which the proportion of parthenogenetic forms is significantly greater than that in untreated rats. Parthenogenesis not only solved the potential problem of mate finding in this nematode, but also allowed it to optimally exploit its host thanks to the flexibility of its reproductive strategy.

Asexual reproduction is also a major reproductive strategy in a wide range of parasites. In unicellular organisms, asexual reproduction is often predominant and may lead to large numbers of offspring. For example, an infection with a few sporozoites of *Plasmodium falciparum*, the causative agent of tropical malaria, may give rise to 10^{11} merozoite stages. In other unicellular parasites, asexual reproduction has been considered as the only way of reproduction, but the recent identification of meiosis-associated genes in some of those protozoans challenges this view. Asexual reproduction is not restricted to unicellular parasites. In some platyhelminths, it is used to amplify the number of individuals early in the life cycle, perhaps as an adaptation to counter the losses incurred during the many transmission steps of a complex life cycle. Digeneans use asexual multiplication to increase larval stages in their snail intermediate hosts. In the case of *Schistosoma mansoni*, it has been calculated that one miracidium can produce 40 000 cercariae through asexual reproductive steps. In some tapeworms, the metacestodes have the ability to reproduce asexually, such as the fox tapeworm *Echinococcus multilocularis*. Several thousand protoscolices can occur in its larval mass, each of which will form a very small and relatively short-lived tapeworm in the intestine of a fox that eats an infected field mouse. In these cases, the parasite has thus shifted a significant part of its reproduction to the asexual phase in the intermediate host.

Further Reading

Combes, C. (2005) *The art of being a parasite*. The University of Chicago Press, Chicago.

Harvey, S.C., Gemmill, A.W., Read, A.F., and Viney, M.E. (2000) The control of morph development in the parasitic nematode *Strongyloides ratti*. *Proc. R. Soc. London, Ser. B*, **267**, 2057–2063.

Kurtz, J. (2003) Sex, parasites and resistance–an evolutionary approach. *Zoology*, **106**, 327–339.

Poulin, R. (2007) *Evolutionary Ecology of Parasites*, Princeton University Press, Princeton, NJ.

Toft, C.A., Aeschlimann, A., and Bolis, L. (1991) *Parasite-Host-Associations. Coexistence or Conflict?* Oxford University Press, London.

Wedekind, C., Strahm, D., and Schärer, L. (1998) Evidence for strategic egg production in a hermaphoditic cestode. *Parasitology*, **117**, 373–382.

Test Questions

1. What is the relationship between genome size, and the reproductive behavior of parasites?
2. Which mechanisms regulate the population density of parasites?
3. Give examples of a reduction of morphological structures in parasites.
4. Why is *Sacculina carcini* regarded as being a drastically simplified parasite?

5. An increase in parasitic worms' body size provides them with what kind of advantage?
6. What is the disadvantage of parthenogenesis?

1.3 The Impact of Parasites on Host Individuals and Host Populations

Many ecologists still believe that animal populations are controlled by food abundance, competition, predation, and abiotic factors. They tend to dismiss parasites as unimportant: parasites are small, hardly visible, and therefore assumed not to unduly affect the host. The opposite is true, however, as recent research has shown conclusively. Even parasitoses with low mortality and morbidity, such as flea or nematode infections, can have a massive impact on the host and its reproductive success – and even affect the structure of host populations as a result. Only by realizing the extent of this effect, can we understand the evolutionary pressure exerted by parasites on their hosts – and also understand the coevolutionary processes that have shaped the interaction between parasites and their hosts.

Depending on the particular type of parasite and the intensity of infection, the effects of a parasitosis vary. These effects are essentially determined by the virulence of the parasite, the susceptibility of the host species, the condition of a host individual, and its instant response situation. While some parasitoses, such as sleeping sickness in humans (caused by *T. brucei*), can induce a very high mortality rate, the impact of other parasite infections is usually so slight that it may be unnoticed. The skin of around 30% of all people is home to eyelash mites (*Demodex folliculorum*), for instance – and they are hardly ever noticed. These widely differing impacts on hosts make it extremely difficult to derive any general conclusion on the harmful effects of parasites. In addition, parasites such as helminths and arthropods are not evenly distributed among individual hosts in a population, and thus not all hosts incur the same effects from the same parasite species. Typically, a small proportion of hosts in the population harbor large numbers of parasites, whereas most of the others harbor few or no parasite (see Section 1.4.2). This aggregated distribution of parasites results from behavioral or physiological variability in the host population, leading to differences among individual hosts in either their exposure to infective stages, susceptibility to infection, or ability to mount immune responses following infection. This is why in what follows, we use some examples to illustrate the impact parasites can have on the performance of their hosts and on the hosts' progeny – and how they can consequently affect entire populations. Although these examples do not illustrate universal effects of parasitism, they nevertheless show the type of impact parasites can sometimes have on their hosts.

In a series of exemplary studies, researchers led by H. Richner from Bern, Switzerland, have illustrated the effects of an infection of great tits with the bird flea *Ceratophyllus gallinae*. Great tits are particularly suitable for such studies, because these cavity nesters are often plagued with fleas and the size of their

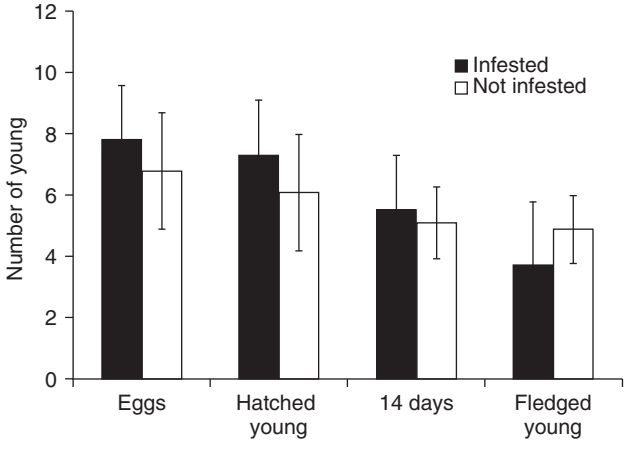


Figure 1.17 Reproductive success of great tits from nests infected/not infected with *Ceratophyllus gallinae*. (According to data from Richner, H., Oppliger, A., and Christie, P. (1993) *J. Anim. Ecol*., **62**, 703–710.)

egg clutches is easily manipulated. Under natural conditions, the number of eggs varies between 6 and 12, but eggs can easily be removed or added to the clutch. Tits also readily accept artificial nest boxes. The boxes are first placed into a microwave oven to remove any flea that is present. A predetermined number of fleas can then be put into each box for experimental purposes.

The effects of infection by ectoparasites are usually considered to be relatively low. The behavior of tits searching for nesting cavities shows, however, that flea infection in a nest box significantly reduces the box's attractiveness to the birds. In the case of heavily infected nest boxes, fleas surround the entrance hole, forming a dark ring, ready to infect the bird immediately. Tits in search of nesting opportunities try to avoid nest boxes like this. If a shortage of nesting sites forces a tit to accept a flea-ridden nesting site, its offspring will have significantly fewer red blood cells. They are also skinnier and their mortality rate is higher than that of tit offspring from nests with no flea infection (Figure 1.17). In fact, more eggs are laid by adults in flea-infected nests than in clean nests to compensate for the higher mortality of the nestlings, but a significantly smaller proportion of those will survive fledging (Figure 1.17). On average, one more young bird will fledge and leave a clean nest than a flea-infected nest. In infected nests, the breeding success rate (measured by the number of fledged young) was only 53% compared with 83% in clean nests. A pair of great tits which adopts a flea-infected box has therefore significantly fewer offspring; infection by these ectoparasites – contrary to the general opinion that they are relatively harmless – considerably diminishes the fitness of their hosts.

The parent birds also suffer from the consequences of flea infection. In the experiments, the indirect effects of infection were studied after the female tits were stimulated to lay an extra egg, corresponding to the situation in a flea-ridden nest. The additional stress resulted in an increase of 30% (from 20% to 50%) in

Figure 1.18 Scottish grouse, *Lagopus lagopus scoticus*.

the prevalence of avian malaria in the females, suggesting that the combined effects of fleas and increased investments in egg production and care caused a drop in vigilance against mosquito bites or resistance to malaria itself. Similarly, the male great tits significantly expanded their foraging area when the brood was artificially increased by two additional hatchlings. The additional effort involved resulted in the percentage of male great tits infected by *Plasmodium* increasing to 40% for flea-free birds and 80% for flea-infested birds. It appeared that few of the infested males survived through to the following year. They therefore had significantly lower chances of reproduction than birds in nests without fleas. Similar experiments with barn swallows and other birds have shown that the Swiss great tits are no exceptions.

The above experiments show the effect of parasitism at an individual level – but the impact on host populations can also be impressive. One famous experiment on host populations was conducted with the Scottish red grouse (*Lagopus lagopus scoticus*), which are widespread in the mountain heaths of the Scottish Highlands (Figure 1.18). The annual *Red Grouse* hunt is a popular social event. It has always been celebrated by Scottish landowners, as guests and tourists shooting grouse on their land provide a tidy income. Records have been kept of the numbers of birds shot each year, providing long-term data on changes in grouse population abundance. These data show that the population densities of grouse vary considerably over time, making hunting almost pointless during some seasons. A long-term study of 175 populations of grouse in different regions of Scotland showed that population density of grouse goes through a cycle with periods varying between 4 and 8 years (Figure 1.19a). Interestingly, the population density of the grouse was negatively correlated with infection intensities by the nematode *Trichostrongylus tenuis*, which also show variations over time. This parasite is monoxenous, so that transmission depends heavily on the density of the bird population. The parasite

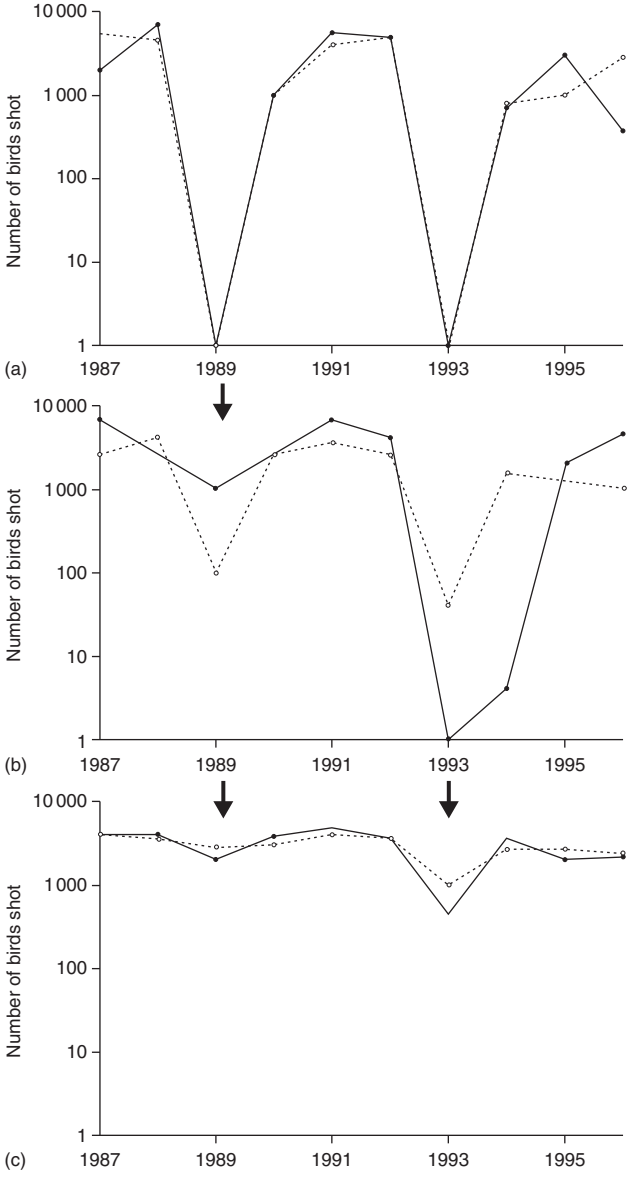


Figure 1.19 Fluctuation in the population of Scottish grouse due to infection with the intestinal nematode *Trichostrongylus tenuis*. Deworming (arrow) of a proportion of the birds prevents the decline of populations. (a) Course of events with two control populations; (b) Course of events in two populations after a single treatment; (c) Course of events in two populations after two treatments. (From Hudson, P.J., Dobson, A.P., and Newborn, D. (1999) *Science*, **282**, 2256–2258 with kind permission of the publisher.)

load of adult birds had a decisive impact: heavy worm infection – as occurs in dense host populations when transmission conditions are favorable – resulted in a high mortality rate of the young birds. The intensity of infection with *T. tenuis* was obviously reaching a point where the fitness of the birds was decimated, resulting in a population collapse.

In order to confirm these relationships experimentally, adult birds from six sample grouse populations were caught with nets before the beginning of the breeding season. The birds in some populations were then given a drug orally to rid them of worms, whereas birds from control populations were not treated. The grouse caught amounted to between 15% and 50% of each bird populations. Even this limited action yielded amazing results – in the treated populations, the deworming of some of the birds prevented the collapse of the populations seen in control populations (Figure 1.19). A mathematical model showed that a single annual deworming of >20% of the animals was sufficient to prevent population declines and ensure a relatively high population density. Therefore, there seems to be a critical infection threshold, above which we observe instability in the host population; it is therefore clear that in this host–parasite system, the parasite regulates the density of the host population. A similar periodicity is also found in some insect populations, which achieve high densities, only to collapse when parasitoids enter the equation. These provide real-world examples of the regulatory capacity of parasites predicted by the classical modeling work of Anderson and May in the late 1970s, and echoing back to the earlier demonstration of the potential of predators to control prey populations provided by Lotka and Volterra in the 1920s.

In cases where populations are regulated by parasites, however, the aforementioned marked periodicity is very rare. This is partly due to the influence of factors obscuring the influence of infection, such as climate, vegetation, and human actions. On the whole, however, density regulation by parasites and other pathogens can play an important role in natural animal populations. It is noteworthy that in some cases, relatively minor changes to the balance can have disproportionately strong impact. This finding is important for the design of control programs: if we can keep the prevalence of parasites below a certain threshold and reduce transmission as a result, a significant decline in parasitosis or even its eradication can be achieved – and this perhaps with only a modest effort.

In view of the serious effect of parasitic infections on the fitness of hosts (described above), it is not surprising that the loss of a parasite can be a huge advantage for a population. Introduced species often bring with them fewer parasites than they harbored in their original habitats. The house sparrow, introduced to North America, is infected by only 35 ectoparasite species on that continent – whereas in its home territory of Europe it has 69 species with which it must contend. The potential increase in fitness resulting from the loss of parasites indicates that invasive species are often more successful than in their original habitat and can spread rapidly as a result. One striking example of this is the European green crab *Carcinus maenas* – it has spread all over the world from its original habitat on the North Sea and Atlantic coasts. In Europe, the growth and spread of this crab is mainly restricted by the parasitic castrator, *Sacculina*

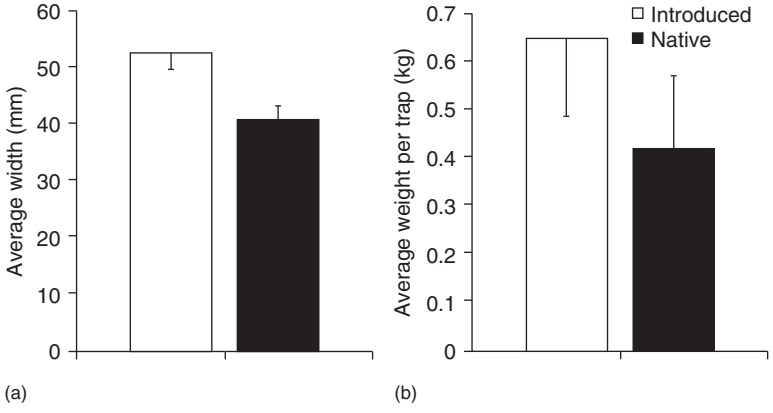


Figure 1.20 Development of green crabs from areas with and without their original parasites. (a) Width of shell. (b) Average weight of crabs in a trap (kg). (From Torchin, M.E., Lafferty, K.D., and Kuris, A.M. (2001) *Biol. Invasions*, **3**, 333–345, with kind permission of the publisher.)

carcini and the feminizing parasitic isopod *Portunion maenada*. These parasites do not occur in the new distribution areas and no native parasitic crustacean has so far switched to exploit *C. maenas*. Certain trematodes, cestodes, and acanthocephalans – the larvae of which exploit *C. maenas* as an intermediate host – do not exist in the new areas of distribution; and copepods and nemerteans, which subsist on crab eggs, are also absent. A loss of this diverse parasite fauna is considered to be a key reason why the green crab is about 30% larger, on average, in its new areas of distribution than in Europe, spreading successfully and in some areas even displacing native species (Figure 1.20).

Conversely, the introduction of a parasite can threaten host populations which have not yet been exposed to it. The mite *Varroa destructor* (see Section 4.2.3.3) was originally an ecoparasite of the eastern honey bee *Apis cerana*, which is relatively resistant to infection. Introduced to Europe with Asian bees in the 1970s, *V. destructor* spread rapidly throughout the populations of the highly susceptible Western honey bee, *Apis mellifera*. The mites suck on bee larvae, impairing their development, eventually leading to infection of the entire brood and crash of a hive's population. It has been shown, however, that resistant genotypes can evolve in populations of Western honey bees which have been exposed to the mites for a lengthy period of time. One striking example of this is the "Primorsky bees." These are honeybees that were introduced into the far eastern regions of Russia by European settlers in the mid-nineteenth century. These bee populations acquired resistance against *V. destructor*, with the result that the development of varroatosis in the hives of the resistant bees takes a different course than that of susceptible bees (Figure 1.21). In the United States and other countries where *V. destructor* has now been introduced, attempts are being made to breed *Varroa*-resistant high-performance bees, using Primorsky bees as the source material.

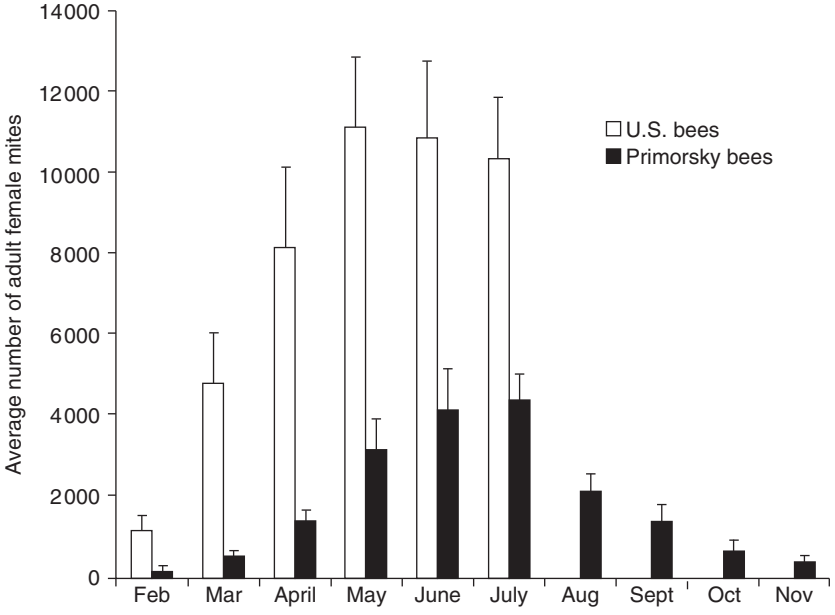


Figure 1.21 Increase of infection with *Varroa destructor* in bees of different susceptibility. White bars: American bees; black bars: Primorsky bees. (Data from Rinderer, T.E., Guzman, L.I., Delatte, G.T., Stelzer, J.A., Lancaster, V.A., Kutznetsov, V., Beaman, L., Watts, R., and Harris, J.W. (2001) *Apidologie*, **32**, 381–394, by kind permission of the publisher.)

The eel nematode *Anguillicola crassus* (order Spirurida) is another introduced parasite now part of a novel parasite/host association. Introduced to Europe in the early 1980s from Asia, it has spread very rapidly in the past few decades, threatening the populations of *Anguilla anguilla*, the European eel (Figure 1.22). It has now also invaded North America, where it infects the American eel, *Anguilla rostrata*. *A. crassus* is originally a parasite of the Japanese eel *Anguilla japonica*. In its adult stage, the parasite lives in the swim bladder of the eel. The life cycle of *A. crassus* includes copepods as intermediate hosts. In an experimental study, Japanese eels – having been fed a standardized number of infective larvae – harbored 7.5 worms on average, with a mean dry weight of 11.8 mg/worm. On average, the European eels that had been infected the same way harbored many more and much larger worms – 24 nematodes, each with a mean weight of 98.3 mg – a testament to the significantly better conditions for development in the new host. The pathogenicity of infection is also much stronger in the European eel, so that infection with *A. crassus* is considered to be a factor in the dramatic decline of eel populations. Since the eels have to make use of currents at very different depths, the swim bladder's role in pressure compensation is absolutely vital – especially during the migration to the spawning grounds in the Sargasso Sea. An interesting fact about the threat of an Asian parasite is the fact that the

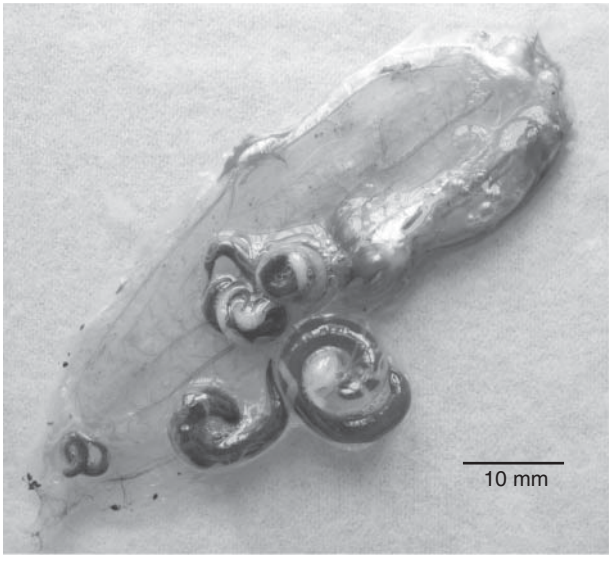


Figure 1.22 *Anguillicola crassus* in the swim bladder of a European eel (*Anguilla anguilla*). (Image: Courtesy of Klaus Knopf.)

radiation of the eel took place from Asia. The European eel diverged from its Asian ancestor and shifted its distribution areas to the west – and it probably lost its nematode parasites during this process. After a considerable delay, *A. crassus* has finally caught up with its host again – and since the eels have largely lost their immune response against these nematodes, this parasite is once again threatening eel populations.

Further Reading

Hatcher, M.J. and Dunn, A.M. (2011) *Parasites in Ecological Communities*, Cambridge University Press, Cambridge.

Knopf, K. and Mahnke, M. (2004) Differences in susceptibility of the European eel (*Anguilla anguilla*) and the japanese eel (*Anguilla japonica*) to the swim-bladder nematode *Anguillicola crassus*. *Parasitology*, **129**, 49–96.

Oldroyd, B.P. (1999) Coevolution while you wait: *Varroa jacobsoni*, a new parasite of Western honeybees. *Trends Ecol. Evol.*, **14**, 312–315.

Taraschewski, H. (2006) Hosts and parasites as aliens. *J. Helminthol.*, **80**, 99–128.

Test Questions

1. What impact does flea infestation have on the fitness of cavity-nesting birds?
2. Why do grouse populations in Scotland regularly collapse?
3. What is the reason for the fact that some introduced animal species have a survival advantage compared to native species?
4. Why does *Anguillicola crassus* pose a threat to European eels?

1.4
Parasite–Host Coevolution

1.4.1
Main Features of Coevolution

The term "**coevolution**" describes the evolution of different species of organisms which evolve in close association and influence one another. The evolution of a species does not occur in isolation; it takes place alongside the evolution of other species within ecosystems. Imagine a deciduous forest or coral reef – in a habitat like this, plants, animals, protists, fungi, and bacteria and viruses not only live together, they also interact and influence one another. Some of these species are very closely coupled, such as parasite and host, and hence the evolution of one is very strongly influenced by the other, and vice versa.

One often used example of coevolution is the joint development of flowering plants and their pollinators, which has led to very specialized reciprocal adaptations. Certain plants, for example, have evolved flower forms that allow access only to specific pollinators. If these pollinators also specialize in this very plant in their search for food, they will in turn evolve the morphological features necessary to obtain nectar from those flowers. This will result in efficient and specific pollination. Both sides benefit from this partnership, which lays the foundation for a mutualistic relationship (even if conflict can arise regarding the exact extent of mutual services). An **antagonistic relationship** exists between parasite and host, however, because the advantage is mainly on the side of the parasite, while the host tries to eliminate the parasite. This antagonism in the coevolutionary relationship creates a unique selection pressure, one which sustainably shapes both parasite and host and may force the parasite into extreme specialization. Extreme specialization, manifested by strict host specificity, is not the exception, but rather the rule in many groups of parasites. However, it must be said that "generalist" parasites with a very wide range of hosts can also be very successful, like the asexual stages of *Toxoplasma gondii*.

One major difference between free-living and parasitic organisms with high host specificity lies in the fact that the evolution of parasites does not take place in a complex environment that is relatively stable due to the large number of possible partners and food sources. A population of lynx that specializes in mountain hares as prey can switch to grouse or other prey only if the hare population is decimated. By contrast, the environment of many parasites provides only limited opportunities, since most parasites specialize in few or often only a single host species. Switching to a different host species is simply not possible for these highly specialized masters of exploitation – at least not on short time scales – and this is why such parasites are forced to adapt very specifically to their hosts, for better or for worse. Parasites also negatively affect their hosts, indirectly forcing them to develop defense mechanisms that can only be avoided if the parasite "invents" new evasive strategies. The evolutionary changes of one partner therefore exert selection pressure on the other partner in the parasite–host relationship. It is this

reciprocal selection pressure that drives the evolutionary arms race between the two antagonists. The traits of pathogens that are under such a high selection pressure evolve extremely fast. Combined with their short generation times and high reproductive potential, this quality puts parasites among the organisms with the highest speed of evolution.

One important aspect regarding coevolution in the antagonistic parasite–host relationship is the fact that for both opponents, the selection pressure comes from several sources:

- On the one hand, parasites are subjected to **selection pressure by the host's defense mechanisms**. A probable response here is the development of evasion mechanisms in the course of evolution. Such mechanisms would disable, undermine, or otherwise thwart the defenses of the host. The host, however, is exposed to **selection pressure applied by the pathogenicity of the parasite** – and the host will inevitably respond to this with, for example, the development of improved defense mechanisms.
- On the other hand, parasites and hosts, like any organism, are also subject to **evolutionary pressure through intraspecific competitors** competing for the same ecological niche and sexual partners. **Evolutionary pressure through interspecific competitors, predators, and environmental factors** is also acting on hosts and parasites.

The combination of evolutionary pressure applied by the antagonist and generated through competition with competitors has far-reaching consequences, because even slight disadvantages – created by an opponent – will cause an organism to fall behind in the race with its competitors; and this may have a much more drastic effect on fitness than would normally be the case.

In coevolution, the constant threat of falling behind in the arms race with the antagonist drives both parasite and host to change – permanently and swiftly. This is an ongoing process, since each opponent forces the other to adapt and this in turn leads to a counteradaptation. Characteristics that expose an Achilles heel to the opponent must be modified. From the viewpoint of the parasite, if its surface structure is recognized by the host's antibodies, thereby allowing the activation of host immune responses, it must react and change its surface structure to survive – only parasites that do this will persist. From the perspective of the host, it responds to fitness reductions caused by the parasite by "inventing" improved defense mechanisms to attack the parasite, despite the latter's new surface structure. Both parasite and host are thus competitors in an evolutionary race, a race in which neither can win a permanent advantage. They are in constant motion, so to speak, without significantly changing their position relative to one another. If one side slackened its efforts, however, the opponent would gain the upper hand. This situation represents the foundation of the "***Red Queen* hypothesis**," coined after a quote from Lewis Carroll's classic, "Alice through the Looking Glass"; the Red Queen tells Alice that she has to run very quickly to stay in one spot, because the surroundings themselves are moving very fast. It is because of these dynamics that

the coevolution of parasites and their hosts is believed to proceed more rapidly than most other evolutionary changes.

The importance of the host–parasite relationship has, however, a different dimension for the two opponents: a parasite is completely dependent on its host and cannot exist without it – so it cannot afford to make any mistake. A mistake like a failed invasion attempt or ineffective protection against immune mechanisms will usually result in the death of the parasite. Conversely, for the host, just one failure of its defenses against one infective stage of a parasite will not necessarily result in death, but may only entail a small reduction in its fitness. Richard Dawkins, in his book "The Selfish Gene," described this difference as the "***life/dinner* principle**" and compared this situation with the relationship between the hare and fox: While the hare will be eaten if it fails to escape just once, it is just another meal for the fox. This is exaggerated, of course; but it is true that parasites are under a great deal more evolutionary pressure than hosts and are therefore forced to adapt even more rapidly and precisely than their counterparts.

The main elements in the scenario for this antagonistic interaction are the individuals. However, evolution – and consequently coevolution – takes place within populations via the differential survival and reproduction of individuals. In a parasitized population, susceptible host genotypes become scarce due to their impaired fitness, but infection-resistant genotypes come to the fore. In this way, the distribution pattern of the host population's genotypes changes under evolutionary pressure from the parasites. Parasites must consequently adapt to such change and alter their own genomes through time. Coevolution in the short term is thus determined by changes in the frequency of genotypes within populations.

Although parasite and host are in permanent competition, the dynamics of the evolutionary process can allow a delicate balance to be created under certain conditions. The host contributes to this compromise – after all, it is often less costly, in terms of fitness, to permit some slight infection than to develop solid defenses with no loopholes. On the part of the parasites, there is a tendency for strains with lower virulence to prevail in the long term, especially if high virulence leads to the early death of the hosts, impairing transmission. A relatively balanced equilibrium may become established if parasites adapt very specifically to their hosts through long coevolution and if the transmission rate is also low.

The result of this reciprocal influence is often an extreme specialization of parasites on one host species, with the parasites becoming highly adapted to that particular host's characteristics. From a phylogenetic perspective, **cospeciation** with their hosts is typical for these species – thus, when subsets of an ancestral host population become segregated and evolve into separate species, the parasites follow their host species by speciating in parallel (Figure 1.23). If new host species are created through geographical isolation or other barriers to gene flow, their parasites adapt to the individual properties of the new host species to such an extent that they themselves form new species during the course of evolution. In such cases, the phylogeny of a group of parasite species becomes a mirror image of that

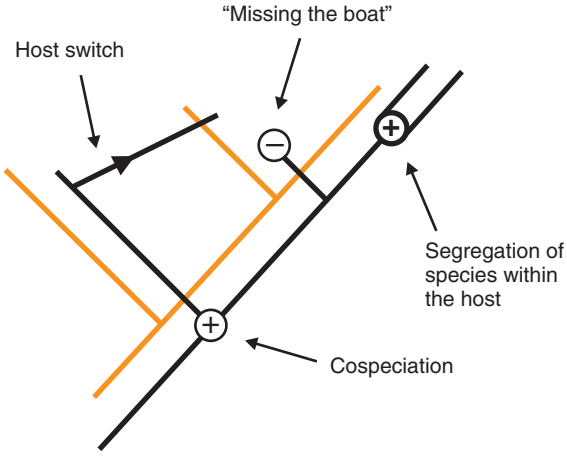


Figure 1.23 Various modes of coevolution of hosts and their parasites. For details see text. Black line: evolutionary line of parasite species; grey line: evolutionary line of host species. (From Paterson, A.M., Palma, R.L., and Gray, R.D. (1999) How frequently do avian lice miss the boat? Implications for coevolutionary studies. *Syst. Biol*., **48**, 214–223 with kind permission of the publisher.)

of the hosts they infect. This pattern, combined with the fact that the speciation of the parasites follows that of the host after a short time lag, has been summarized in "Fahrenholz's rule." The relationships of parasites should therefore enable conclusions to be drawn about the relationships of their hosts – and before the birth of modern-day phylogenetics, this was indeed the case. The relationships of ducks with flamingos and of old world camels with new world, llama-like camelids were thus substantiated (inter alia) by the relationships among their ectoparasites.

However, parasites do not always follow their host species over evolutionary time. Since the distribution of parasites in host populations is not uniform, a few host individuals from a small founder population may be free of one particular parasite. A study on introduction to New Zealand as alien fauna shows that this event occurs very frequently: only three of 18 bird species introduced by humans had the same number of bird lice species as they had in their area of origin; all others had fewer parasite species. Host species that evolve from such founder populations may be free of the corresponding parasite species – or in other words, the parasite has *missed the boat*. Conversely, specialized parasites may also adapt to a new host species, particularly if it is closely related to their own host and offers them similar living conditions. This is termed a *host switch* – it consists of the parasite species colonizing a new host species while remaining capable of exploiting its original host.

Most of our current knowledge of phylogenetic patterns of coevolution comes from studies of ectoparasites such as bird lice, which are extremely specialized for their respective host species. Across all parasite taxa, it remains unclear whether coevolution has proceeded mostly by cospeciation following Fahrenholz's rule,

by repeated host switching, or through a mixture of both. In particular, we know relatively little about the speciation of parasites which are pronounced generalists, infecting a variety of host species, such as *Toxoplasma gondii*.

Finally, we note that according to many evolutionary biologists, coevolution with pathogens may have decisively contributed to one of the most fundamental aspects of life on Earth. One hypothesis – the subject of some debate – is that selection pressure from pathogens has strongly favored hosts capable of maintaining genetic flexibility and is responsible for the **evolution of sexual reproduction.** According to one rival hypothesis, the genetic recombination generated by sex is mostly beneficial for the purging of adverse mutations.

Whether these hypotheses are correct or not, it can be said that the intermixing of the genome resulting from sexual reproduction provides two different individuals with the opportunity to create offspring with new and unique genotypes through recombination. This is essential for the continued evolutionary refinement of new defenses against pathogens. On the contrary, sexual reproduction has one marked disadvantage: 50% of the population (the males) produces no offspring; essentially, they only make their sperm available, yet require many resources for growth. A population of parthenogenetic females would seem better off, as all offspring could themselves produce offspring. Despite this huge disadvantage, the fact that sexual reproduction has prevailed in most higher organisms indicates the extreme importance of genetic variation, presumably as an important requirement for defense against pathogens.

1.4.2 Role of Alleles in Coevolution

Adaptation occurs gradually through **mutation and selection** in the course of evolution. The mutations, however, evolve in random, undirected manner and usually have a disruptive character; this is why mutants normally have a lower level of fitness than the wild type. The relatively few successful mutations that provide a selective advantage, however, are of great importance. If we consider only short periods of time, stable mutations that provide selective advantages do not occur often enough to explain the rapid adaptation of parasites to their hosts. Moreover, often only a combination of several mutations has a lasting effect on the parasite–host relationship. Combinations like this occur much more rarely than successful single mutations. In addition to the emergence of mutations, their distribution throughout the population is therefore a crucial factor for coevolution. If there are mechanisms that help to spread the relatively rare beneficial mutations efficiently, populations of parasites or hosts can respond quickly to new conditions.

In order to understand the rapid adaptations that occur during the course of a coevolutionary process, it is not enough to simply analyze the genes of individuals – we must study their frequency distribution in populations of parasites or hosts. Such studies show that populations are composed of very different genotypes. Typically, there are different alleles for each single gene. Adaptations to the

coevolution partner arise through the **selection of alleles** within a population, so that **changes in allele frequencies** can be observed, while new successful mutations occur only rarely.

Host populations consist of individuals that exhibit a spectrum of host characteristics that are more or less suitable for any particular parasite of a given species. Similarly, the parasite population is also assumed to consist of a range of different genotypes. It could therefore be argued that the success of infection and the progression of parasite development and reproduction depend on which genotype combinations of the parasite and of the host encounter one another. Experiments involving controlled exposure with bacterial pathogens and other parasites have confirmed the importance of this "matching" of genomes.

If the infection success of parasitic worms (or arthropods) in a population of hosts is examined, clear inequalities among individual hosts will become apparent. A negative binomial distribution of worm loads is typical – while few hosts harbor a large number of worms, most host individuals have few or very few worms, or no worms at all (Figure 1.24). This uneven distribution of parasites among hosts is in part due to chance events, as different host individuals will not encounter the same number of parasitic infective stages. However, high susceptibility to helminth infection is frequently found in certain families and not others, therefore genetic predisposition exists and contributes to uneven worm burdens among hosts. In a host population, there is a pool of different alleles, which define the host qualities of any individual. These hereditary characteristics are modified by other factors, of course, such as environmental conditions and current constitution.

Analysis of the genetic compatibility of a parasite–host combination is complicated by the fact that in most cases, several genes have an influence at the same time. Situations where individual genes play a crucial role are illustrated by the drug resistance of parasites or by host diseases such as sickle cell anemia (see below). In these cases, the spread of the corresponding alleles in a population

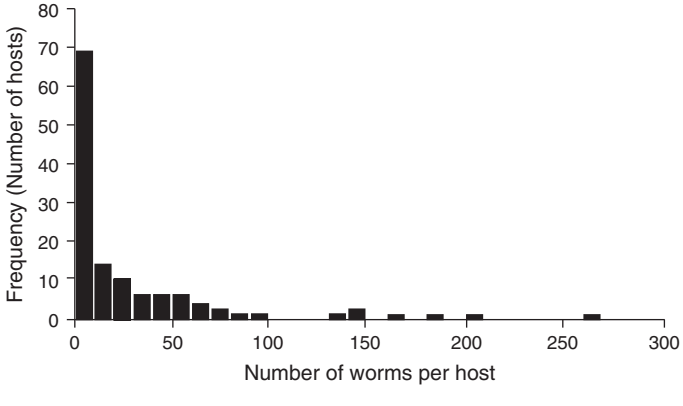


Figure 1.24 Negative binomial frequency distribution of hookworms in a human population in Papua and New Guinea. A few individuals have many worms – but the majority of individuals have few or no worms. (From Pritchard *et al.* (1990), Parasitology 100, 259–267.)

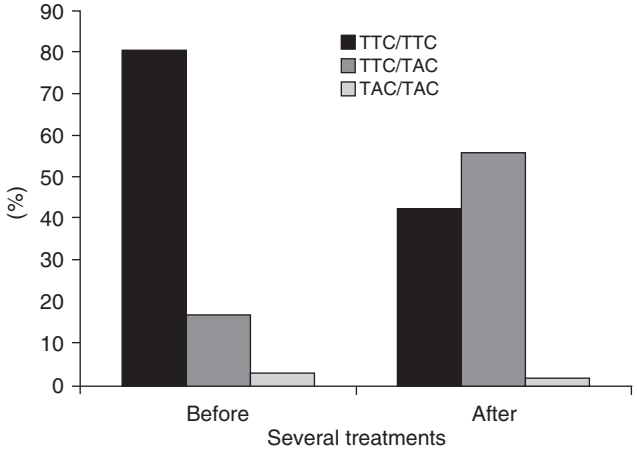


Figure 1.25 Shift of allele frequencies of the β-tubulin gene of small strongyles parasitic in horses after several treatments with benzimidazole. The replacement of phenylalanine with tyrosine in position 200 of the protein leads to drug resistance. Heterozygous genotypes increase in frequency under the pressure of the drug treatment. (According to data from G. Samson-Himmelstjerna.)

can be traced. Studies like this have confirmed that rapid adaptation to a new drug/new pathogen can occur within a few generations through changes in allele frequencies.

For example, the shift of allele frequencies in populations of parasites under evolutionary pressure is well illustrated by the resistance of gastrointestinal nematodes to the drug class of benzimidazoles (Figure 1.25). Their active agents create a long-lasting bond with the β-tubulin of the parasite; the bond prevents the formation of microtubules, ultimately leading to the death of the worms. The amino acid 200 of the β-tubulin, a phenylalanine, is crucially important for the bonding process. In a population of small strongyles parasitic in horses that has never been treated with benzimidazole, there is always a high percentage of worms in which the β-tubulin gene has a phenylalanine (TTC/TTC) in both alleles at position 200. A much smaller percentage of nematodes is heterozygous, where one allele codes for phenylalanine and the other for tyrosine (TTC/TAC), while very few individuals are homozygous for tyrosine in position 200 (TAC/TAC). Under the pressure of drug treatment, this balance changes rapidly. After only a few generations, the number of homozygous TTC/TTC parasites fell drastically, the frequency of the heterozygous TTC/TAC worms increased sharply and the homozygote TAC/TAC type remained almost constant. Therefore, it is clear that when under the pressure of the drug treatment, the β-tubulin allele with tyrosine at position 200 provides a distinct advantage to the heterozygous forms – and worms with this combination of genes can therefore prevail. A low-frequency allele in the parasite population is therefore "brought forward" – and because it provides a selective advantage in the presence of the drug, it can quickly spread through sexual recombination.

1.4.3 Rareness Is an Advantage

Individuals in a population have different alleles, as the above example with the β-tubulin allele shows. Frequently occurring alleles probably provide a selective advantage, while the rarity of other alleles suggests that they currently provide no advantage. So why do a population's rarely occurring alleles not simply dwindle away? The maintenance of inferior alleles in the population for an event that might occur later is not compatible with the selective pressure under which all organisms exist. Understanding this question provides a basic cornerstone for understanding coevolution itself.

A closer look reveals that the rare, unconventional alleles of a host can also provide selective advantages in its current situation, simply because they do not fit into the typical host genetic landscape to which parasites have adapted. Here we assume that parasites must overcome two major obstacles in order to reproduce

- infection success, persistence, and reproduction in the host;
- transmission of the offspring to a new host.

For most parasites, the probability of the offspring finding a suitable new host is ver small. This chance is increased, however, when the genotype of the parasite enables the infection of a frequent host type – and the result of this is that pathogens which are compatible with a frequent host genotype and which specialize in that host genotype will prevail. Rare or aberrant genotypes of hosts therefore enjoy relative protection from pathogens. The selection pressure exerted by pathogens on the "normal type" provides a selective advantage to aberrant hosts in intraspecific competition. These conditions can follow a dynamic pattern, as captured by the "*Red Queen*" hypothesis. A negative frequency-dependent selection causes a cyclic change of genotypes in the populations of host and parasite, as schematically shown by Schmid-Hempel in the "coevolution wheel" (Figure 1.26).

Under certain circumstances, pathogens can decimate one of a host population's frequently occurring genotypes in which they have specialized – and to such an extent that the genotype can even become rare. In a case like this, previously aberrant host genotypes would now be expected to come to the fore and proliferate, subsequently forming a large proportion of the population. Parasites that are still specialized for the rarefied host genotype now have less chance of finding a suitable host and must adapt to specialize for the host type which now prevails under the current conditions. The parasites change their target, so to speak. After a reversal like this, the previously predominant host genotype (now in the minority) is relieved of the pressure of parasite infection and again enjoys a selective advantage.

This rarity advantage also benefits the parasite – the defenses of the host have to focus on the prevailing parasite genotype, so rare parasitic genotypes consequently enjoy a selective advantage. The dynamics of this coevolutionary cycle can be compared with a predator–prey relationship, in which predators that specialize

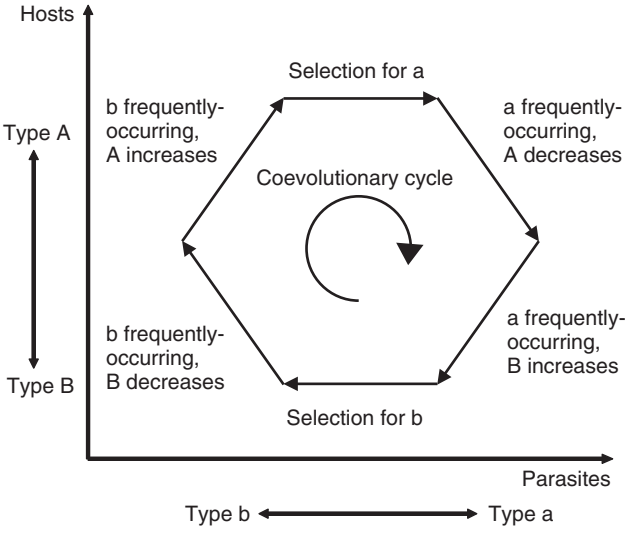


Figure 1.26 In the simplest case assumed here, with two types of hosts (A, B) and parasites (a, b), type a parasites can infect the hosts of type A and correspondingly, type b parasites can infect the hosts of type B. Infection leads to a decrease in the frequency of the corresponding type in the host population (fitness loss) and to an increase in the corresponding type in the parasite population (fitness gain). If, for example, there were currently many hosts of type B, but few parasites of type b, parasites of this type would have a high selective advantage. Selection of the type b now occurs causing — with a time delay — an increase in type b's frequency in the population. The various frequencies of host and parasite types alternate accordingly during the course of a cycle. In the longer term, however, none of the types disappear from the population, as each type is "protected" against elimination by negative frequency-dependent selection. From Schmid-Hempel, P. in Allgemeine Parasitologie (2006). Eds. Hiepe, Lucius, Gottstein, Parey in MVS Medizinverlage Stuttgart.

in one specific, very common prey animal first decimate its population, and then switch to a different species of prey. This allows the decimated prey species to recover. In the case of the host–parasite relationship, however, evolutionary pressure is reciprocal, so allele frequencies of both parasite **and** host are subject to changes.

1.4.4 Malaria as an Example of Coevolution

There is hardly a disease which more clearly illustrates the sequence and the consequences of host–parasite coevolution than malaria. All four human pathogenic *Plasmodium* species (*P. vivax*, *P. ovale*, *P. malariae*, and *P. falciparum* see Section 2.6.3). are human-specific; their evolution is therefore tied exclusively to humans. In the past, only the natural defense mechanisms of humans held the parasites at bay – but now our defense arsenal has been expanded by mosquito repellents,

insecticides, and, especially, drugs against which the parasites are evolving evasion mechanisms. The development of drug resistance is a very good example of how parasites evade the selection pressure exerted by the host.

The history of *Plasmodium*'s drug resistance begins with the use of chloroquine. It was first synthesized in 1934 by the German chemist Andersag and accumulates in the parasitophorous vacuole, preventing the neutralization of toxic heme formed as a by-product of the parasite's breakdown of hemoglobin. Heme is normally aggregated into polymers and then stored in the food vacuole as an insoluble complex with proteins. Chloroquine inhibits the aggregation; the free heme is then toxic to the parasite. Resistance to chloroquine is caused by a mutation in the gene of the transporter protein cg2 (=crt). This gene enhances the discharge of the active ingredient, detoxifying the parasite. According to current opinion, the known mechanisms of chloroquine resistance are derived from two independent mutations, which arose in 1957 in Asia and 1959 in South America. They have since spread around the world (Figure 1.27). Since the parasites develop resistance to avoid the effects of such specific drugs, the use of combination preparations (mixtures of two or more drugs with different active mechanisms) is now preferred. However, multiple mutations can also evolve and confer resistance, even against drug combinations.

Just as drug-resistant genotypes prevail under selective pressure in the plasmodia population, rare mutations also spread throughout human populations in malaria-endemic regions – if these mutations provide resistance to *Plasmodium*. The *P. falciparum* infection exerts a particularly strong selective pressure due to its high mortality rate. This infection is responsible for 40% of child deaths in some parts of Africa. In such regions, malaria – as a selective factor – has affected

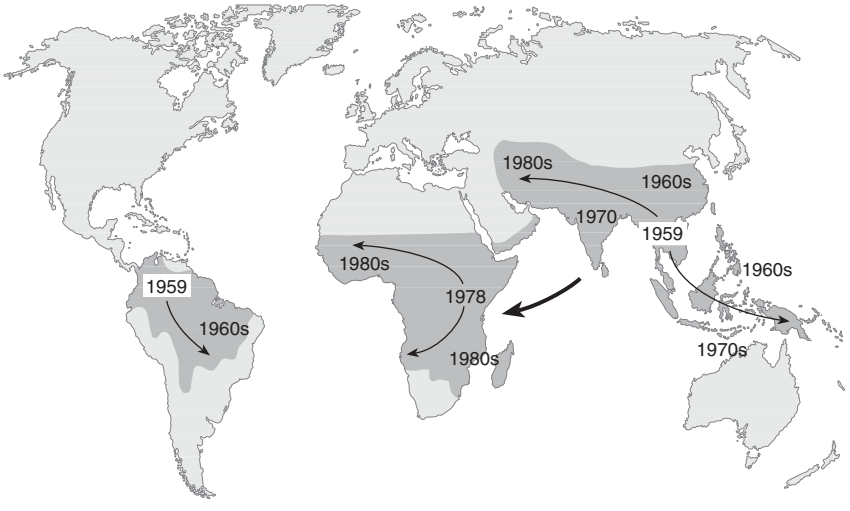


Figure 1.27 Chronological sequence of the worldwide spread of chloroquine resistance in *Plasmodium falciparum*. (Data from X. Su *et al.* (1997) Cell 91, 593–603.)

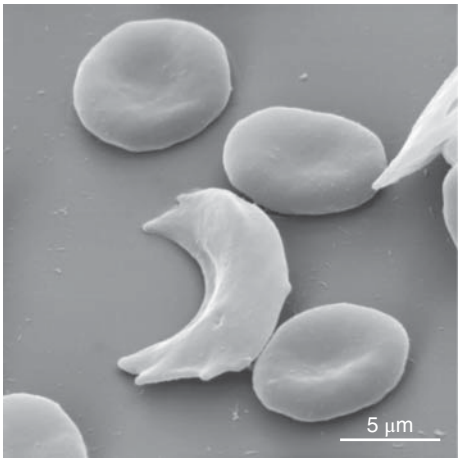


Figure 1.28 Sickle cell erythrocyte. (EM image: Courtesy of Eye of Science.)

humans as much as tuberculosis has affected the genome of the inhabitants of temperate climates.

One impressive example of the change of allele frequencies in humans as an adaptation to malaria is **sickle cell anemia**. This hereditary disease is caused by the amino acid valine replacing the glutamine at position 6 of the hemoglobin's β chain. With reduced oxygen tension, the peptide chains of the sickle cell hemoglobin form elongated polymers, causing deformation of the erythrocytes, which then take on a crescent shape (Figure 1.28). The red blood cells of sickle cell anemia sufferers also exhibit membrane changes and are relatively rigid. Since they are more rigid than normal erythrocytes, they get stuck in capillaries. Plasmodia cannot multiply as efficiently in these abnormal red blood cells as they would in normal erythrocytes, and therefore grow more slowly.

People who are homozygous for the sickle cell gene (HbSS) suffer from chronic hemolytic anemia, closure of capillaries, localized necrosis, and greatly increased susceptibility to bacterial infections. These symptoms are so severe that only 20% of HbSS patients reached adulthood before specialized medical centers were established in Africa. By contrast, there is hardly any clinical difference between heterozygous (HbAS) persons and those with normal hemoglobin (HbAA). However, heterozygous individuals possess 60–90% protection against severe malaria. They also have fewer parasites in their blood and the prevalence of parasitemia is lower. The heterozygous genetic trait probably exerts its main effect in early childhood: it appears that the poorer growth of the parasites allows the development of a more efficient immunity in infants aged up to 16 months.

The geographical frequency of the sickle cell disease correlates with the spread of tropical malaria (Figure 1.29). In some endemic areas, up to 40% of the population is heterozygous and manifests the HbS trait. The prevalence of the HbS trait in malaria-free areas, however, is negligibly low. So why is the frequency of

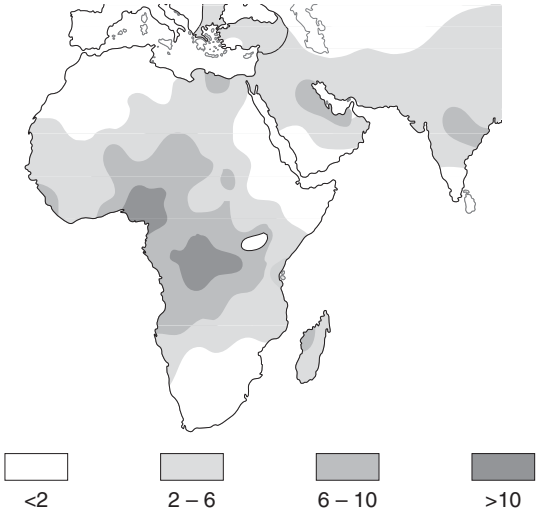


Figure 1.29 Frequency of HbS alleles (in %). The distribution of sickle cell anemia correlates with the spread of *Plasmodium falciparum*. (Compiled from various sources.)

the sickle cell gene not much higher in endemic areas? Models have ascertained that a balanced polymorphism is involved here – in malarial regions and under certain conditions, heterozygous carriers of the gene benefit from its protection against malaria and have a selective advantage, so their fitness is relatively high. This is why the frequency of the gene increases.

However, as the proportion of HbAS individuals increases, so does the probability that they will produce homozygous HbSS-type offspring that cannot survive. This reduces the genetic fitness of HbAS individuals; and under these conditions, it is comparatively advantageous to possess the HbAA genotype, although it does involve susceptibility to malaria. This trade-off results in a balance in which the proportion of persons with sickle cell disease correlates with the selective pressure induced by *P. falciparum*.

Other mutations that affect red blood cells have a similar effect: Abnormalities in the hemoglobin (e.g., hemoglobin C, various forms of thalassemia), enzyme defects (glucose-6-phosphate-dehydrogenase deficiency), or changes in transport proteins (in the case of Melanesian ovalocytosis) lead to severe handicaps or death in homozygotes, while heterozygous genotypes – in contrast to unchanged genotypes – have the advantage of immunity to malaria (Table 1.3). Alleles like this spread throughout endemic areas (even remaining prevalent in the human populations of former distribution areas some time after the disease has disappeared from the population) before ultimately subsiding. This is why the **α+ Thalassemia** (from the Greek *thálassa* = the [Mediterranean] sea), the most common single-locus hereditary disease of the inhabitants of the former distribution areas of malaria, is widespread in the Mediterranean region.

Table 1.3 Some gene polymorphisms which cause resistance to *Plasmodium* infections in humans.

Protein	Disease	Mutation	Protection
Hemoglobin	Sickle cell anemia	Repl. in β chain at position 6 (valine)	Heterozygote: 60–90%
Hemoglobin	Hemolytic anemia	Repl. in β chain at position 6 (lysine)	Heterozygote: up to 74% Homozygote: up to 86%
Hemoglobin	α+-Thalassemia	Deletion, limited production of α chain	Heterozygote: up to 34% Homozygote: up to 60%
Glucose-6-phosphate dehydrogenase	Favism (conditional hemolytic anemia)	Various mutations in the G-6-PD gene	Heterozygote: up to 46% Hemizygote: up to 58%
Band 3 protein	Melanesian ovalocytosis	N-terminal extended CD233 (bicarbonate transporter)	Protection against severe malaria

Susceptibility to malaria is determined by not only polymorphisms of hemoglobin but also immune responses. This is reflected in **polymorphisms of cytokine genes**, for example, mutations in the promoter region of the cytokines TNF-α and IL-10 or the inducible nitric oxide synthase. In addition, malaria has a strong influence on the **MHC make-up** of people living in endemic areas. In high-transmission areas, alleles that permit an efficient presentation of plasmodia peptides occur frequently – for example, the allele HLA B53 binds extremely efficiently to a peptide of the antigen LSA1 of *P. falciparum*, which is formed by liver cell stages. This is believed to permit the sensitization of cytotoxic T cells that can kill infected liver cells. Rare in Europe, this MHC genotype is associated with protection against the symptoms of severe malaria.

Further Reading

Dawkins, R. (1976) *The Selfish Gene*, Oxford University Press.

Dawkins, R. and Krebs, J.R. (1979) Arms races between and within species. *Proc. R. Soc. London, Ser. B*, **205**, 489–511.

Howard, R.S. and Lively, C.M. (1994) Parasitism, mutation and the maintenance of sex. *Nature*, **367**, 554–557.

Liu, W., Li, Y., Learn, G.H. *et al.* (2010) Origin of the human malaria parasite *Plasmodium falciparum* in Gorillas. *Nature*, **467**, 420–425.

Loker, E.S. and Hofkin, V. (2015) Parasitology: A Conceptual Approach.*Garland Science.*

Morran, L.T., Schmidt, O.G., Gelarden, I.A., Parrish, R.C., and Lively, C.M. (2011) Running with the Red Queen: host-parasite coevolution selects for biparental sex. *Science*, **333**, 216–221.

Poulin, R., Skorping, R., and Poulin, R. (eds) (2000) *Evolutionary Biology of Host-Parasite-Relationships: Theory Meets Reality*, Developments in Animal and Veterinary Sciences, Elsevier, Amsterdam.

Price, P.W. (1980) *Evolutionary Biology of Parasites*, Princeton University Press, Princeton, NJ.

Schmid-Hempel, P. (2011) *Evolutionary Parasitology*, Oxford University Press, Oxford.

Segal, S. and Hill, A.V.S. (2003) Genetic susceptibility to infectious disease. *Trends Microbiol.*, **11**, 445–448.

Thompson, J.N. (1994) *The Coevolutionary Process*, University of Chicago Press, Chicago, IL, London.

de Vienne, D.M., Refrégier, G., López-Villavicencio, M., Tellier, A., Hood, M.E., and Giraud, T. (2013) Cospeciation vs host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution. *New Phytol.*, **198**, 347–385.

Test Questions

1. How do parasites exert selective pressure on their hosts?
2. How do hosts exert selective pressure on parasites?
3. Why is intraspecific competition often the strongest form of competition?
4. Why are parasites much more dependent on their host than the other way around?
5. What circumstances cause parasites to adapt to their hosts and not vice versa?
6. What does the "Red Queen" hypothesis imply?
7. Give an example of a host switch.
8. What mechanism ensures the rapid spread of resistance in host or parasite populations?
9. Why do a host population's rarely occurring alleles not simply dwindle away?
10. Which genetic diseases cause resistance to malaria?

1.5 Influence of Parasites on Mate Choice

The previous chapters have demonstrated that parasites diminish the condition of their hosts and their genetic fitness. Therefore, hosts would benefit if they could recognize and avoid infected conspecifics to avoid infection by directly transmitted parasites. Infected animals are also less suitable as sexual partners than healthy ones, as they could be less efficient in rearing the offspring and their offspring might inherit their susceptibility for parasites. What signals could allow an animal to discern whether a potential sexual partner is infected or not, and whether it carries suitable or less suitable genes? This is where **sexual selection** comes into play, a selection mechanism that relies among other things on conspicuous signals. Darwin rightly indicated that many animal species express conspicuous traits that are apparently disadvantageous and should therefore theoretically be counterselected. The expression of such **ornaments**, for example, the antlers of deer or the peacock's tail (Figure 1.30), costs much energy and in many cases renders the animal much more visible or vulnerable to predators. Heavy and bulky ornaments would also exhaust their carrier, reducing its fitness at first sight. In spite of this, elaborate ornaments prevail in the males of many animal species, as they provide distinctive advantages in the context of the choice of sexual partners.

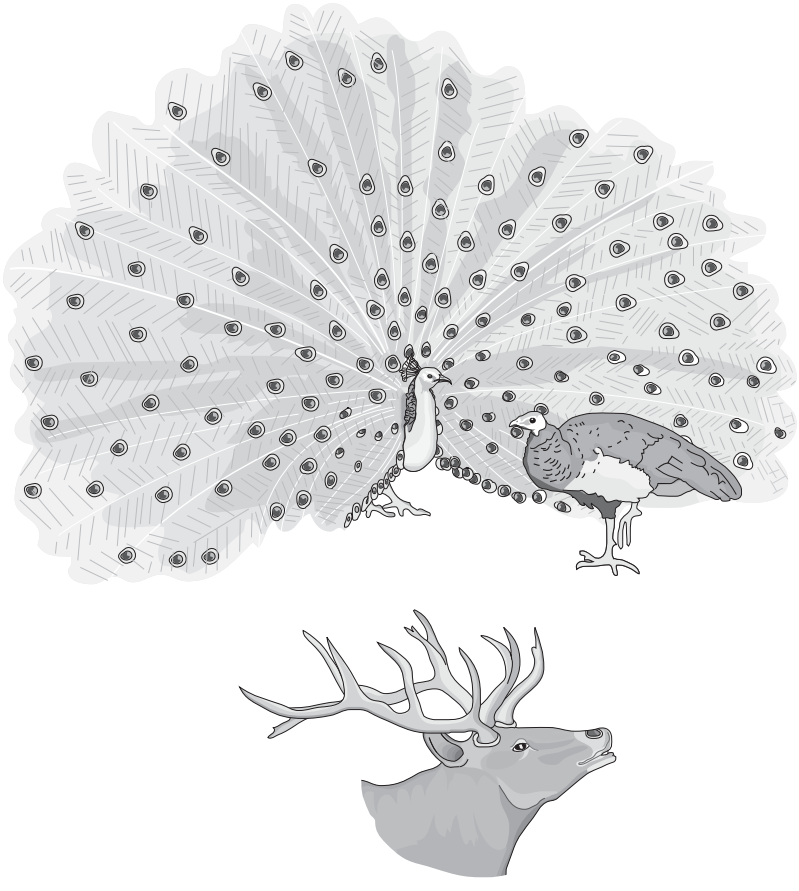


Figure 1.30 Ornaments such as the peacock's tail or deer antlers signal the genetic quality, including resistance against infections, of males to females.

According to a widely accepted hypothesis, costly ornaments are expressed by males as an indicator that these males can survive in spite of the handicap due to a good genetic constitution (**handicap principle**). In some animal groups, it is conspicuous decorative feathers, bright colors or the energetic songs and displays of males that determine which animals are finally chosen by females (**female choice**). In other animal species, for example, ungulates or seals, the selection is mainly driven by fights between competitors, the winner of which copulates with the most females (**male – male competition**). Both strategies are neither mutually exclusive nor can be strictly separated, since, for example, the dance of attractively plumed gallinaceous birds does also feature aggression among males, and vice versa the fights of deer also have an element of display, through which the males present themselves to the females. However, in both cases, a good physical condition is a prerequisite to win the favor of the females. Males with a poor physical condition, for example, due to malnutrition or infection, are disadvantaged and their genes

will not prevail. Therefore, ornaments are "**honest signals**," which allow females to choose specifically between potential sexual partners. A parasite infection usually reduces the physical condition of males, and may also reduce the expression of ornaments. The preference of females for attractive partners therefore leads to the success of males that are resistant to infections, allowing them to pass on this genetic capacity to their offspring.

Before we address the mechanisms of mate choice more closely, we must discuss why in many cases females play the decisive role in the choice of the sexual partner. Irrespective of its gender, each individual strives to pass on to the next generation as many copies of its genome as possible. However, there is a significant gender-specific difference: Females usually invest relatively much energy in each single descendent, since they produce relatively few but large egg cells, eggs, or live offspring in comparison to the many small sperms produced by the males. Consequently, females are dependent on choosing the father of their offspring very carefully, to assure that their relatively few descendents have good chances of survival and reproduction. With this selection, females optimize their genetic fitness. Conversely, males should be less choosy regarding which females get their sperm, and seek to fertilize several females to produce many descendents. In simple terms, one could say that, regarding their choice of sexual partners, females focus on quality, whereas males go for quantity. In this context, it is necessary for females to check the health status of the male similar to an army doctor during physical examination of recruits.

Various explanations can account for the choices made by females:

- Avoidance of infection of the female and the offspring (transmission avoidance model).
- Provision of safety for the family and efficient acquisition of food by a healthy father (resource provisioning model).
- Optimization of genetic quality (good-genes model).

The good-genes model is nowadays the most widely invoked to explain mate choice, but the importance of the other factors is also accepted. It is assumed that females check the quality of males based on a variety of signals, and choose a partner with the condition and disposition to produce many high-quality offspring. In this context, resistance against parasites is an important factor. Information on the quality of the male is, among other routes, transmitted by visual and acoustical signals, and also through odors. Importantly, such key triggers do not only allow an assessment of the actual health status of the male, but also of the genetic quality of a candidate. Therefore, a female can check the qualities of a male in the sense of the good-genes model to find an optimal partner that matches its genotype and allows production of offspring that are resistant to parasites.

How does a female achieve **direct discrimination** between infected and non-infected conspecifics? Experiments with mice revealed that females decide based on information from the odor of urine whether a male mouse is infected with the nematode *Heligmosomoides polygyrus* or not. The same has been shown for infections with the apicomplexan parasite *Eimeria vermiformis* and other pathogens.

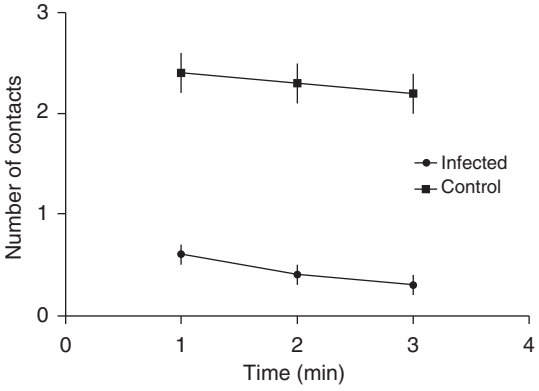


Figure 1.31 Lower attractiveness of infected males: female mice sniff at the urine of healthy male mice significantly longer than at the urine of males infected with *Eimeria vermiformis*. (According to Kavaliers and Colwell (1995) Proc. Roy. Soc. B. 261, 31–35.)

This is not surprising in itself as mice extract many chemical signals from the urine of their conspecifics. The experiments revealed that healthy females had an aversion to the urine of infected males (Figure 1.31). They avoid these males and prefer uninfected animals for mating. Males, too, discerne between infected and healthy females by the odor of their urine and prefer uninfected female partners. Because females play a more important role in mate choice, their behavior is more decisive for mating and reproduction. Many similar studies suggest that the health status plays a key role in partner choice in other animals as well.

Studies with knock out mutants allowed to identify a key gene involved in the analysis of male infection status by female mice. Females with an inactivated oxytocin gene could not discern *H. polygyrus*-infected males from healthy ones. Oxytocin is a neurohormone that, among other things, is effective in creating a bond between sexual partners, or between parents and their offspring. Therefore, it seems as if the uptake of odor is followed by a rating that is mediated by oxytocin, and which decides between attraction or aversion. Oxytocin-deficient female mice cannot efficiently rate the quality of a potential male partner by its odor, and therefore instead copy the choices of other females regarding the selection of males, as revealed by mate choice experiments.

Indirect discrimination between male candidates based on their ornaments has also been very well demonstrated experimentally. As the genetic traits for the expression of ornaments seem to be linked to resistance genes, ornaments inform about not only the actual state of health but also the genetic quality of an animal. A female that chooses an attractive male therefore also chooses at the same time a partner that is likely to pass on resistance to parasites to their offspring. This **parasite-mediated sexual selection** is considered one of the driving forces in the coevolution of parasites and their hosts (Figure 1.32). Parasite-mediated sexual selection was first convincingly described in 1982 by Hamilton and Zuk. Analysis of data regarding parasite infection of American song birds revealed that males of

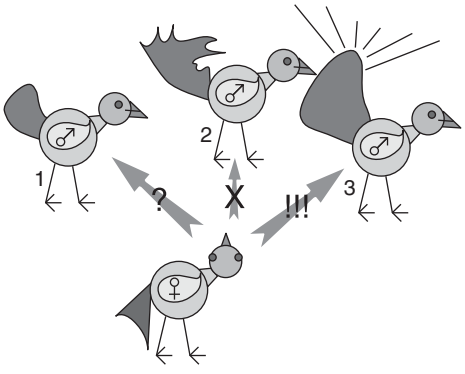


Figure 1.32 Parasite-mediated sexual selection. A female is rejecting male 1, since its poorly developed tail does not allow a statement on its parasite burden. The female rejects male 2, since its ruffled tail signals a bad health status. Male 3 is accepted due to its healthy looking attractive tail. (According to Clayton, D.H. (1991) *Parasitol. Today*, **7**, 329–334, by courtesy of the publisher.)

those species that are heavily parasitized by *Plasmodium* and related Hematozoa, as well as filarial nematodes, show the most conspicuous plumage colors. Since then, numerous experimental studies have corroborated the connection between expression of ornaments and resistance to parasites.

Some of the most convincing studies on parasite-mediated sexual selection were performed by Milinski and his group on the three-spined stickleback, a small freshwater fish. The experiments revealed that in this species, decorative colors are of great importance for the choice of a male by a female. In three-spined sticklebacks, the male builds a nest composed of plant materials and foam into which it lures the female to lay her eggs. In order to achieve this goal, it performs a dance consisting of a fixed sequence of behavioral elements: in the presence of a female ready to produce eggs, the male swims steeply upward and then falls back toward the nest with faltering movements. At the same time, it displays its bright red flanks to the females. With this mating dance (if successful), the female is lured closer to the nest, in which it deposits its eggs, which are then fertilized by the males (Figure 1.33). If the female is not interested in the male, it will swim away after a short time.

In a laboratory setting, the attractiveness of a male for a female has been shown to correlate with the length of time during which the female shows interest in the male's dance, from which it was separated by a glass pane. Males with extensive red color attracted the attention of female fish for significantly longer than the weakly colored males. The bright red color indicates the health state of the fish. The intensity of color declines in male sticklebacks that are infected with the ciliate parasite *Ichthyophthirius* (see Section 2.6.5.2). The key experiment consisted in comparatively determining the reaction of stickleback females to males before and after an infection with *I. multifiliis*. Male fish were significantly less attractive after they had undergone an infection and therefore showed diminished

Figure 1.33 Stickleback dance: The stickleback male tries to impress a female that is ready to lay eggs (noticeable by her bulging belly) through its red underbelly and lures the female into the nest. (According to J. Münzing in Grzimeks Tierleben (1970) Verlag Kindler Zürich.)

coloration (Figure 1.34). Consequently, male fish resistant to *I. multifiliis* have an advantage over their infected rivals, and their genotypes have a better chance to spread in the stickleback population.

The ornamental red coloration of male sticklebacks is produced by high concentrations of carotenoids in their skin. As the production of these pigments is relatively energy-intensive, red color is predominantly expressed by healthy animals. As the coloration indicates the actual state of health, it is known as a "**honest signal**" that allows females to choose healthy sexual partners. However, in the stickleback case, a rating based on coloration only allows a female to detect an actual infection, but not necessarily the genetic quality of a male. If coloration were the only criterion for choice, females might choose males that actually do not harbor a current infection, but are intrinsically highly susceptible due to their genetic disposition.

Further experiments have revealed that female sticklebacks not only rely on the coloration of male fish, but also check their genetic quality based on olfactory signals, as shown in choice tests. Male sticklebacks were kept in separate aquaria and only the "conditioned" water, containing soluble molecules released by the fish, was offered to females. It was shown that females exhibit individual preferences for water in which particular males had been maintained, probably due to olfactory signals of the males as key factors. Analysis of MHC genes showed that females preferred males whose MHC genes complemented their own genotype optimally, such that the probability of producing offspring with resistance to parasites was high. Such mate choice based on odors is not restricted to fish, but was also shown for mammals and even for humans. In the latter, too, odors convey information

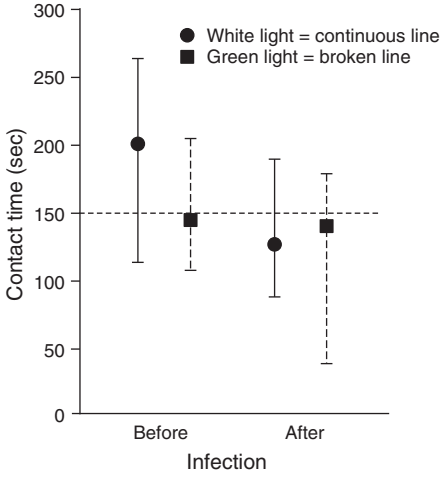


Figure 1.34 Time spent by female sticklebacks with male before and after infection with *I. multifiliis*. Before infection, males seen in white light (continuous line) are more attractive than in green light (broken line), because green light extinguishes the bright red color of males. Infection reduces the red color, such that males are less attractive, and attraction is similar in white and green lights. (Created from data of Milinski, M. and Bakker, T.C.M. (1990) *Nature*, **344**, 330–333.)

on the MHC type of a potential partner. In choice experiments, women preferred odors of men whose MHC genes optimally matched their own genotype, such that their potential offspring would have good genes allowing efficient defense against pathogens.

The ranking of mates based on criteria that provide information on infection with parasites and/or the quality of the genotype allows the production of resistant offspring with a much higher efficiency than random mate choice. Apart from the aforementioned odors and ornaments, many other signals can contribute to the optimal choice of sexual partners. However, it should be remembered that parasites may also subvert such signals to increase their chances of being transmitted. Taken together, the existence of highly sensible communication systems suitable for the choice of partners resistant to parasites demonstrates the key importance of these pathogens in evolution.

Further Reading

Andersson, M. and Simmons, L.W. (2006) Sexual selection and mate choice. *Trends Ecol. Evol.*, **21**, 296–302.

Beltran-Bech, S. and Richard, F.J. (2014) Impact of infection on mate choice. *Anim. Behav.*, **90**, 159–170.

Ehmann, K.D. and Scott, M.E. (2002) Female mice mate preferentially with non-parasitized males. *Parasitology*, **125**, 461–466.

Hamilton, W.D. and Zuk, M. (1982) Heritable true fitness and bright birds: a role for parasites? *Science*, **218**, 384–387.

Kavaliers, M., Choleris, E., and Pfaff, D.E. (2005) Genes, odours and the recognition of parasitized individuals by rodents. *Trends in Parasitology*, **21**, 423–429.

Möller, A.P. (1994) *Sexual Selection and the Barn Swallow*, (ed. A.P. Möller), Oxford University Press, Oxford.

Vyas, A. (2013) Parasite-augmented mate choice and reduction in innate fear in rats induced by *Toxoplasma gondii*. *J. Exp. Biol.*, **216**, 120–126.

Test Questions

1. Which functions do ornaments have?
2. What is the handicap principle?
3. What are the potential disadvantages for a female and her offspring of mating with males showing weak ornaments?
4. Why is the male stickleback so colorful?
5. How do animals estimate the best fit of a partner's MHC genes with their own?

1.6 Immunobiology of Parasites

All parasites induce a variety of innate and adaptive immune responses – but they are not necessarily eliminated by these defensive reactions of the hosts. Parasites are in fact gifted immunologists that have developed successful strategies during the course of evolution – and these strategies help them evade host immune defenses and manipulate their hosts' immune systems. The ineffectiveness of some defense reactions previously led to the misconception that hosts do not develop immunity to parasites. Currently, it is known that many **effector mechanisms** of hosts kill parasites efficiently or at least limit their spreading. However, "successful" parasites that are well adapted to their hosts have efficient **evasion mechanisms**, which they use to thwart immune responses – a situation that often results in a stalemate. If malfunctions in the immune system disrupt this balance, some pathogens that are normally harmless can multiply unhindered, causing severe disease or even death ("opportunistic infections").

In order to understand the immunobiology of parasite infections, we must consider the fact that individual genetic differences always occur within populations. As far as the host is concerned, immune effector mechanisms vary, giving them the ability to ward off parasites. Within a parasite population, the ability to evade effector mechanisms probably differs between individuals. Therefore, the course of the infection in many parasitoses varies greatly on an individual basis according to the different genotypes of host and parasite. In addition, there exist typical patterns for many parasite infections. In some host–parasite associations, clinically apparent infections occur only rarely, while the majority of the host population limits the infection to a subclinical level (e.g., in infections with *Leishmania donovani*). By contrast, the majority of the host population in highly endemic areas is infected with the filarial nematode *Onchocerca volvulus* and nearly all individuals will have clinically relevant infections, but with significantly varying degrees of severity – while most individuals will have relatively low worm burdens, a few

will be "worm-ridden." These examples show that parasites always encounter a range of hosts with different degrees of susceptibility. Conversely, hosts are faced with a range of parasite genotypes with different degrees of virulence.

Depending on these differences, the symptoms of parasitoses can vary widely indeed. In particular, marked individual differences occur when the clinical outcome of a parasite infection is determined by immunopathology, that is, when immune responses significantly influence the symptoms.

Parasites are not only at the mercy of the immune system – in many cases, they use it for their own purposes, like the regulation of their population density. In a particular type of immunity – **premunition** – or "concomitant immunity," protection against superinfection with the same parasite exists in the presence of infection (Figure 1.35). In many helminth infections, established worms evade the host immune responses – but infective stages do not have this ability and are eliminated. Also, in protozoan infections, for example, in the case of *Toxoplasma gondii* infections, immune responses induced by tissue cysts protect against new infections, with the result that competing conspecifics cannot colonize the host and the parasite burden remains limited. The term premunition has now been largely superseded by *concomitant immunity*, a term that originated in tumor research. In the case of intense infections with worms, yet another mechanism of population regulation (the *crowding effect*) results in the individual parasites remaining, on average, small and producing few offspring – the host suffers no undue stress and this consequently benefits the parasite in the longer term. Immune responses can also be exploited by parasites to transport their offspring to the outside world (see *Schistosoma mansoni*, section 3.1.1.5) and in some parasitoses it has been shown that host cytokines act as growth factors for the parasites, so well-adapted pathogens parasitize not only the host but also the host's immune system.

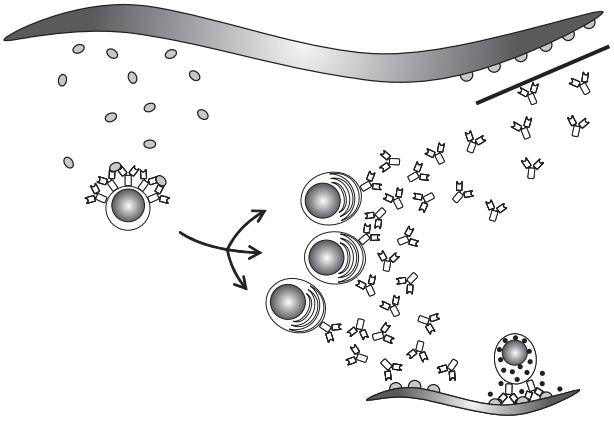


Figure 1.35 Schematic representation of premunition. The antigens of worms (gray ovals) induce immune responses that eliminate infective larvae; but established worms block these responses with immune evasion mechanisms (represented by a bar). (By R. Lucius (1996) in Allgemeine Parasitologie (2006). Eds. Hiepe, Lucius, Gottstein, Parey in MVS Medizinverlage Stuttgart.)

This portrayal of the interaction between parasite and host at the level of the immune system will be limited here to parasites of vertebrates, although similar processes occur in invertebrate hosts. Typical patterns will be illustrated through examples of infections which have been intensively studied due to their medical and economic relevance. Since a comprehensive description of the functioning of the immune system is beyond the scope of this chapter, a textbook of immunology should be consulted where necessary.

1.6.1 Defense Mechanisms of Hosts

Hosts mount very different innate and adaptive immune responses against different parasites, depending on the different stimuli of the pathogen, the cells or organs affected, the duration of infection, and other factors. Even when infected with a single species of parasite, different immune responses can be induced, since each development stage may trigger specific reactions or can colonize immunologically distinct compartments within the host. For example, the liver stages of the malaria parasite *Plasmodium falciparum*, see Section 2.6.3.5) can be eliminated by cytotoxic T cells, while the blood stages of the same parasites are being attacked by antibodies. Therefore, the overall result of a parasite infection is usually a very complex suite of immunobiological processes.

1.6.1.1 Innate Immune Responses (Innate Immunity)

The infective stages of pathogens first trigger innate immune responses in a host. Here, molecular structures are detected as foreign to the host, but typical of pathogens, such as bacterial cell wall components or characteristic DNA sequences, or double-stranded viral RNA. The general term used for these structures is ***pathogen-associated molecular patterns*** (PAMPs). PAMPs are recognized by host receptors located on the cell surface or inside the cells. These receptors are referred to as ***pattern-recognition* receptors** (PRRs), such as Toll-like receptors (TLR) and Nod receptors. The binding of PAMPs to PRRs triggers specific signal chains, resulting in the activation of the cells and leading to the activation of effector reactions and the attraction of inflammatory cells. Pathogen molecules can also be detected by the complement system, which can cause the attraction of inflammatory cells and the destruction of foreign cells by soluble factors. Innate immune reactions like these often kill a large proportion of the parasite. For example, as many as 80% of the infective larvae are killed in some helminth infections. At the same time, these early-occurring, nonspecific immune responses also set the course for the imprinting of the later-occurring adaptive immune response.

One typical configuration of innate immunity against parasites is the activation of dendritic host cells, which in turn sets off a chain of reactions (Figure 1.36). PAMPs activate the dendritic cells and the latter produce cytokines (IL-12, IL-18, TNF-α, and possibly also IL-4), chemokines, and other factors that attract other cells chemotactically. The cytokines produced by dendritic cells often activate

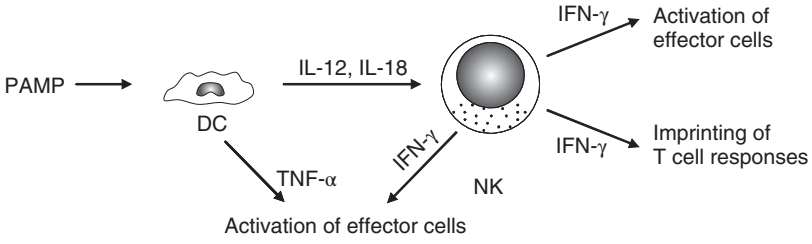


Figure 1.36 Example of the triggering of innate immunity. In dendritic cells (DC), parasite molecules (PAMPs) initiate an activation process, which leads to the formation of IL-12 and IL-18. In natural killer cells (NK), this leads to the production of IFN-γ, which affect other cells. For details, see text. (By R. Lucius In Allgemeine Parasitologie (2006) Eds. Hiepe, Lucius, Gottstein, Parey in MVS Medizinverlage Stuttgart.)

natural killer (NK) cells. These cells secrete IFN-γ, which, in turn, activates other cells. Relatively few PAMPs of parasites have been discovered to date. In the case of trypanosomes, plasmodia and Toxoplasma, glycolipid anchors of proteins have been identified as triggers that lead to the production of IL-12, IL-18, and TNF-α through binding to TLRs, with subsequent induction of IFN-γ. When amplified by TNF-α, this cytokine can trigger parasite-infected cells to kill their intracellular pathogens. Simultaneously, the nascent adaptive immune responses are instructed towards a proinflammatory Th1 direction (see below). However, some PAMPs (such as certain lipids from schistosomes) result in the production of IL-4 by dendritic cells and this promotes the less inflammatory Th2-type immune responses.

The activation of the effector cells by IFN-γ and other cytokines varies depending on the cell type. In the case of macrophages, activation causes (among others) the upregulation of phagocytosis and the production of reactive oxygen products (*oxidative burst*). Neutrophils and eosinophils release cytotoxic molecules stored within granules. The granules' cytotoxic molecules attack the pathogens, but also damage the host's own tissues. Even in the case of epithelial cells or fibroblasts, the activation by cytokines may result in changes in cell metabolism, resulting in the killing of parasites. Intracellular parasites can, for example, be defeated by changes in the tryptophan or iron metabolism. Just a single cytokine can trigger an extensive defense program, for example, the action of IFN-γ regulates more than 1000 genes of host cells.

The importance of a recently defined group of innate leukocytes, innate lymphoid cells (ILCs), has been confirmed in parasitic infections. These cells are highly potent cytokine-producing cells and comprise at least three major subsets. NK cells are also members of this group and the different populations are believed to work in concert with cells of the adaptive immune system in promoting different types of immune responses. They often play very important roles in the initiation of immune responses at barrier surfaces, for example, the skin and gut, where they expand in numbers in response to growth factors released by cells found at these sites (e.g., epithelial cells) following infection and damage.

The traditional roles of many innate cells are also increasingly recognized following the use of sophisticated techniques for analyzing cell function such as flow cytometry and gene expression. Macrophages adopt different functional capabilities depending on whether they are activated by particular cytokines, for example, M1 macrophages activated by IFN-γ and M2 macrophages by IL-4.

Furthermore, granulocytes such as eosinophils, neutrophils, basophils, and mast cells have been identified as regulatory cells that can modify their environment and the immune response through the secretion of cytokines such as IL-4, IL-13, TNF-α, and others.

1.6.1.2 Acquired Immune Responses (Adaptive Immunity)

The nature and intensity of initial innate immune responses have a major impact on the direction of the acquired immune response, which develops in the days following infection. The early cytokine response also influences the reaction of T cells, which are sensitized in lymph nodes near the infection site. Here, T helper cells play a dominant role through their function in orchestrating the qualitative and quantitative aspects of immune responses. Cytotoxic T cells play a more specialized role in targeting host cells with intracellular infection. The T helper cells are sensitized by presentation of parasite-derived peptides in the context of MHC II by dendritic cells and other antigen-presenting cells. The presentation of such peptides to the T cell receptor – in conjunction with costimulation by other molecules – activates the T cells to divide and produce cytokines. Depending on how a dendritic cell was triggered in the early phase of infection by PAMPs, the cell steers the differentiation of T cells in different directions (see below) and the dividing T helper cells (Th) take on a different phenotype (Figure 1.37).

- Th1 cells are characterized by the production of IFN-γ and other cytokines. They activate macrophages and other cells to kill intracellular pathogens; in humans, this leads to the formation of IgG2 and IgG3 antibody classes (IgG2a and IgG2b in mice), which are in turn efficiently detected by many effector cells. Strong overall inflammatory responses are the result, and these often damage the host tissue ("immunopathology").
- Among other messenger substances, Th2 cells produce IL-4 as a typical cytokine. This mainly triggers B cells to grow, and as a result greatly stimulates the production of antibodies, particularly of the classes IgG1, IgG4, IgA, and IgE in humans (IgG1, IgE, and IgA in mice). Mast cells and eosinophils are also stimulated to divide and are subsequently activated. Th2 responses thus move the immune response in a direction that is particularly suited to the destruction of worms, by IgE and eosinophils, for instance. This too can result in damaged host tissues ("Th2 inflammation"). These inflammatory responses, however, tend to be weaker than Th1 responses.
- Th17 cells produce IL-17 and IL-22, which induce recruitment of neutrophils and stimulate epithelial cells to produce antimicrobial effector molecules. They are involved in epithelial and mucosal immunity, but also in many autoimmune diseases.

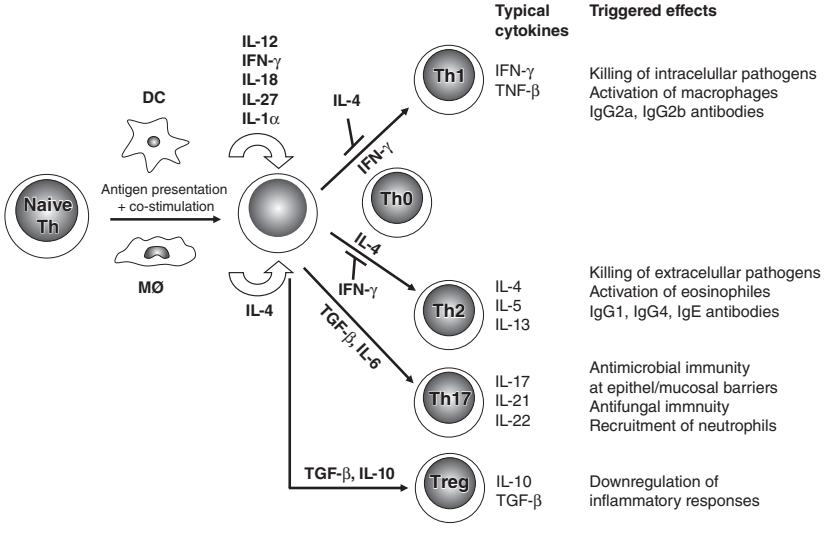


Figure 1.37 Differentiation of T cell subpopulations in the mouse. The differentiation is significantly influenced by the context of antigen presentation, in particular by the cytokine signals of antigen-presenting cells during the sensitization of T cells. In the presence of IL-4, there is a tendency to imprint Th2 cells (typical for helminth infections), producing a specific pattern of cytokines. Similarly, the presence of other cytokines results in Th1, Th17, or Treg responses. For details, see text. From Lucius, R. In Allgemeine Parasitologie (2006) Eds. Hiepe, Lucius, Gottstein, Parey in MVS Medizinverlage Stuttgart.

- Regulatory T cells produce anti-inflammatory cytokines such as IL-10 and TGF-β. Their main task is the specific downregulation of excessive immune responses.

In a number of well-studied infections, Th1 responses develop in the early stages of a parasite infection. These responses limit the spread of the parasite, but switch to Th2-type responses during the course of a chronic infection. Often a crucial factor during the course of an infection is the correct sequence of immune responses. If switching to less inflammatory Th2 responses is insufficient during the chronic phase of infection, for example, the host may suffer from severe immunopathology.

1.6.1.3 Scenarios of Defense Reactions Against Parasites

Just which effector mechanisms attack and eliminate parasites depends greatly on the size and location of pathogens and is also determined by the compartment (e.g., skin, intestine, and blood) in which they live. Intracellular protozoa are thus targeted by different immune responses than large extracellular parasites such as helminths. In most cases, there is not merely a single suitable effector mechanism – several or many components of the immune system work together. The effectiveness of immune responses is also decisively determined by the immune evasion mechanisms of the parasites (see below):

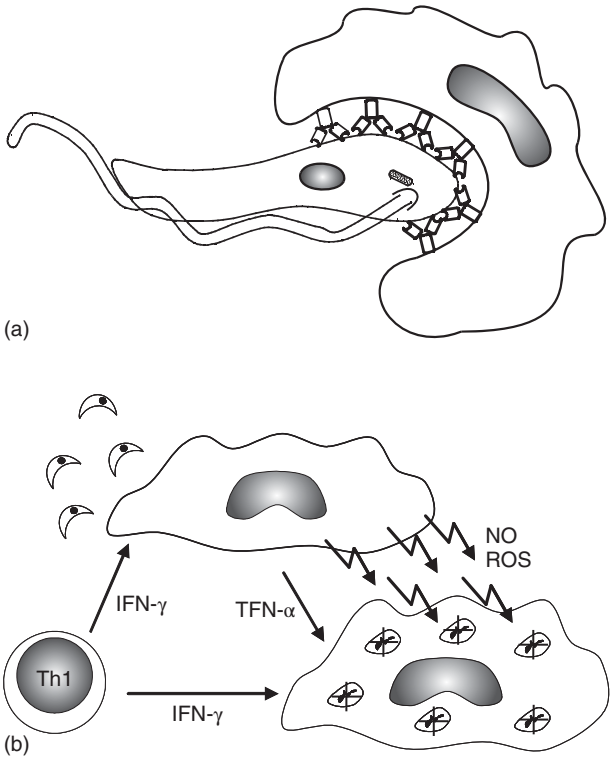


Figure 1.38 (a) Immune attack on extracellular protozoan parasites. Surface-bound antibodies make the parasite detectable for effector cells, resulting in phagocytosis. This process can be amplified by complement activation. (b) Killing of intracellular parasites. Th1 cells stimulate the host cell with IFN-γ to kill their intracellular parasites. IFN-γ can also result in the activation of macrophages that use their effector molecules to kill intracellular parasites in neighboring cells. From Lucius, R. in Allgemeine Parasitologie (2006). Eds. Hiepe, Lucius, Gottstein, Parey in MVS Medizinverlage Stuttgart.

- Small extracellular parasites can often be controlled by humoral immune responses alone (Figure 1.38a) – so probably the great majority of *Leishmania* from an early infection are repelled by activation of complement on the alternative pathway. Antibodies can prevent the adhesion of pathogens to host cells, agglutinate parasites, or make them detectable for phagocytes, mechanisms which, for example, eliminate many merozoites of plasmodia. Antibodies which activate complement are very efficient – the activation results, for example, in the elimination of *T. brucei* trypomastigotes in the blood by phagocytes.
- Intracellular parasites are of course shielded from antibodies and complement, but they can still be reached by several effector mechanisms. If presentation of parasite epitopes in the MHC I context occurs on the surface of the host cell, the cell can be killed by cytotoxic T cells – liver stages of *Plasmodium* can be eliminated in this way, for instance. Under certain circumstances, infected

host cells may kill their intracellular pathogens themselves by either producing cytotoxic molecules (e.g., reactive oxygen products or NO) or, if they have been activated by exogenous factors such as IFN-γ or TNF-α, by changing metabolic pathways (Figure 1.38b). A "kill" from the outside can also take place when nearby effector cells secrete cytotoxic molecules that diffuse into the infected cells, killing intracellular parasites.

- In order to defend against the relatively large helminths, however, the joint effort of several components of the immune response is usually necessary. The classic defense against worms is *antibody-dependent cellular cytotoxicity* (ADCC) – here, antibodies, possibly reinforced by complement activation through the classical pathway, make the surface of the parasites detectable for effector cells, which can then attack. In this way, eosinophils, neutrophils, or macrophages bind to the worms and release their effector molecules on to the surface of the parasite, harming it (Figures 1.39 and 1.40).
- The IgE-dependent degranulation of mast cells plays an important role in the defense against helminths in the intestines. Products of mast cell granules (primarily histamine) make the capillaries and epithelia permeable, attracting eosinophils, which in turn attack the worms. The release of certain peptides can activate peristalsis and initiate massive mucus production, expelling parasitic worms from the gut with a mechanism (*rapid expulsion*) that exhibits similarities to allergic reactions (Figure 1.41). Antibodies which gain access to the intestines can also lead to ADCC reactions.

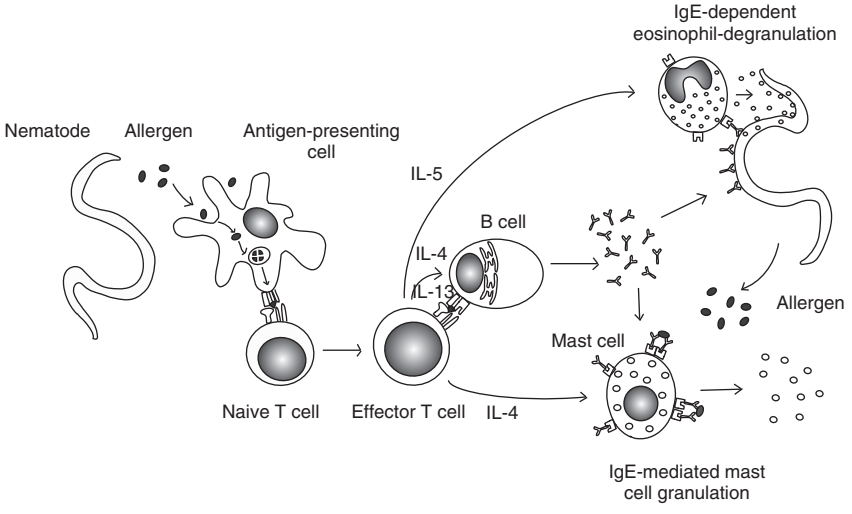


Figure 1.39 Immune attack on worms. The allergens secreted by worms induce IgE antibodies, which make the worms detectable for attacking eosinophils. The IgE-dependent degranulation of mast cells facilitates the recruitment of eosinophils. For details, please see the text.

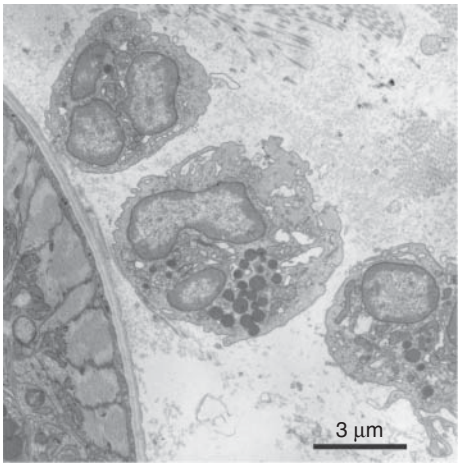


Figure 1.40 Eosinophils attack a third-stage larva of the filarial nematode *Acanthocheilonema viteae* in the tissue of a gerbil. (EM image: Department of Molecular Parasitology, Humboldt Universität.)

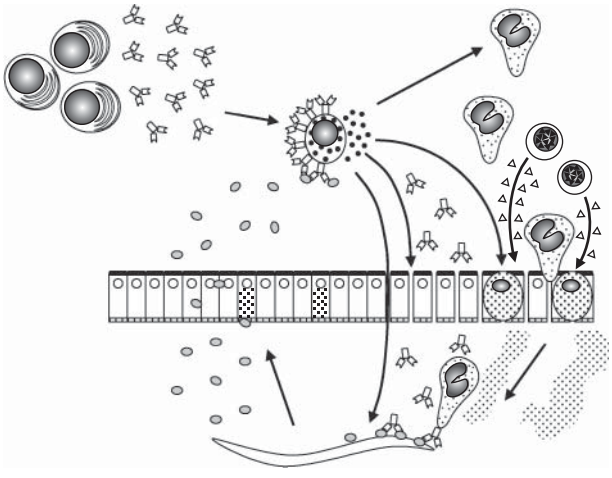


Figure 1.41 Immune attack on nematodes in the intestine. Worm antigens (*gray ovals*) passing into the sensitized tissues of the host that have already formed IgE antibodies, result in the degranulation of mast cells. The released mast cell products attract granulocytes and myeloid cells that release cytokines (black triangles), stimulate epithelial turnover and goblet cells to produce mucus. These factors loosen the association of epithelial cells, creating permeability for antibodies, eosinophils, and the effector molecules of mast cells. Some mast cell products also act directly on worms. The combination of these effects results in the rapid expulsion of nematodes. For details, see text. (From Lucius, R. in Allgemeine Parasitologie (2006). Eds. Hiepe, Lucius, Gottstein, Parey in MVS Medizinverlage Stuttgart.)

- The saliva of hematophagous arthropods contains various compounds, which can trigger allergic reactions of both rapid and delayed types. This can prevent the parasites from taking blood.

1.6.1.4 Immunopathology

The symptoms of many parasitoses are characterized by pathological reactions caused by immune responses. Since the immunoreactivity of individuals is very varied depending on individual genetic predisposition and environmental influences, immunopathology is also varied – as is the clinical picture of the infection as a result. In host–parasite associations that have a long evolutionary history, the reactions are often limited, so serious diseases tend to be rare.

A common cause of immunopathology is **excessive inflammatory reactions**, where cytotoxic effector molecules are released. These not only damage the parasites, but also harm the surrounding host tissue (collateral damage). The constant stimulation by PAMPs (which are derived from persistent parasites) can thus cause chronic inflammatory responses that lead to the damage of host tissue. One example of this is the chronic infection of heart muscle cells (cardiomyocytes) by *Trypanosoma cruzi*, see Section 2.5.8). This leads to permanent myocarditis (Figure 1.42). This, in turn, causes the long-term degeneration of areas of the heart muscle, which may lead to muscle fatigue and rupture.

Diseases caused by immune complexes are another frequently occurring element of immunopathology: antibodies bind to parasite antigens, when they are released in great numbers in malaria infections, for instance. These immune complexes circulate in the blood, and are preferentially deposited in narrow vessels with high pressure and flow speed. They then activate complement, initiating the attraction of inflammatory cells and causing tissue damage. In the glomeruli of the kidney, this process can cause immune complex glomerulonephritis with chronic kidney problems, such as those that frequently occur in malarial or filarial

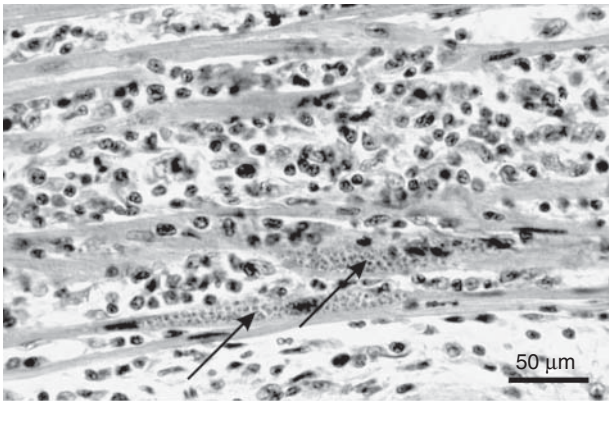


Figure 1.42 Myocarditis in Chagas disease. The muscle fibers have been infiltrated by inflammatory cells. Arrows: amastigote stages of *Trypanosoma cruzi* in muscle cells. (Image: Archive of the Department of Molecular Parasitology, Humboldt University, Berlin.)

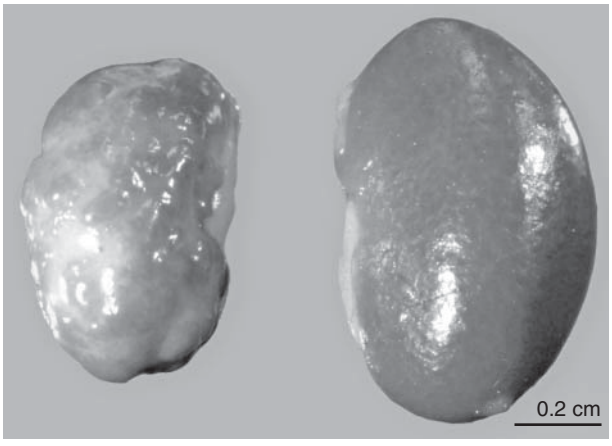


Figure 1.43 Kidney damage due to immune complex-mediated inflammation in gerbils infected with the filarial nematode *Acanthocheilonema viteae*. Left, damaged kidney; right, healthy kidney. (Image: Richard Lucius.)

infections (Figure 1.43). Small immune complexes can also escape from capillaries and activate complement in tissue, resulting in perivascular inflammations. In the case of *T. brucei* infections, perivascular inflammations are assumed to be a major factor in the development of sleeping sickness.

In many parasitoses, **immunosuppression** is the result of defects in the formation or function of leucocytes caused by parasite-related immunomodulation or exhaustion of particular immune responses. This can lead to increased susceptibility to other infections. In the case of *Leishmania donovani* infections, for example, a general depression of cell-mediated immune responses is observed. Together with other factors such as malnutrition, this depression is considered to underlie secondary infections (measles, pneumonia, tuberculosis, and others), which are a common cause of death. In the case of infections with trypanosomes and *Leishmania*, macrophages emerge with strong immunosuppressive properties. They produce prostaglandin E2, for instance – and this strongly impairs the ability of the lymphocytes of infected individuals to proliferate. Another type of immunosuppression takes place after massive stimulation of B cells by B cell mitogens of *Trypanosoma cruzi*. Here, the simultaneous stimulation of all the B cells impedes specific antibody responses from developing. In addition, the constant stimulation of T or B cells by parasite antigens can lead to clonal exhaustion – a mechanism that has been held responsible for immunosuppression in malaria patients.

1.6.2 Immune Evasion

Parasites can only colonize an immunocompetent host if they avoid, thwart or alter its immune responses. This capacity is known as **immune evasion**. In most

parasite–host systems with a long coevolutionary history, every host effector mechanism has its counterpart in a parasite evasion mechanism, so effector and evasion mechanisms cancel each other out to some extent. This balance prevents the host from controlling parasite infections completely, as it must limit its effort because of trade-offs with other functions. Excessive investments in immune defense would not necessarily result in improved net fitness for the individual. A host that perfects its defenses at the cost of reproduction probably has a lower level of fitness than a conspecific which takes a residual risk of infection into account. On the contrary, investment in evasion mechanisms is of ultimate importance for a parasite, as their failure would be lethal (see also life/dinner principle, 1. 4. 1). The existence of very efficient immune evasion mechanisms also explains why it is difficult to develop antiparasite vaccines.

The spectrum of evasion mechanisms ranges from simple immune response-avoiding tactics and the circumvention of effector mechanisms to interfering with the control of the immune system (Table 1.4). The establishment of parasites in cells, tissues, or organs characterized by low immune responses is considered an

Table 1.4 Examples of effector mechanisms of hosts and corresponding immune evasion mechanisms of parasites.

Effector mechanisms	Evasion mechanisms	Pathogens
Activation of complement	Complement inhibitors in the surface membrane	*Trypanosoma cruzi*, *Schistosoma mansoni*
Oxidative burst of macrophages	Inhibition of macrophage activation and detoxification of reactive products through LPG	*Leishmania*
Antibodies	Intracellular lifestyle	*Trypanosoma cruzi*, *Leishmania*, Apicomplexa, *Trichinella*
Antibodies	Cutting of the FC ends by specific proteases	*Schistosoma mansoni*
Antibody-dependent complement-mediated cytotoxicity through Kupffer stellate cells	Antigenic variation	*Trypanosoma brucei*
Antibody-dependent cellular cytotoxicity by eosinophils	Induction of connective tissue nodule, permanent migration through the tissue	*Onchocerca volvulus*, *Loa loa*
Cytotoxic T cell responses	Survival in cells without or with little MHC I on the surface, Reduction of MHC I	*Plasmodium* (erythrocytes), *Toxoplasma* (neurons)
Inflammation caused by Th1 responses	Polarization of the T cell response to Th2	*Schistosoma mansoni*, filarial nematodes
Inflammation caused by Th2 responses	Inhibition of cell activation through IL-10 produced by various immune cells	Filarial nematodes

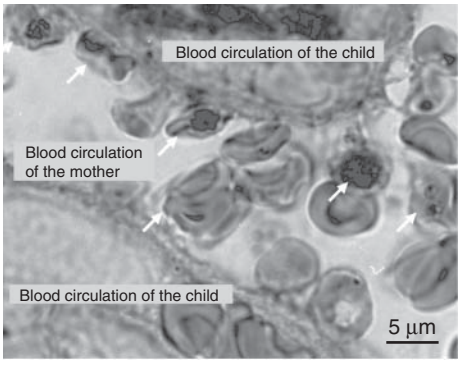


Figure 1.44 Cytoadherence. *Plasmodium falciparum*-infected erythrocytes (arrows) in a maternal blood vessel of the placenta. (Image: Courtesy of Mats Wahlgren.)

avoidance strategy. For instance, erythrocytes infected with *Plasmodium falciparum* prefer to adhere to the placenta's capillary walls. This is attributed to the fact that this environment is "immunoprivileged" (Figure 1.44) owing to immunosuppressive mechanisms active in this organ to prevent rejection of the fetus. The preference of some parasites for the central nervous system (e.g., tissue cysts of *Toxoplasma gondii*, see Section 2.6.2.6) is also attributed to less efficient immune responses in that environment. An intracellular localization can protect parasites from immune responses to a certain extent, as the spatial partitioning exempts them from being recognized by antibodies. Inflammatory cells can also be kept at bay to some extent through tissue barriers, a strategy that is pursued by some parasitic worms. The filarial nematode *Onchocerca volvulus*, see Section 3.3.4.14), for example, induces the formation of coarse connective tissue nodules, in which the convoluted female worms (up to 50 cm long) are ingrown, while the males are mobile. Very few inflammatory cells usually exist in these nodules, suggesting that the dense nodule tissue together with other mechanisms prevents effector cells from accessing them (Figure 1.45).

Parasites can also avoid effector mechanisms if they constantly keep on the move, preventing the effector cells from attacking effectively. It is quite conceivable that skin-dwelling microfilariae of the filarial nematodes *O. volvulus* and *Mansonella streptocerca* actually cast off effector cells during their constant migration through the connective tissue – and the same probably applies to the adult stages of the filarial nematode *Loa loa* that migrates through subcutaneous tissue.

Parasites can also incorporate host molecules into their surface, thereby escaping detection by the immune system. The best known example of this *antigen disguise* is displayed by adult *Schistosoma mansoni*: These worms incorporate among others MHC molecules, blood group antigens, and complement protein into their outer surface layer.

Some small parasites, which are genetically flexible due to rapid division rates, can evade the antibody responses of the hosts by varying their surface antigens

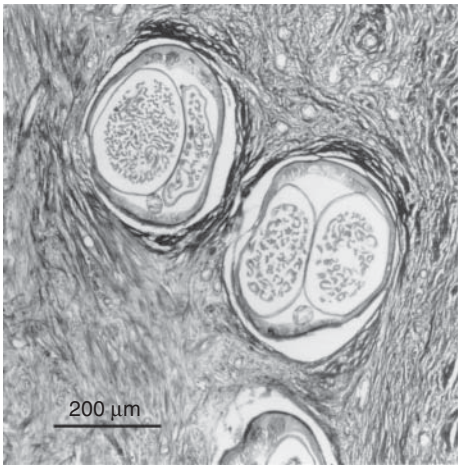


Figure 1.45 Sections of *Onchocerca volvulus* females in skin nodules. Note the absence of inflammatory cells in the connective tissue. (Image: Department of Molecular Parasitology, Humboldt University, Berlin.)

(trypanosomes, plasmodia, *Giardia*). The best example of antigenic variation is illustrated by *Trypanosoma brucei*. It has a large family of genes, which encode variable surface glycoproteins, and different variants of these antigens are expressed approximately every 10 days – so any specific antibody response will quickly become obsolete and prove futile, with the parasites managing to remain one step ahead of the immune response. The specific aspects of antigenic variation in individual parasites will be discussed later in this book.

One key strategy of immune evasion is the inactivation of effector molecules. The inhibition of complement activation is vital for many single-cell organisms and worms – three different complement inhibitors have been found in *Trypanosoma cruzi* alone. Antibodies can be rendered ineffective by highly specific proteases secreted by parasites, and cytotoxic effector molecules of immune cells, such as reactive oxygen and nitrogen products, are counteracted by increased production of detoxifying parasite enzymes (glutathione-S-transferase, glutathione peroxidase, catalase, etc.).

By secreting specifically–acting products, parasites can also interfere with the cytokine network, enabling them to modulate local or systemic host immune responses. Some parasites manage to suppress proinflammatory Th1 immune responses (which are potentially dangerous to the pathogens) in favor of less aggressive Th2 immune responses (see *Taenia crassiceps*, Section 1.7.2). In filarial infections, studies have identified several secreted parasite products that alter the activation and cytokine production of immune cells, downregulating inflammatory responses. It is therefore assumed that helminths and other parasites interfere with the central switching points of the immune system, altering the host's "immunological phenotype" to suit their purposes.

1.6.3
Parasites as Opportunistic Pathogens

Individuals with an intact immune system ("immunocompetent") can repel or limit many potentially pathogenic organisms. By contrast, if important components of the immune system fail, some protozoa, helminths, and arthropods can establish themselves, causing diseases that either do not occur in immunocompetent individuals or are limited or controlled by their immune system. Pathogens that occur exclusively or predominantly in immunocompromised hosts are termed **opportunistic pathogens**. This category includes many important parasites of humans and animals.

Since the spread of HIV/AIDS in the 1980s, the importance of opportunistic pathogens has increased dramatically, because AIDS patients usually die from infections that would normally pose no threat to immunocompetent individuals. In the case of AIDS, the decrease in the number of T helper cells colonized and destroyed by the virus is the main cause of immunosuppression in HIV-infected individuals. There is a clear correlation here between the number of $CD4^+$ cells and susceptibility to different pathogens (Figure 1.46).

Similarly, immunosuppression through other causes can also increase susceptibility to pathogens. Transplant recipients are threatened, for example, since they are chemically immunosuppressed to reduce rejection reactions. The chemotherapy or radiation therapy of tumors may also be associated with immunosuppression, increasing the risk of infection. The unborn and the old are also more susceptible to certain infections, since the immune system of the fetus is not yet developed and the immune system's efficiency decreases with age. Malnutrition (and particularly the protein deficiency usually associated with it) is also a widespread cause of immune deficiency – and this is why many infectious diseases in tropical developing countries are more frequent and severe.

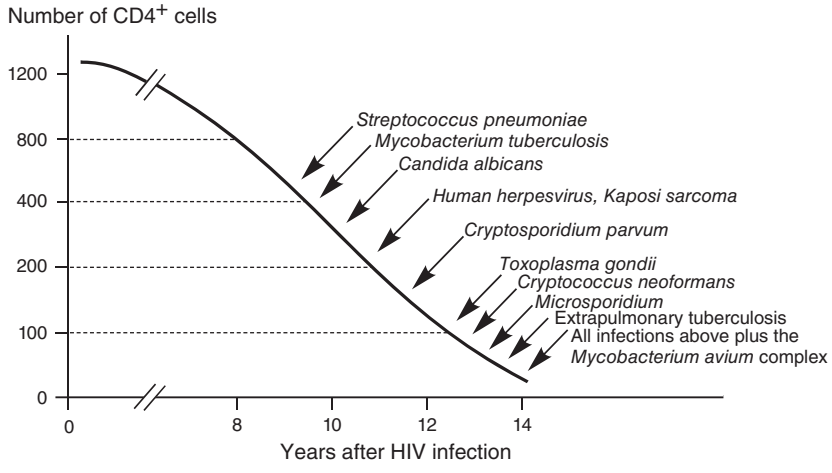


Figure 1.46 Occurrence of opportunistic infections depends on the density of $CD4^+$ T cell per µl of blood. (Composed from various sources by W. Presber.)

As opportunistic pathogens, ***Leishmania*** (see Section 2.5.9) have become very important in relation to the AIDS pandemic. Many clinically healthy persons are probably latently infected in endemic areas, but the parasites cannot spread under the pressure of the immune response. It is believed that such silent infections are clinically relevant in persons suffering from immunosuppression, due to AIDS, for instance. The probability of developing a visceral leishmaniosis is therefore 100–2300 times higher in AIDS patients than in immunocompetent people infected with *Leishmania* – and the fact that around 70% of all reported cases of leishmaniosis in Spain and Portugal involve HIV patients is explained by the reactivation of latent infections.

Among Apicomplexans, one important pathogen-causing opportunistic infection is ***Cryptosporidium parvum***, see Section 2.6.2.1). This parasite can cause severe bouts of diarrhea in AIDS patients – and such bouts can be the direct cause of death. Unfortunately, there is no specific treatment for cryptosporidiosis, as drugs acting against other Apicomplexans are not effective against *C. parvum*. ***Cyclospora cayetanensis***, an *Isospora*-like parasite of tropical climes, may also cause severe diarrhea in immunocompromised people. In healthy persons, infections with ***Toxoplasma gondii***, see Section 2.6.2.6) mostly run subclinical courses with flu-like symptoms that heal after several weeks: under the pressure of the immune response, tissue cysts form preferentially in the brain. These cysts contain long-term resting stages, the bradyzoites. If the level of immune responses decreases (e.g., as a result of HIV infection), the dormant stages can be activated and tachyzoites then differentiate, spreading locally into the surrounding tissue. Inflammation and tissue damage in the brain can cause large and potentially lethal lesions (Figure 1.47). *Toxoplasma* infections passed from mother to fetus can also

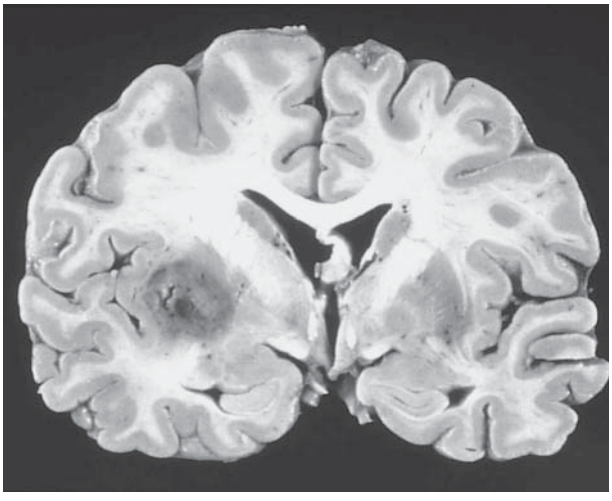


Figure 1.47 Reactivated toxoplasmosis in the brain of an AIDS patient. Note the necrotic area in the left hemisphere. (Image: Courtesy of Julio Martinez.)

cause varying degrees of fetus damage. In the first trimester of pregnancy, a *Toxoplasma* infection usually results in the death of the embryo or an abortion, while later infections can cause severe defects.

In immunocompromised individuals, infections with ***Balantidium coli*** (see Section 2.6.5.1) and ***Entamoeba histolytica*** can cause severe bouts of diarrhea – and in the case of amoebic infections, extraintestinal infections in HIV-infected patients may occur more frequently than in immunologically healthy individuals. *Acanthamoeba*, the pathogen that causes granulomatous meningoencephalitis, is also more common in immunocompromised individuals.

Certain helminths and arthropods can also be opportunistic pathogens. The nematode (***Strongyloides stercoralis***, see Section 3.3.4.1) can cause severe infections in immunocompromised people. One peculiarity in this parasite's life cycle is noteworthy: *S. stercoralis* is capable of autoinfection, which is unusual for helminths (see Section 1.2.3). Great numbers of larvae occur in immunosuppressed hosts. During their migrations through the host's body, the larvae can cause life-threatening inflammations and various changes in organs like the lungs and brain.

Eyelash mites, ***Demodex folliculorum*** and ***Demodex brevis*** (see section 4.2.4.1), can also play an opportunist role. These mites, living inconspicuously in hair follicles and the sebaceous glands of the hair follicles can cause skin diseases in immunocompromised individuals. The itch mite ***Sarcoptes scabiei*** causes a higher-grade disease in immunocompromised patients. Large areas of the body can be affected and the disease can develop into the severe form known as Norwegian scabies.

1.6.4
Hygiene Hypothesis: Do Parasites Have a Good Side?

The last half of the twentieth century has seen a steady increase in allergic and inflammatory diseases in developed countries, but not, however, in less developed countries. According to many scientists, this is due to the decline of infections in countries with highly developed hygiene (the "hygiene hypothesis"). It has been suggested that the relatively rare occurrence of childhood infections in industrialized countries together with other factors would leave regulatory circuits of the immune system untrained. As a consequence, overshooting inflammatory responses in adults would become more frequent. This hygiene hypothesis has an additional aspect, as infections with pathogens may also downregulate inflammatory responses. In particular, helminth infections have a significant impact on allergic and inflammatory reactions, as recently revealed by epidemiological data, animal experiments, and clinical trials.

For instance, in Africa and South America, children infected with *Schistosoma mansoni* or with the hookworm *Necator americanus*, respectively, were dewormed in carefully controlled studies and checked for allergic skin reactions. Dewormed children had significantly more allergic skin reactions to house dust mite allergen – thus infestation with parasitic worms clearly protects against this

type of allergy. However, only chronic worm infections have this effect – weaker, temporary worm infections can even increase susceptibility to allergies – so it is obvious that long-term helminth infections can alter the regulation of the immune system in such a way that the tendency to allergies (and other inflammatory reactions) is reduced.

This alleviation of allergic diseases is seen today as a positive side effect of the evasion mechanisms through which parasitic worms block IgE-mediated immune attacks of the host. IgE-mediated mast cell degranulation and IgE-mediated binding and degranulation of eosinophils are classical defense reactions against worms. In allergic infections, these same reactions – elevated levels of IgE, eosinophilia, and activation of mast cells – are triggered by environmental allergens such as pollen, molecules in cat hair, or dust mites. In a sensitized individual, when allergens like these are detected by IgE antibodies bound to the surface of basophils or mast cells via specific Fc receptors, an immediate reaction takes place, in which the cells release histamine, chemotactic substances, and cytokines. The subsequent reactions lead to swelling, redness, and itching. Later stages of the allergy are characterized by the accumulation of cells, which are mainly eosinophil granulocytes that cause tissue destruction through the release of their granules. These allergic symptoms can affect the skin, the mucous membranes of the eyes and nose, for example, or the lungs in the form of allergic asthma.

So, which evasion mechanism of parasitic worms could prevent the onset of these allergic symptoms? Four main causes have so far been discussed:

- Inefficient degranulation of basophils and mast cells due to changes in antibody responses. Helminth infections usually stimulate the production of nonspecific IgE. This results in the "dilution" of allergen-specific IgE on the surface of effector cells. At the same time, IgG4 production can be strongly stimulated and compete for epitopes, intercepting antigens before they come in contact with IgE-sensitized basophils and mast cells.
- The secreted products of parasites could prevent the attraction and activation of effector cells, for example, by cleaving eotaxin, an attractant and activator of eosinophils.
- The induction of regulatory T cells, which downregulate inflammatory responses with the IL-10 and TGF-β cytokines they produce.
- The induction of regulatory macrophages, which inhibit downstream activation of immune cells and effector mechanisms.

Can the anti-inflammation effects caused by helminth infections be used to influence undesirable immune reactions such as allergies and inflammations? Various animal models have shown that colon inflammation, autoimmune diabetes, asthma, gastritis, and experimentally induced brain inflammation may indeed be reduced by nematode infection or nematode products. Positive effects like this could be harnessed if it were possible to decouple them from the harmful effects of a parasite infection.

The following example shows that this is indeed possible: on the basis of data from studies in animal models, clinical studies have been conducted with patients

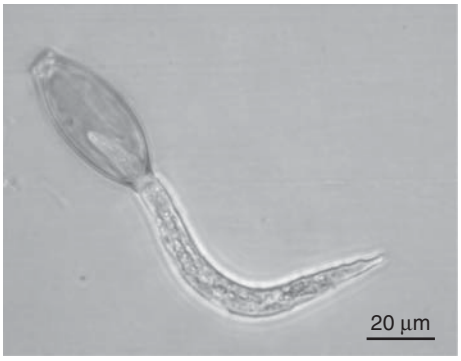


Figure 1.48 Hatching larva of *Trichuris suis*. (Image: Courtesy of Ovamed.)

suffering from the chronic inflammatory bowel diseases known as ulcerative colitis and Crohn's disease. Eggs of the pig whipworm *Trichuris suis* (Figure 1.48 see also Section 3.3.3.2) were administered to the subjects at regular intervals. The larvae of *Trichuris suis* hatch in the human intestine and die there, since they do not reach sexual maturity in what for them is an unsuitable host. During the course of their brief development, they reduce inflammatory responses, with the result that a marked improvement of the disease was observed in a significant proportion of patients.

These patients do not suffer ill effects from the parasite, since the larvae die at an early stage. The spreading of the parasites is also excluded, because the worms do not produce eggs. It is thus possible to take advantage of the positive effect of a parasitosis – without suffering any negative impact. Further studies must show whether or not a similar treatment can also affect allergies and other inflammatory diseases. As parasite molecules involved in downregulation of immune responses have been characterized, treatments based on defined molecules might become available in the future.

Further Reading

van den Biggelaar, A.H., van Ree, R., Rodrigues, L.C., Lell, B., Deelder, A.M., Kremsner, P.G., and Yazdanbakhsh, M. (2000) Decreased atopy in children infected with *Schistosoma haematobium*: a role for parasite-induced interleukin-10. *Lancet*, **356**, 1723–1727.

Crompton, P.D., Moebius, J., Portugal, S., Waisberg, M., Hart, G., Garver, L.S., Miller, L.H., Barillas-Mury, C., and Pierce, S.K. (2014) Malaria immunity in man and mosquito: insights into unresolved mysteries of a deadly infectious diseases. *Annu. Rev. Immunol.*, **32**, 157–187.

Farrar, J., Hotez, P., Junghanns, T., Kang, G., Lalloo, D., and White, N.J. (2013) *Manson's Tropical Diseases*, 23rd edn, Elsevier Publishers, 1360 pp.

Grencis, R.K. (2015) Immunity to helminths: resistance, regulation, and susceptibility to gastrointestinal nematodes. *Annu. Rev. Immunol.*, **33**, 205–231.

Lynch, N.R., Hagel, I., Perez, M., Prisco, M.C., Lopez, R., and Alvarez, N. (1993) Effect of anthelminthic treatment on the allergic reactivity of children in a tropical slum. *J. Allergy Clin. Immunol.*, **92**, 404–411.

Maizels, R.M. (2005) Infections and allergy – helminths, hygiene and host immune regulation. *Curr. Opin. Immunol.*, **17**, 656–661.

McKenzie, A.N., Spits, H., and Eberl, G. (2014) Innate lymphoid cells in inflammation and immunity. *Immunity*, **41**, 366–374.

Melendez, A.J., Harnett, M.M., Pushparaj, P.N., Wong, W.S.F., Tay, H.K., McSharry, C.P., and Harnett, W. (2007) Inhibition of FceRI-mediated mast cell responses by ES-62, a product of parasitic filarial nematodes. *Nat. Med.*, **13**, 1375–1381.

Solbach, W. and Lucius, R. (2005) Parasite evasion (incl. helminths), in *Topley and Wilson – Microbiology and Microbial Infections*. Immunology Volume (eds S. Kaufmann and M. Steward), Edward Arnold, London.

Summers, R.W., Elliott, D.E., Urban, J.F., Thompson, R.A., and Weinstock, J.V. (2005a) *Trichuris suis* therapy for active ulcerative colitis: a randomized controlled trial. *Gastroenterology*, **128**, 825–832.

Summers, R.W., Elliott, D.E., Urban, J.R., Thompson, R., and Weinstock, J.V. (2005b) *Trichuris suis* therapy in Crohn's disease. *Gut*, **54**, 87–90.

Wilson, M.S., Taylor, M.D., Balic, A., Finney, C.A., Lamb, J.R., and Maizels, R.M. (2005) Suppression of allergic airway inflammation by helminth-induced regulatory T cells. *J. Exp. Med.*, **202**, 1199–1212.

Test Questions

1. How are pathogens recognized by innate immune responses?
2. How can intracellular parasites be killed by their host cells?
3. By which mechanisms can intestinal helminths be expelled very rapidly?
4. Which type of immune response is induced by insect saliva?
5. Give an example of inflammatory disease caused by persisting parasites.
6. Which organ is frequently damaged by immune complex diseases?
7. How can nematodes isolate themselves spatially from immunoreactive compartments of the body?
8. Give an example of disguise with host antigens and examples of antigen variation.
9. What is the danger of latent toxoplasmosis in immunocompromised individuals?
10. What is the relationship between allergy and helminth infections?

1.7 How Parasites Alter Their Hosts

With their relatively long generation times, eukaryotic parasites tend to exploit their hosts for long periods. In order to create an optimal niche, they modify the morphology, metabolism, immune reactions, and/or behavior of their hosts. Using highly specific mechanisms, the pathogens alter properties of their host – or in other words, they modify its phenotype. Therefore, parasite genomes do not stop at encoding their own phenotype; parasite genes are also expressed through modifications of the host phenotype. Evolutionary biologist Richard Dawkins compared this capability of parasites with the ability of beavers to change a landscape to their advantage by building dams and coined the term "**extended**

phenotype." Many spectacular changes such as parasitic castration or bizarre behavioral alterations of the host provide examples of the importance of this concept. More subtle modifications are found in almost all parasite infections, so we can generally assume that well adapted parasites manipulate their hosts to optimize their own survival, reproduction, and transmission. The following chapter presents examples of this phenomenon. Modifications of host-defensive reactions have already been discussed in Section 1.6.

1.7.1 Alterations of Host Cells

Many intracellular parasites alter their host cell in a spectacular way – and the mechanisms they use to do this remain mostly unknown. For example, the transformation of host cells by stages of cyst-forming coccidia can result in extreme enlargement – *Sarcocystis gigantea* produces tissue cysts of up to 15 mm in length within the pharynx muscle cells of sheep (Figure 1.49). The survival of these modified cells – which are almost completely filled by parasite stages – necessitates a substantial reprogramming of the host cell. In a less obvious way, *Toxoplasma gondii*, see Section 2.6.2.6, Fig. 2.57). modifies its host cell by attracting mitochondria and the endoplasmic reticulum toward the parasitophorous vacuole and by inducing the formation of a thin wall around tissue cysts. The molecular processes underlying such changes are studied by -omics techniques such as transcriptomics, proteomics, and metabolomics, but these studies are still restricted to model parasites. Such work indicates that intracellular parasites interfere with transcription regulation and signal transduction in a targeted manner. For instance, *T. gondii* releases specific phosphatases that activate particular transcription factors, which, in turn, regulate cytokine expression of the host cell. A far-reaching modification can also be achieved without influencing gene-regulatory networks, as shown by the example of *Plasmodium falciparum* modifying its red blood host cell, which is devoid of a nucleus. *P. falciparum* creates within the erythrocyte's cytoplasm a network of membranes, through which proteins are transported to the surface of the host

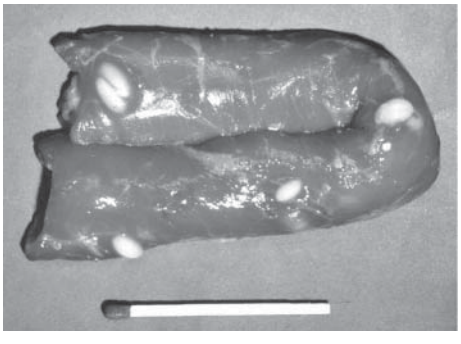


Figure 1.49 Muscle cyst of *Sarcocystis gigantea* on the pharynx of a sheep. (Image: Archive of the Department of Molecular Parasitology, Humboldt University, Berlin.)

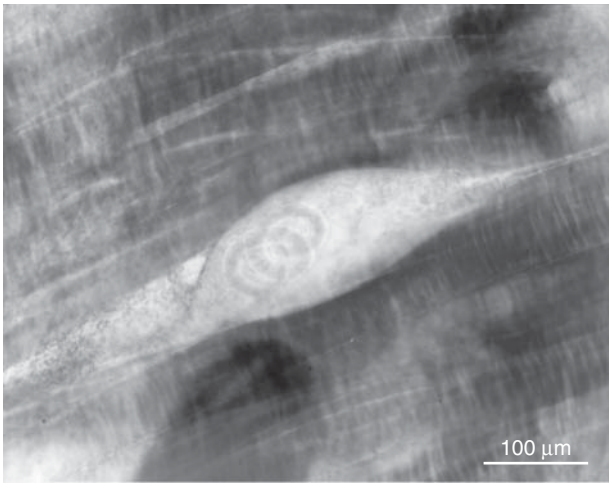


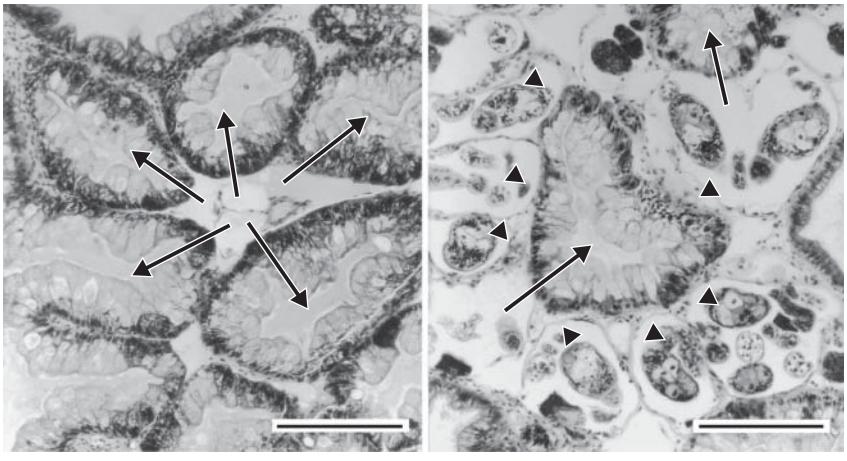
Figure 1.50 Nurse cell first-stage larva of *Trichinella spiralis* in the muscle tissue of an infected rat. (Image: Archive of the Department of Parasitology, University of Hohenheim.)

cell to allow adhesion of infected erythrocytes to blood vessel endothelia. These mechanisms are dealt with in more detail in other chapters.

The ability to alter host cells is not limited to single-celled organisms. The first larval stage of the nematode *Trichinella spiralis*, see Section 3.3.3.2) penetrates a muscle fiber and reorganizes the fiber in such a way that it grows to several times its original size (Figure 1.50). A collagen sheath is also formed and the resultant nurse cell is supplied with nutrients from newly formed blood vessels. In the case of *T. spiralis*, parasite proteins have been found in the nucleus of the host cell – thus, it is assumed that the intracellular nematode larva interacts with transcription regulation to alter the cell activity. A similar ability to reprogram the host cell has also been demonstrated in plant-parasitic nematodes. These examples show that parasites possess strong mechanisms to reprogram their host cell in very targeted ways.

1.7.2 Intrusion into the Hormonal System of the Host

Digenetic trematodes provide particularly compelling examples of the intrusion of parasites into the hormone metabolism of their hosts. Trematodes have inhabited their molluscan hosts since the Paleozoic era about 570 million years ago – and thanks to this prolonged coevolution, they have evolved the ability to exploit their hosts to a very great extent. This is impressively demonstrated in the study of infected snails: the largest organ in mollusks, the hepatopancreas, is normally brown, but in infected individuals of many species, it is very bright-colored. This is caused by the almost total replacement of the tissue by trematode stages (sporocysts, rediae, cercariae, Figure 1.51). The gonads of infected snails are often reduced or even missing altogether because the parasites have castrated



(a) (b)

Figure 1.51 Cross section of the liver of a *Biomphalaria glabrata* snail infected with *Schistosoma mansoni* (b) and the liver of a control animal (a). Note the degree of displacement of liver tissue (arrows) by parasitic stages (arrow heads). (Scale = 150 µm). (Image: Department of Molecular Parasitology, Humboldt University, Berlin.)

their hosts, either hormonally or mechanically. In trematodes with sporocyst stages, hormonal castration usually occurs, while species with rediae can actually eat the gonads. This "parasitic castration" diverts resources from the host snail's own reproduction to the production of parasite stages. Egg laying by the snail is either limited or completely stopped in favor of cercariae production.

However, this is not the only consequence of castration. In the dwarf pond snail *Galba truncatula*, infection with *Fasciola hepatica*, see Section 3.1.1.8) larvae results in not only complete castration but also enhanced growth of the snail, with the result that parasitized snails achieve a significantly greater weight than uninfected control animals ("parasitic gigantism," Figure 1.52). This seems paradoxical, since the rediae of *F. hepatica* actively consume host tissue. However, the loss of the gonads seems to allow diversion of resources normally allocated to reproduction and makes them available for somatic growth, allowing other host tissues, including the shell, to achieve greater than normal sizes. In addition, the loss of parts of the host is obviously offset by the biomass of the parasite.

Evidence of molecular mechanisms that cause hormonal castration is found in *Trichobilharzia ocellata* infections of *Lymnaea stagnalis*. This pond snail regulates its growth, metabolism, and reproduction activity by means of peptide hormones produced by specialized nerve cells in the brain. It has been found that infection causes a marked change in the snail's hormone pattern. An obvious key element in these alterations is the defense peptide schistosomin, which is released into the hemolymph shortly after contact with the parasite occurs. Schistosomin suppresses the female gonadotropic neurohormone calfluxin. The decrease in hormone levels inhibits the rate of protein synthesis in the snail's albuminous

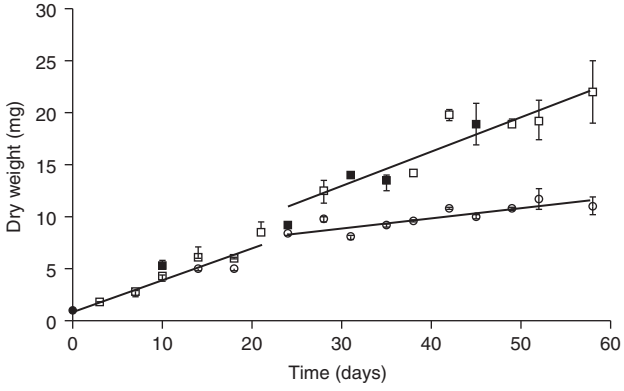


Figure 1.52 Growth of the snail *Galba truncatula* after infection with *Fasciola*, expressed by increase in dry weight. Uninfected snails (*circles*) begin to produce eggs after 20 days and grow only slightly. Infected snails (*squares*) are castrated, produce no eggs, and continue to grow. (From Wilson, R. A. and Denison, J. (1980) Z. Parasitenkd. 61, 109–119.)

gland, reducing it to <1% of the initial rate. Since the albuminous gland produces the highest proportion of egg protein, this intrusion leads to a drastic reduction in egg production.

Metacestodes also effectively regulate the hormonal system of mammalian hosts, as illustrated by a series of studies on *Taenia crassiceps* infection in mice (Figure 1.53). *T. crassiceps* is a cestode of foxes and dogs; its metacestodes develop in the abdominal cavity of rodents and multiply there asexually. Mice can thus be easily infected with metacestodes intraperitoneally – and the growth and the effects of metacestodes on the host can then be studied. In the early stages of the infection, *Taenia crassiceps* reproduces faster in female mice than in male mice.

In later stages of the infection, the male mice also provide good conditions for the parasites. This shift is due to the feminization of the males by the parasites. In the case of a prolonged infection, male mouse testosterone levels drop to 10% of their initial value, while the levels of estradiol increase up to 200 times their normal amount. Here the benefit for the parasite lies in the modulation of immune responses, which are partly controlled via the endocrine system. A female-oriented hormone system favors the development of the immune responses of the less aggressive Th2 type. This response allows better growth of the metacestodes than the Th1-oriented immune responses of uninfected males. Estradiol also acts as a growth factor by favoring the growth of the metacestodes.

Feminization by *T. crassiceps* involves the interaction of the immune and endocrine systems. A key event here is the production of IL-6 in cells of the testes, which, in turn, induces the expression of aromatase P-450. This enzyme causes testosterone to be converted to estradiol. IL-6 also boosts the production of the follicle-stimulating hormone (FSH), which in turn increases the expression of aromatase. The importance of IL-6 is underpinned by the fact that IL-6 *knock-out* mice are not feminized by the parasite.

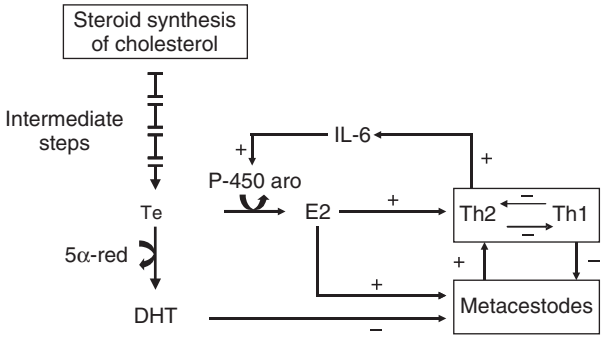


Figure 1.53 Suggested mechanism of the feminization of male mice by metacestodes of *Taenia crassiceps*. The metacestodes stimulate the immune system toward a Th2-oriented response, leading to the expression of IL-6 in cells of the testes. IL-6 stimulates the expression of the enzyme aromatase p-450 (P-450 aro), which converts testosterone (Te) to estradiol (E2) rather than converting it into dihydroxytestosterone (DHT) by means of 5α-reductase (5α-red). Estradiol in turn acts as a growth factor for metacestodes and favors Th2 immune reactions. (From Morales-Montor, J. and Larralde, C. (2005) *Parasitology*, **131**, 287–294.)

The consequences of infection with *T. crassiceps* metacestodes are serious for the male mouse, because its sexual activity ceases and its male dominance behavior changes completely. Feminization thus represents a hormonal castration, the purpose of which is to optimize asexual reproduction of metacestodes in the peritoneal cavity of mice. The metacestodes of other tapeworms are also dependent on the hormone levels of their hosts for their growth, as demonstrated by infections with metacestodes of the pig tapeworm *Taenia solium*. In boars, the prevalence and intensity of metacestode infection is significantly lower than in sows, but this ratio is balanced when boars are castrated. Even in the human population, women are more frequently seropositive and have higher titers of anti-*Taenia* antibodies, an indication of an increased prevalence of cysticercosis. However, it is not known whether tapeworms also interfere with the hormonal system of their human hosts.

1.7.3 Changing the Behavior of Hosts

Many parasite infections bring about changes in the behavior of hosts. It has often been documented that behavioral changes of infected intermediate hosts make it easier for final hosts to catch and/or ingest the intermediate hosts. In some cases, infected intermediate hosts have reduced escape responses and are probably simply exhausted – and this poorer condition increases chances of falling prey to the final host. Any time the behavioral changes of an infected host result in an increase in parasite fitness, natural selection will favor parasites capable of sophisticated manipulation of host behavior. The available evidence shows that in such cases, parasites often repurpose the reactions the host needs for its defense, healing or

recovery. In order to achieve this aim, pathogens often seem to affect the processing of stimuli, resulting in atypical reactions such as impaired movement, a reduction in reaction speed, or decreased photophobia. Parasites can even induce the appearance of completely new phenotypic traits (either behavioral or morphological) in their hosts, with positive consequences for parasite fitness. Examples of host manipulation extend to parasites with all types of transmission modes.

1.7.3.1 Increase in the Transmission of Parasites by Bloodsucking Vectors

Studies on insects that serve as vectors of *Plasmodium* and, *Leishmania*, have shown that the probability of transmission is greatly increased by changing the behavior of vectors. Due to the specific effects of parasites, infected insects bite victims more often, increasing the probability of transmission.

Leishmania (Section 2.5.9) develop into infective promastigote forms in the midgut of sandflies. While the fly is sucking in its meal of blood, they have to migrate through the sand fly's proboscis into the skin of the vertebrate host, against the incoming flow of blood. This difficult migration is enabled by manipulating the valve section (*Valva cardiaca*) which lies between the sandfly's midgut and the pharynx. This segment of the intestinal tract is part of the foregut and is lined with a chitinous membrane. The parasites secrete chitinases that damage the membrane, preventing the valve from closing properly, and allowing the parasites to migrate from the midgut into the pharynx, from where they reach the host tissues with the saliva. As the sand fly's bloodsucking process becomes inefficient due to the defect in the valve it interrupts its blood meal after only a short time and – still hungry for blood – flies off to land on a new host where it once more attempts to feed and spreads the parasites. In experiments with laboratory animals, nonparasitized flies only bit victims once or twice, while infected flies bit at least three times. The maximum number of bites observed was 26, of which 11 resulted in *Leishmania* infections of the animals.

Plasmodium-infected *Anopheles* mosquitoes also bite more often than uninfected control insects – and the number of bites increases in relation to the number of sporozoites in the salivary glands. Experiments have shown that the apyrase content in the saliva of infected mosquitoes is significantly reduced. Mosquitoes use this enzyme to feed from host blood vessels. It inhibits the aggregation of platelets, the first step in blood coagulation. Apyrase is therefore essential in the bloodsucking process. The reduction of the apyrase content is thought to reduce the success rate of bloodsucking attempts – but since sporozoites are transmitted with each injection of mosquito saliva, the transmission potential of mosquitoes is greatly enhanced by the parasite's actions.

1.7.3.2 Increase in Transmission Through the Food Chain

In many parasite life cycles, the infection of the definitive host occurs through the consumption of infected intermediate hosts that are the definitive hosts' natural prey. Closer analysis shows that many parasites do not passively wait for the capture of the intermediate host, but increase the likelihood of predation by changing the intermediate host's behavior.

One simple way to increase the chances that the intermediate host gets captured is to weaken it. A weak animal often detaches from its social group, reacts more slowly, and has less pronounced defense reactions, making it easy prey for predators. Metacestodes of the small fox tapeworm *Echinococcus multilocularis*, see Section 3.1.2.18) first grow in the liver, but develop later in other organs of the abdominal cavities of field mice, voles, and muskrats. Animals with advanced-stage infections show decreased mobility and less pronounced escape responses, greatly increasing the likelihood of being taken by the fox definitive host. Similarly, studies of *Echinococcus granulosus* have documented that the infection of moose with metacestodes of this tapeworm increases the probability of falling prey to wolves. This is particularly pronounced when hydatids are located in the lungs of the moose, preventing the animal from breathing properly during its headlong flight from the wolf pack.

In many cases, it is difficult to determine whether the weakening of an intermediate host is merely a by-product of parasite infection or adaptive manipulation by the parasite. However, the localization of the parasite stages and the severity of their pathological impacts often point to the action of natural selection, and suggest that the parasite have adapted to infect a particular tissue to induce a specific disability in the intermediate host. For example, the larvae of the nematode *Tetrameres americana* encyst in the muscles of grasshoppers. This restricts the insect's mobility, and the definitive bird host finds it easier to catch the slower, infected grasshoppers than the faster uninfected insects. Similarly, the metacercariae of the trematodes *Curtuteria australis* and *Acanthoparyphium* sp. encyst within the foot muscle of their bivalve intermediate hosts, impairing their ability to burrow into the sediment (Figure 1.54). This leaves the bivalves exposed to predation by oystercatchers, the parasite's definitive host. Field experiments have confirmed that heavily infected bivalves stranded on the sediment surface are several times more likely to be eaten by oystercatchers than bivalves that successfully burrow under the sediment. Impairment of the bivalve is essential to allow the parasites to reach the intestine of a bird, in which they leave their cyst (Figure 1.55) and complete their life cycle.

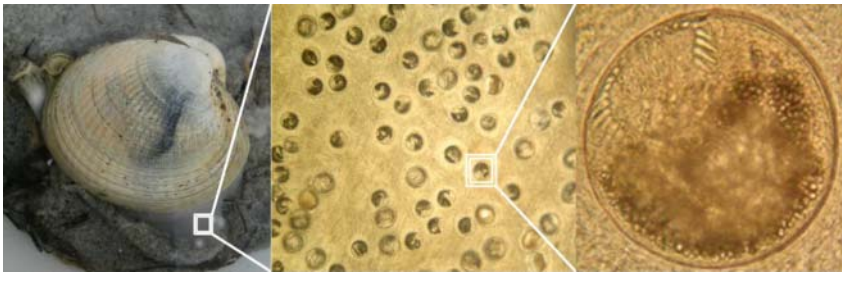


Figure 1.54 Metacercariae of *Curtuteria australis* and *Acanthoparyphium* sp. encysted in the foot tissue of the bivalve *Austrovenus stutchburyi*. (Image: Tommy Leung and Robert Poulin, University of Otago.)

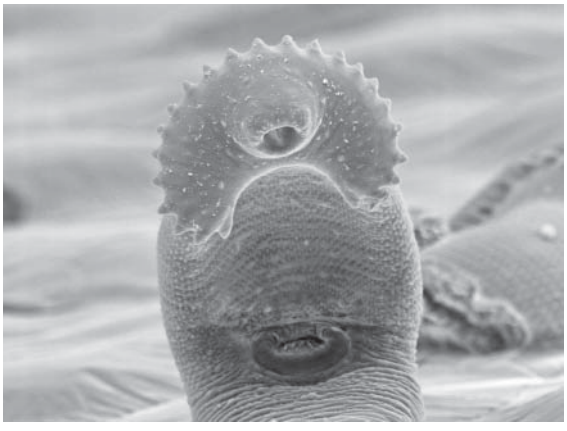


Figure 1.55 Scanning electron micrograph of a recently excysted metacercaria of *Acanthoparyphium* sp. (EM image: Haseeb Randhawa, Matthew Downes, and Robert Poulin, University of Otago.)

Not surprisingly, infection of the central nervous system can also lead to behavioral changes that facilitate transmission to the definitive host. The metacestode of the tapeworm *Taenia multiceps* settles in the brain or spinal canal of sheep, which can lead to behavioral changes. Infected sheep leave the herd and run around in circles with erratic movements, easily falling victim to the definitive hosts, which in this case are wolves or wild dogs. The metacestode consists of a cyst of a few centimeters in diameter, which contains multiple protoscolices ("coenurus"). The space occupied by this cyst in the host's central nervous system leads to pressure atrophy and subsequent deficiency symptoms, which cause the atypical behavior.

An infection of the brain of intermediate hosts can also lead to very specific behavioral changes without the need for any massive space-occupying process. This is demonstrated by *Toxoplasma gondii* infections (see Section 2.6.2.6). Bradyzoites function as long-lived resting stages of *T. gondii*, typically residing in the brain in modified host cells (Figure 1.56). Compared with control animals in maze tests, infected mice learned how to find a food reward much more slowly, and they also had poorer memory. However, they are more active, more curious, and less sensitive to light than the control animals. Importantly, the smell of cat urine is attractive to infected mice and rats – in contrast to the pronounced aversion shown by infection-free animals for that odor (Figure 1.57). This change in odor preference is highly specific. It has been explained by the concentration of brain cysts in the amygdala, an area of the brain that is involved in the development of anxiety and emotional evaluation. The behavioral changes induced by *Toxoplasma* probably make it easier for cats to catch infected rodents. Other research has even suggested a link between *Toxoplasma* infection and personality changes in human beings. However, these changes appear very subtle, and it remains to be seen whether or not a causal link can be established by further investigation.

A change in behavior can also be achieved without directly affecting the brain of the host. For instance, consider the Acanthocephala (thorny-headed worms see

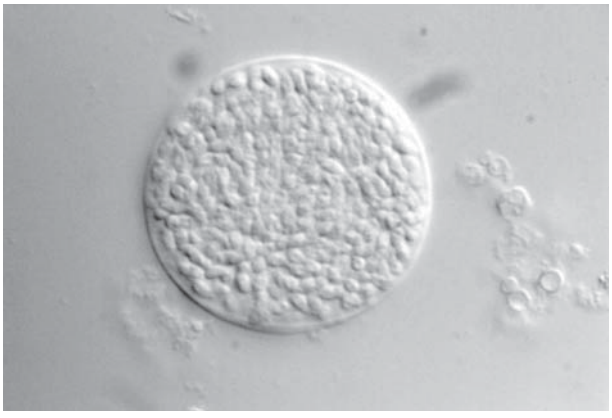


Figure 1.56 Bradyzoites of *Toxoplasma gondii* in a tissue cyst from the brain of a mouse. (Image: Archive of the Department of Molecular Parasitology, Humboldt University, Berlin.)

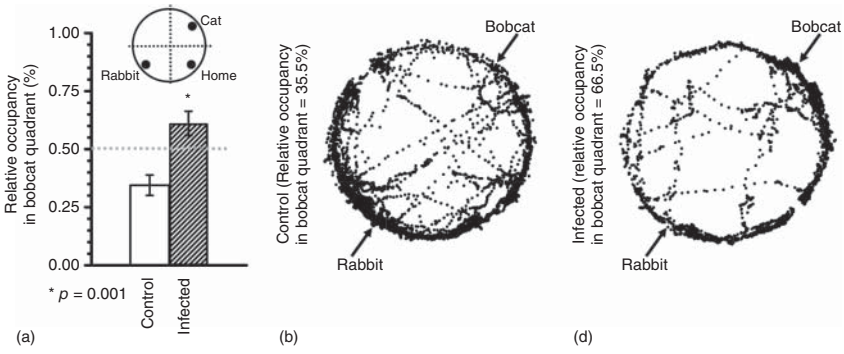


Figure 1.57 Behavioral alteration of rats infected with *Toxoplasma gondii*. Uninfected and infected rats were exposed to bobcat urine and rabbit urine in a circular arena. (a) Control animals visited the quadrant laced with bobcat urine significantly less often as compared with infected rats. (b, c) Representative scatter blots showing the movements of a control rat (left) and infected rat (right) within the arena. (From Vyas, A., Kim, S.-K., Giacomini, M., Boothroydt, J.C., and Sapolsky, R.M. (2007) *Proc. Natl. Acad. Sci. U.S.A.*, **104**, 6442–6447, with kind permission of the publisher.)

Section 3.2). The adult worms live in the intestines of vertebrates, while the cystacanth larvae develop in the hemocoel of invertebrates, usually crustaceans.

These cystacanth stages can induce distinct behavioral changes in arthropods that facilitate transfer to the definitive host. In the case of infections with *Plagiorhynchus cylindraceus* (definitive host: birds, e.g., starlings; intermediate host: woodlice), infected intermediate hosts often leave their damp hiding places and look for bright areas with low humidity, where starlings can find and ingest them more easily than uninfected woodlice. In the case of the spiny-headed worm, *Moniliformis moniliformis*, infected cockroach intermediate hosts run

more slowly, but are more active for longer periods – and when light suddenly falls upon them, they move around more than uninfected cockroaches, making them easy targets for rats, their definitive hosts.

Such behavioral changes can be very precisely controlled by parasites, as illustrated by a comparative study of the behavioral changes induced by three acanthocephalan species that exploit dabbling ducks and/or diving ducks as definitive hosts. The intermediate host for all three acanthocephalans was the amphipod *Gammarus lacustris*. Cystacanth stages of the three spiny-headed worms each induced different behavioral changes in the same amphipod – and each of the behavioral change specifically facilitated transmission to the respective definitive host (Figure 1.58). Uninfected amphipods avoided light, burying themselves in the bottom mud layer when disturbed, while infected individuals lost their aversion to light, moving to upper water layers that were not so dark. Amphipods infected by *Polymorphus paradoxus* preferred to remain at the surface. When disturbed, they swam along the surface or clung to a plant with a rigid posture – they were then easily noted and caught by dabbling ducks or muskrats.

When equal amounts of infected and uninfected amphipods were put into a pool with ducks, the birds ingested 68% of the infected amphipods, and only 19% of uninfected individuals.

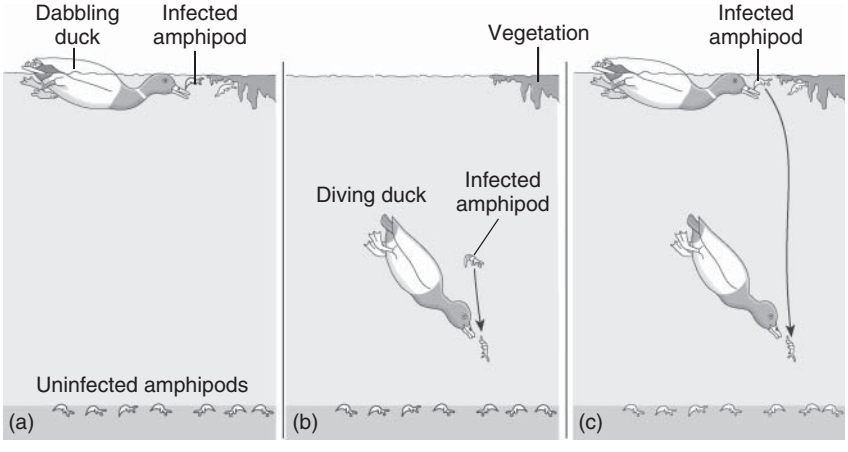


Figure 1.58 Behavioral changes in amphipods after infection with three different species of spiny-headed worms. Uninfected amphipods are primarily bottom dwellers, burying themselves in the mud when danger threatens. (a) Amphipods infected with *Polymorphus paradoxus* prefer the surface of the water, anchoring themselves rigidly in the aquatic vegetation when threatened, where they are taken by dabbling ducks (e.g., mallards). (b) Amphipods infected with *P. marilis* prefer better-lit areas in zones of average depth, where they are taken by diving ducks. (c) Amphipods infected by *Corynosoma constrictum* float on the surface. When danger threatens, however, they swim down to deeper zones, where they are taken by both dabbling and diving ducks. (From Moore, J. (1984) *Sci. Am.*, **250**, 2–89, by kind permission of the publisher.)

By contrast, amphipods infected with *Polymorphus marilis* remained at an average depth, swimming downward when disturbed. They did not, however, hide in the mud at the bottom and were thus easily taken by diving ducks, their definitive hosts. In the case of the third acanthocephalan species, *Corynosoma constrictum*, infected amphipods floated on the surface of the water. When disturbed, some of them swam downward and were taken by both dabbling and diving ducks, in the gut of which they attain sexual maturity. Each parasite species induced behavioral changes in the amphipod that matched very well with the foraging behavior of their target definitive host, indicating that the parasites' actions are extremely host-specific.

The mechanisms underlying this behavioral change were partially elucidated in the case of *P. paradoxus.* Injections of serotonin, a neurotransmitter, induced the same behavior in uninfected amphipods as seen in fleeing infected animals, while other neurotransmitters had no effect. A study of the neurons of the infected amphipods later found an increase of serotonin-containing vesicles at specific synapses. Acanthocephalans synthesize very little serotonin themselves, so this indicated increased serotonin synthesis in the host caused by parasite-secreted substances – and since serotonin levels rise in other invertebrates as a result of immune responses, it is generally believed that *P. paradoxus* exploits this reaction, reinforcing it to specifically manipulate the behavior of its intermediate host. Since that early study, several other researchers have identified alterations in neurotransmitter levels as a likely mechanism underlying behavioral changes in intermediate hosts ranging from crustaceans to fish.

Another example of the deliberate manipulation of intermediate hosts by acanthocephalans comes from *Pomphorhynchus laevis*, a spiny-headed worm that uses predatory fish as definitive hosts, and the amphipod *Gammarus pulex* as intermediate host. In experiments, infected intermediate hosts preferred the area of an aquarium occupied by a perch – but uninfected amphipods showed a strong aversion to the perch's presence. This preference was based on olfactory and not visual stimuli, suggesting a change in the perception of smell – and in nature, this should lead to more efficient transmission of cystacanth stages to the predator.

The list of such examples of manipulation of host behavior by parasites transmitted via predation is growing each year. These involve host and parasites from numerous taxa; indeed, among parasites, host manipulation is known to be used by a wide range of parasites using transmission through the food chain, including protozoans, trematodes, cestodes, nematodes, and acanthocephalans. The independent evolution of adaptive alteration of host behavior in many separate lineages of parasite suggests a case of convergent evolution – the problem of transmission by predation has been solved in similar ways by different parasites with similar life cycles.

1.7.3.3 Introduction into the Food Chain

Sometimes, a parasite must be transmitted by predation from an intermediate host to a definitive host, even if the two hosts do not belong to the same food chain. These situations can lead to astonishing evolutionary achievements, whereby a

parasite can induce such profound changes in its intermediate host's behavior or appearance that it fools the definitive host into ingesting an item not normally in its diet. An example of this truly manipulative behavior comes from trematodes of the family Dicrocoeliidae. *Dicrocoelium dendriticum* (see Section 3.1.1.11), the "lancet fluke," which is widespread in Europe and North America, and *Dicrocoelium hospes*, a liver fluke which occurs in sub-Saharan Africa, both inhabit the bile ducts of ruminant herbivores. The first intermediate host is a land snail and ants act as the second intermediate hosts. Since ruminants ingest ants only accidentally at best, the transmission from the ant to the definitive host is only made possible by a radical change in the ants' behavior. Uninfected ants, if weather permits, roam outdoors during the day and return home to their nest at dusk – but infected ants show a very different behavior.

Ants of the genus *Formica* infected with *D. dendriticum* do not return to their nest with the onset of dusk – rather they climb grass stems and plants near the nest and bite into them with their mandibles (Figure 1.59). They cannot free themselves when disturbed, since spasms keep their mandibles firmly closed – this way, there is an increased likelihood that they will be accidentally ingested by herbivores feeding on grass in the early morning hours. The metacercariae encysted in the ant's abdomen then reach the small intestine of the definitive host, and exit their cyst to colonize the bile ducts of the liver. Infected ants can only open their mandibles when the temperature increases – they then mix with their nest mates and behave normally during the middle part of the day. Ants of the genus *Camponotus* infected with *Dicrocoelium hospes* also climb up plants – they do not

Figure 1.59 *Dicrocoelium dendriticum*-infected *Formica* with mandibles firmly clenched on a blade of grass. (EM image: Courtesy of Eye of Science.)

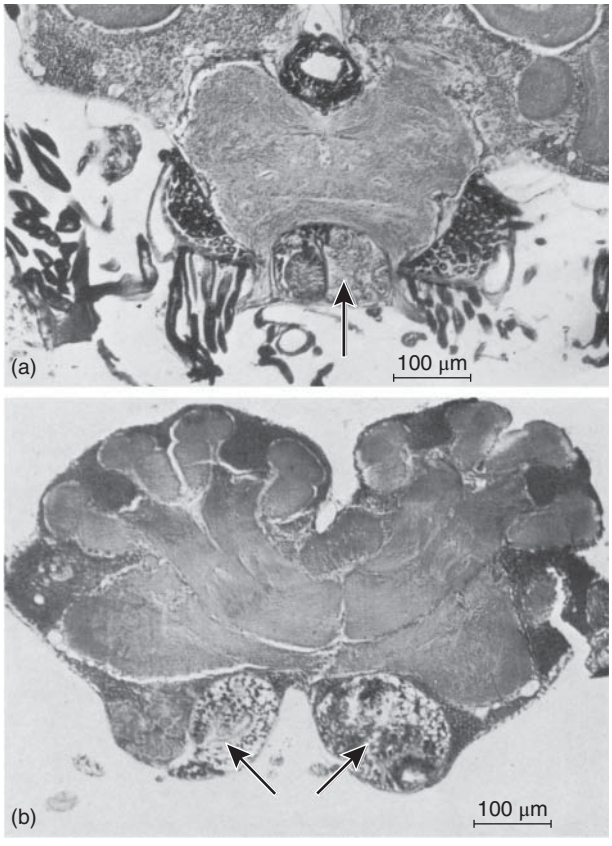


Figure 1.60 Specific localization of the brain worms of *Dicrocoelium dendriticum* and *Dicrocoelium hospes*. In *D. dendriticum* infection, a brain worm usually penetrates into the subesophageal ganglion (a). In *D. hospes* infection, two brain worms are usually localized in the antennal lobes of the supraesophageal ganglion (b). Arrows: Brain worm. (Images: Courtesy of Thomas Romig.)

bite into them, however, but simply sit there day and night. They are fed by passing nestmates and only leave their post temporarily when severely disturbed. The probability of an infected ant being eaten by a definitive host is thus much higher than that of an uninfected individual, since it should be proportional to the time spent at the tip of grass blades or on leaves of plants.

It is cercariae that are responsible for this behavioral change. They penetrate specific areas of the ant brain, where they usually remain without a cyst wall. *D. hospes*-infected *Camponotus* ants usually harbor two "brain worms" in certain areas of the supraesophageal ganglion, the antennal lobe (Figure 1.60). By contrast, the single brain worm of *D. dendriticum* lies in the subesophageal ganglion of the *Formica* ant.

In the 1960s, Hohorst and his team studied the exact sequence of a *D. dendriticum* infection of *Formica pratensis* and found that cercariae ingested by the ant first drill through the wall of the stomach and close the resulting wound. All cercariae first migrate toward the head. When a larva has entered the brain of the ant, the others migrate back into the abdomen to encyst there. The change in behavior occurs around 30 days after infection, when the encysted metacercariae are infective to the final host. After being ingested by a suitable host, the brain worm itself probably not develop into a liver fluke, because it is not protected by a cyst wall when it is digested (Figure 1.60). However, other metacercariae can survive and grow into adult flukes.

This is not the only example of a parasite creating a new predator–prey association for the benefit of its own transmission. When infected with the Dicrocoeliid trematode *Brachylecithum mosquensis* (definitive host: redwing; first intermediate host: land snail; second intermediate host: *Camponotus* ant), the behavior of ants changes drastically. While uninfected ants strictly avoid light, infected intermediate hosts run around conspicuously in a circle on sunlit stones. They also have a strong abdominal distension, the bright connective tissue of which shimmers between the segment boundaries. For some reason, the infected ants are taken by redwings, which do not normally eat them. Studies on *B. mosquensis* have also shown that a metacercaria with a slightly different morphology lies either in or near the brain. A similar behavioral modification is caused when ants are infected by an insect pathogen, the fungus Enthomophthora (Zygomycetes). It forces the ant to climb up plants in a similar manner. The ants do not, however, cling to the plant by their mandibles, but are permanently glued to the substrate by hyphae of the fungus. The exposure of infected ants facilitates the dissemination of fungal spores.

How does such a complex behavioral change in dicrocoeliids develop? It is particularly amazing because in the above examples, the altruistic sacrifice of the brain worm is in seeming contrast to the usual pattern of Darwinian evolution. The brain worm triggers the process, but does not gain any fitness advantage for itself, because it is digested and cannot reproduce.

This apparent contradiction is resolved when we realize that all the cercariae entering an ant are probably descended from the same miracidium and are therefore genetically identical. These have been produced asexually in the snail first intermediate host, and transmitted as a group to the same ant. In such cases, the fitness advantage of the entire clone (inclusive fitness) is what really matters – and the full cost is paid by the sacrifice of a single individual with an identical genotype.

There are other cases in which a parasite creates a new predator–prey association to ensure its transmission, by duping a predator into eating what appears to be its normal prey. For instance, trematodes of the genus *Leucochloridium* (Section 3.1.1.6) must be transmitted by ingestion from a snail intermediate host to a bird-definitive host, but bird species that are suitable as hosts for this parasite feed on insects, not snails. In order to overcome this difficulty, the trematode alters the appearance of its snail host: it causes its tentacles to appear swollen and very colorful, and induces them to pulsate rapidly. To a bird in

search of prey, these presumably look like caterpillars, and the bird is fooled into ingesting several parasite larvae as it gobbles up the "caterpillar." Similarly, the nematode *Myrmeconema neotropicum* must be transmitted by ingestion from an ant-definitive host, probably to a frugivorous bird that spreads the nematode eggs with its feces. The solution: the nematode radically changes the color of the ant's gaster from black to bright red and causes the ant to hold its gaster in an elevated position. The parasite thus induces its host to mimick a small red berry that attracts hungry birds. These examples further illustrate how strong selective pressures to complete the life cycle can drive the evolution of "extended phenotypes" in parasites.

1.7.3.4 **Changes in Habitat Preference**

There is another circumstance in which parasites can benefit by modifying the behavior of their host, and that is when the habitat of the host does not overlap with the habitat in which the parasite must emerge from the host. Nematomorphs, or Gordian worms (hairworms), are the best-known examples of this type of manipulation. These worms are larval parasites that develop in arthropods, while the short-lived hairworm adult stages are not parasitic, and mate in water. The hosts of hairworms are often terrestrial arthropods such as beetles, grasshoppers, and praying mantises. Adult hairworms themselves are bound to freshwater – and getting to water is ensured by the mature parasites inside the host. These are typically three to four times longer than their arthropod host, and they force the host to leap into water (Figure 1.61). The worms then break out of the drowned host and look for a sexual partner at the bottom of the body of water, where a "Gordian knot" consisting of several worms may form. Larvae hatch from the eggs produced from the mating and reach their arthropod hosts, either directly or via a paratenic host. The remarkable nature of hairworm infections is the induction of a behavior (throwing themselves in water) that does not occur in the behavioral repertoire of the normal insects. One well-studied model system is the infection of *Meconema thalassinum* – an oak bush cricket commonly

Figure 1.61 Oak bush cricket *Meconema thalassinum* from which the Gordian worm *Spinochordodes tellini* emerges. (Image: from http://en.wikipedia.org/wiki/Spinochordodes_tellinii.)

found in southern France – with the hairworm *Spinochordodes tellinii*. In this parasite–host system, the behavioral change induced by the parasite occurs only at night. Infected crickets jump into the water – and the factors that trigger this behavioral change have been narrowed down relatively accurately.

Proteome analysis of brains of infected versus uninfected crickets revealed that numerous insect proteins were differentially expressed. The analysis revealed that the leads to production of several proteins with similarity to signal transduction molecules, which could control important brain functions. It is therefore assumed that hairworms secrete products to directly influence the central nervous system of the crickets.

Other pathogens of insects are also capable of modifying habitat selection by their host in order for the parasite to reach a location that is optimal for spreading its offspring. These include nematodes of the family Mermithidae, which have a life cycle similar to that of hairworms and induce the same type of water-seeking in their hosts; and the fungus *Cordyceps* spp., which causes infected insects to climb to the top of vegetation, thus providing the parasite with an ideal perch from which to release its spores into the wind. Several parasitoid wasps are also capable of causing marked changes in their host's behavior and microhabitat choice, in ways that ensure the safety of the parasitoid's emerging pupae.

Further Reading

Adamo, S.A. (2013) Parasites: evolution's neurobiologists. *J. Exp. Biol.*, **216**, 3–10.

Baldauf, A.S., Thünken, T., Frommen, J.G., Bakker, T.C.M., Heupel, O., and Kullmann, H. (2007) Infection with an acanthocephalan manipulates an amphipod's reaction to a fish predator's odours. *Int. J. Parasitol.*, **37**, 61–65.

Biron, D.G., Marché, L., Ponton, F., Loxdale, H.D., Galeotti, N., Renault, L., Joly, C., and Thomas, F. (2005) Behavioural manipulation in a grasshopper harbouring hairworm: a proteomics approach. *Proc. R. Soc. London, Ser. B*, **272**, 2117–2126.

Flegr, J. (2013) Influence of latent *Toxoplasma* infection on human personality, physiology and morphology: pros and cons of the *Toxoplasma*-human model in studying the manipulation hypothesis. *J. Exp. Biol.*, **216**, 127–133.

Hoek, R.M., van Kesteren, R.E., Smit, A.B., de Jong-Brink, M., and Geraerts, W.P.M. (1997) Altered gene expression in the host brain caused by a trematode parasite: neuropeptides are preferentially affected during parasitosis. *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 14072–14076.

Hughes, D.P., Brodeur, J., and Thomas, F. (eds) (2012) *Host Manipulation by Parasites*, Oxford University Press, Oxford.

Koella, J.C. (1999) An evolutionary view of the interactions between anopheline mosquitoes and malaria parasites. *Microbes Infect.*, **1**, 303–308.

Loker, E.S. and Hofkin, V. (2015) *Parasitology: A Conceptual Approach*, Garland Science.

Moore, J. (2013) An overview of parasite-induced behavioral alterations – and some lessons from bats. *J. Exp. Biol.*, **216**, 11–17.

Poulin, R. (2010) Parasite manipulation of host behavior: an update and frequently asked questions. *Adv. Study Behav.*, **41**, 151–186.

Romig, T., Lucius, R., and Frank, W. (1980) Cerebral larvae in the second intermediate host of *Dicrocoelium dendriticum* (Rudolphi, 1816) and *Dicrocoelium hospes* Looss, 1907 (Trematodes, Dicrocoeliidae). *Z. Parasitenkd.*, **63**, 277–286.

Thomas, F. and Poulin, R. (1998) Manipulation of a mollusc by a trophically transmitted parasite: convergent evolution or phylogenetic inheritance? *Parasitology*, **116**, 431–436.

Yanoviak, S.P., Kaspari, M., Dudley, R., and Poinar, G. (2008) Parasite-induced fruit mimicry in a tropical canopy ant. *Am. Nat.*, **171**, 536–544.

Test Questions

1. What is the meaning of the term "extended phenotype" in the context of a parasite infection?
2. Give examples of the alteration of host cells by intracellular parasites.
3. Which types of parasitic castration are found in snails?
4. Why may trematode-infected snails become larger than uninfected individuals?
5. How does *Taenia crassiceps* alter the phenotype of its host?
6. How can parasite-induced damage of the mouthparts of bloodsucking insects lead to an increased transmission of parasites?
7. How does infection with larval tapeworms lead to a host being more easily attacked and eaten?
8. How does *Toxoplasma gondii* manipulate its rodent host?
9. How do the cystacanth larvae of certain Acanthocephala manipulate amphipods?
10. Which stage of the lancet liver fluke influences the life cycle by behavioral change of infected ants?

2
Biology of Parasitic Protozoa

Richard Lucius and Craig W. Roberts

The Biology of Parasites, First Edition. Richard Lucius, Brigitte Loos-Frank, Richard P. Lane, Robert Poulin, Craig W. Roberts, and Richard K. Grencis.

2.1 Introduction

The term protozoa (Greek: protos = the first, zoon = animal) is used as a collective name for heterotrophic, eukaryotic single-celled organisms of different lineages. The term is not a category of taxonomy, but is used in the field of parasitology to delineate single-celled parasites from helminths and arthropods. We take this concept into account by dedicating a separate chapter to the protozoa. The importance of parasitism is shown by the fact that out of about 40 000 described protozoan species, approximately 8000 are parasitic. Those include the causative agents of diseases such as malaria, sleeping sickness, Chagas, leishmaniosis, amebiosis, and toxoplasmosis, and of major livestock diseases such as Nagana, theileriosis, and babesiosis order eimeriosis.

A phylogenetic tree of eukaryotes (Figure 2.1) shows that the phylogenetic distance between the taxa of the parasitic protozoa dealt with in this chapter is very large. Indeed, genome data and findings of cell biology show that in terms of phylogenetics, humans and maize, for example, are more closely related than

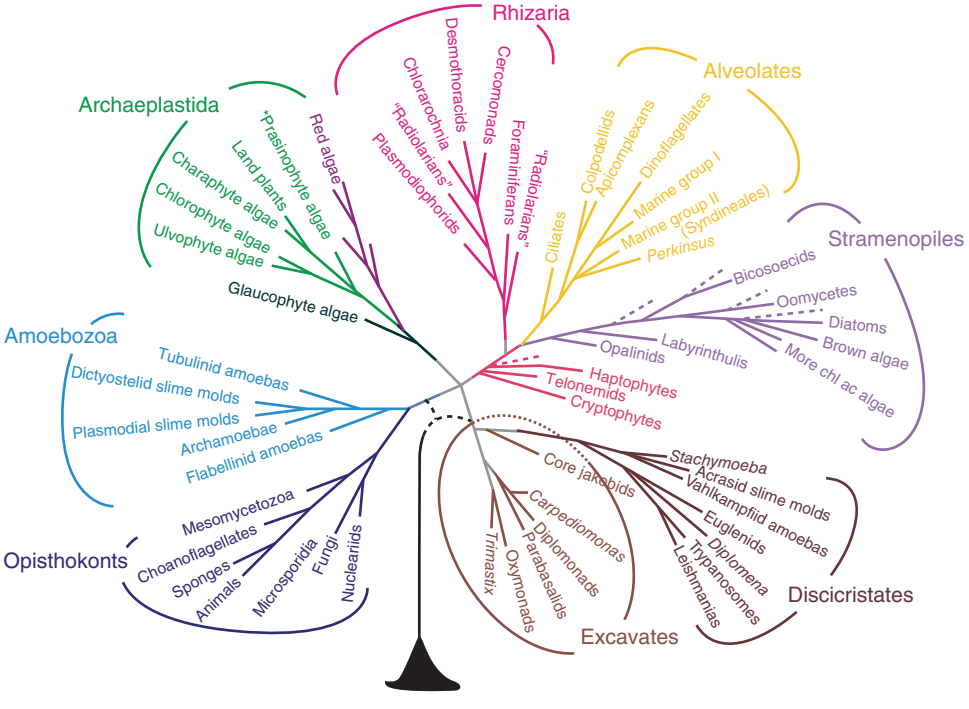


Figure 2.1 Phylogenetic tree of the eukaryotes. (Baldauf, S.L. (2003) *Science*, **300**, 1703–1706, with kind permission of Science Publishers.)

trypanosomes and *Plasmodium*, the malaria parasite. The protozoa – and this pertains also to the taxa at the basis of the tree of life – are not "primitive." In contrast to former concepts, based mainly on morphological characteristics, newer analyses revealed that many of the basal taxa show properties of higher organisms, such as the presence of organelles of endosymbiotic origin or of genes that originate from endosymbionts. Many parasitic protozoa have a comparatively small genome. This is not per se a primordial feature, but often a result of the parasitic life style, which allows a reduction of metabolic pathways due to exploitation of the host organism ("reductive evolution"). There is, however, no stringent correlation between genome size and parasitism, as some parasites have remarkably large genomes. For example, the genome of *Trichomonas vaginalis* (size 176 Mb, 59 681 protein coding genes) accounts among the larger protozoan genomes, but is dwarfed by others, for example, the genome of the free-living *Entamoeba proteus* with a size of 290 000 Mb. The protozoa also do not easily fit into other preformed categories: for example, many apicomplexa contain an endosymbiotic organelle of plant origin, the apicoplast, and have therefore some traits of plant origin.

Consequently, this chapter covers organisms with very different morphology, metabolism, tissue tropism, and pathogenicity. However, they also exhibit several common features such as the fact that they generally replicate relatively quickly, usually occur in large numbers, and are genetically relatively flexible. These properties facilitate adaptation of parasitic protozoa to new conditions and allow them to cope with host immune responses or other challenges. Moreover, the fact that all parasitic protozoa share a common niche, the living host, results often in interesting convergences. Nevertheless, it is remarkable how different the life cycles, life styles, or mechanisms of host exploitations are – a challenge for students who aspire to cover the field of parasitology as a whole. Getting such an overview is eased by modern systematics based on DNA sequences, but the phylogeny of protozoans is not yet completely resolved such that new data sets as well as better bioinformatic methods lead to continuous regroupings. In addition, the taxonomic levels of groups are often unclear. Therefore, we do not focus on systematics in this chapter, but mainly refer to the different relatively homogenous groups of protozoa (referred to as "phyla" hereafter) without providing a detailed account of taxonomy.

Further Reading

Adl, S.M., Simpson, A.G., Farmer, M.A. *et al.* (2005) The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *J. Eukaryot. Microbiol.*, **52**, 399–451.

Adl, S.M., Simpson, A.G., Lane, C.E. *et al.* (2012) The revised classification of eukaryotes. *J. Eukaryot. Microbiol.*, **59**, 429–493.

Baldauf, S.L. (2003) The deep root of eukaryotes. *Science*, **300**, 1703–1706.

2.2 Metamonada

- Intestinal parasites or free-living organisms in a low-oxygen environment
- Even number of flagella
- Lack mitochondria, but can have remnant termed mitosome
- Lack Golgi apparatus
- Some have double sets of organelles

Metamonads are among the most ancient single-celled organisms and are often considered to be a model of simple eukaryotic cells. The phylum includes free-living organisms and parasites. Each species may possess different even numbers of flagella. Metamonads are aerotolerant anaerobic organisms. Once thought to have evolved without mitochondria, metamonads are now known to possess evolutionary related remnant organelles, termed mitosomes with reduced biochemical abilities. Phylogeny demonstrates the presence of mitochondrial derived genes in their nuclei confirming secondary loss of a conventional mitochondrion. Similarly, Golgi structures are not easily discerned, but they possess genes known to encode Golgi proteins. The parasites of the order Diplomonadida are characterized by a **duplication** of their **organelles**.

2.2.1 *Giardia lamblia*

Giardia are single-celled parasitic organisms that are frequently found in humans, livestock, and wild animals. There are currently six confirmed species (*Giardia agilis, Giardia ardeae, Giardia psittaci, Giardia microti, Giardia muris*, and *Giardia lamblia*; synonymous with *Giardia intestinalis* and *Giardia duodenalis*), none of which seem to be highly host-specific. *G. lamblia* has a global distribution and is the only species responsible for disease in humans, but can also be found in other mammals including companion animals and livestock. It has been subdivided into eight assemblages designated A – H according to DNA polymorphisms. There are now proposals for designating groups of these as separate species based on their host distribution.

The parasites colonize the surface of the upper intestine using their ventral fibrillar disk to facilitate attachment to the epithelium (Figure 2.2). Of the various existing assemblages, A and B are found in humans as well as animals (cats, dogs, ruminants, pigs, and horses). It is therefore thought that these are spread by zoonotic transmission, whereas assemblages C – G have only rarely been found in humans and assemblage H was only found in marine mammals. Approximately 3% of apparently healthy European human population excrete cysts, while 3 – 7% of dogs are infected. Infection occurs by feco-oral transmission and prevalence rates vary geographically and are higher in warmer climates, where sanitation is low.

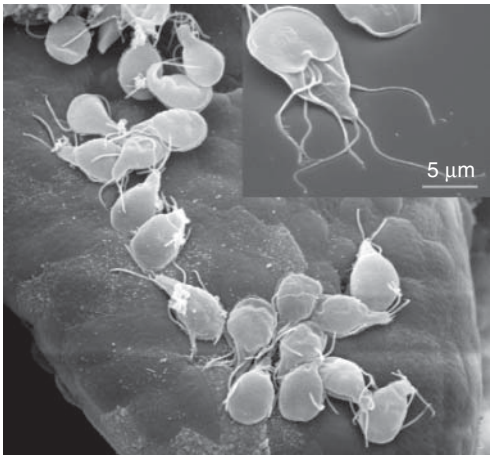


Figure 2.2 *Giardia lamblia* trophozoites on the intestinal epithelium of a mouse. Inset: Underside of the parasite. (image: Department of Molecular Parasitology, Humboldt Universität.)

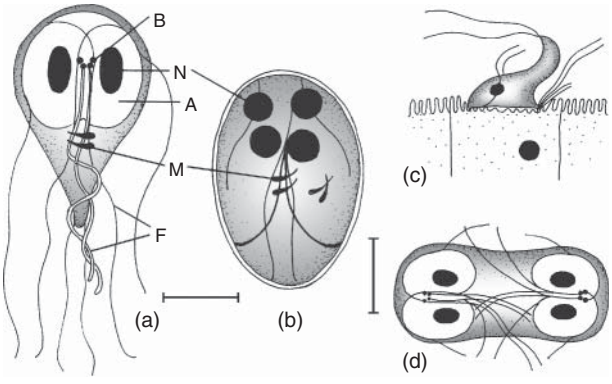


Figure 2.3 *Giardia lamblia*. (a) Trophozoite. (b) Quadrinucleated cyst. (c) Trophozoite with its adhesive disk attached to the microvilli margin of an intestinal epithelial cell. (d) Trophozoite, during fission. A, adhesive disk; B, basal body of flagella; F, flagella; M, median body; N, nucleus. The scale is 5 µm for (a) and (b), 10 for (c) and (d). (Based on several authors.)

Infection occurs through the ingestion of quadrinucleated cysts, each of which releases two binucleated trophozoites (Figure 2.3) in the duodenum. These are between 10 and 20 µm long, their shape resembling a pear split lengthwise. Each trophozoite has four pairs of flagella with $9 \times 2 + 2$ microtubule arrays originating near the anterior cell pole and running along inside the cell. Two pairs of flagella protrude from the cell body in the central region, while another pair is in a lateral position. The fourth pair of flagella runs along a ventral groove that functions as a cytostom. This fourth pair serves to propel food toward the cytostome. The other

three flagella pairs perform tumbling, twisting movements reminiscent of falling leaves.

Two sickle-shaped inclusions, the median bodies, consist of tubulin-like material. It is thought that Giardia feed on epithelial mucus, bacteria, and detritus, breaking down sugars through glycolysis. They multiply by longitudinal division.

During their journey down the intestine with the chyme, the trophozoites form cysts measuring 8–18 µm in response to their exposure to an increasingly alkaline pH and bile salts. After a short maturation period, the cysts become infectious and can survive outside the host for several weeks. Initially the cysts have two nuclei, but ultimately develop four. They also contain sickle-shaped median bodies and flagella fragments. The cyst wall contains a high proportion of N-acetylgalactosamine. Cysts are environmentally resistant and are transmitted through polluted surface water or from one person to another.

The pathogenicity of the Giardia infection varies between infected individuals. Most people with Giardia infection remain asymptomatic, some develop acute diarrhea and others chronic diarrhea. Typically, acute diarrhea lasts about a week, during which time several million cysts are excreted. Chronic disease can last several months. In most cases, patients recover completely from infection. However, severe diarrhea, flatulence, abdominal pain, and malabsorption leading to weight loss and vitamin deficiencies can occur. Fat absorption, in particular, seems to be adversely affected. Giardia may also colonize the gall bladder. Occasionally, Giardia infections can last for years.

The *G. lamblia* genome comprises 6470 protein-encoding genes distributed over five chromosomes. It is a tetraploid organism, with both nuclei containing identical sets of genes, both of which are transcriptionally active. During cell division, daughter nuclei are evenly distributed over daughter cells. Although it is conceivable that one of the nuclei could be redundant, no single-nucleus Giardia have been found, even after long culturing periods. Energy is generated via fermentation of glucose and amino acid metabolism, particularly arginine and aspartate. Mitosomes, evolutionary reduced mitochondria, appear to be important in Fe–S cluster assembly rather than oxidative phosphorylation. Although the genome includes genes required for meiosis, no sexual behavior has been observed and it is thought that reproduction is by asexual binary fission. Giardia can be transformed by various methods: genes can be knocked out, while proteins can be overexpressed with reporter molecules.

A previous infection with Giardia imparts a reduced risk of reinfection and the likelihood of reduced symptoms, if reinfected. This indicates a role for the adaptive immune system in providing protection. Consistent with this, humans with Bruton's X-linked agammaglobulinemia, who are unable to make antibodies, are highly susceptible to disease. *G. muris* infection of mice is often used as an animal model to study the immune response. Secretory IgA antibodies would appear important in murine models and have been demonstrated to kill trophozoites. Murine models also support a role for IL-6 and mast cells.

Giardia have developed antigenic variation as an important immune evasion mechanism. Giardia has approximately 250 variant-specific surface protein

(VSP) genes. The VSPs that coat the surfaces of trophozoites and their flagella are cysteine-rich, bind zinc and other metal ions, and are extremely resistant to proteases. They vary in molecular weight from 20 to 200 kDa and contain tandem repeats of amino acids. The population of Giardia in a given patient at a given time shows some diversity, as several variants may be present at the same time.

Further Reading

Ankarlev, J., Jerlström-Hultquist, J., Ringquist, E., *et al.* (2010) Behind the smile: cell biology and disease mechanisms of *Giardia* species. *Nat. Rev. Microbiol.*, **8**, 413–422.

Cacciò, S.M. and Ryan, U. (2008) Molecular epidemiology of giardiasis. *Mol. Biochem. Parasitol.*, **160**, 75–80.

Monis, P.T., Caccio, S.M., Thompson, R.C. (2009) Variation in *Giardia*: towards a taxonomic revision of the genus. *Trends Parasitol.*, **25**, 93–100.

Morrison, H.G., McArthur, A.G., Gillin, F.D., *et al.* (2007) Genomic minimalism in the early diverging intestinal parasite *Giardia lamblia*. *Science*, **317**, 1921–1926.

Poxleitner, M.K., Carpenter, M.L., Mancuso, J.J., *et al.* (2008) Evidence for karyogamy and exchange of genetic material in the binucleate intestinal parasite *Giardia intestinalis*. *Science*, **319**, 1530–1533.

Gargantini, P.R., Serradell, M.D., Ríos, D.N., Tenaglia, A.H., Luján, H.D. (2016) Antigenic variation in the intestinal parasite *Giardia lamblia*. *Curr. Opin. Microbiol.*, **10**, 52–58.

Roxström-Lindquist, K., Palm, D., Reiner, D., *et al.* (2006) *Giardia* immunity – an update. *Trends Parasitol.*, **22**, 26–31.

Ryan, U. and Cacciò, S.M. (2013) Zoonotic potential of *Giardia*. *Int. J. Parasitol.*, **43**, 943–956.

Tovar, J., Leon-Avila, G., Sanchez, L.B., *et al.* (2003) Mitochondrial remnant organelles of *Giardia* function in iron-sulphur protein maturation. *Nature*, **426**, 172–176.

Test Questions

1. How many flagella does *Giardia* possess?
2. How does *Giardia lamblia* attach to the intestinal epithelium?
3. How does *Giardia lamblia* ingest food?
4. What properties have the main surface protein of *Giardia lamblia*?
5. How does *Giardia lamblia* evade antibody responses?
6. Which immune mechanism eliminates *Giardia lamblia*?
7. How is *Giardia lamblia* transmitted?

2.3 Parabasala

- Commensals or parasites in the intestine and genitourinary tract.
- Axostyle (= axle rod) organelle providing support and helping movement
- Flagella, some with undulating membranes
- No mitochondria, presence of hydrogenosomes
- Food ingestion through pinocytosis

Parabasala are aerotolerant anaerobes, which provides ideal properties for a life as symbionts or parasites in the intestine or genitourinary systems. Many members of this phylum are endosymbionts of insects, including wood-digesting single-cell endosymbionts of termites, while others are causative agents of common diseases. They are characterized by their parabasal body composed of parabasal filaments and a modified Golgi apparatus. Different species of Parabasala vary in the number of flagella they carry. Typically, one flagellum runs along the body and acts as a trailing flagellum, while the others are usually free-floating. Through the length of the cell, runs an axostyle, a rod-like structure, which consists of microtubules. Precisely, the axostyle runs through the length of the cell and partially surrounds the nucleus. In some species, however, the axostyle may have undergone secondary reduction. Other species have further intracellular supporting and locomotor structures. The parasites generate energy through the fermentation of sugar and through the metabolism of certain amino acids. They lack conventional mitochondria, but contain another type of endosymbiont organelles, termed hydrogenosomes. These small organelles are surrounded by a double membrane and break down pyruvate through fermentation, releasing elemental hydrogen. The parasites are mostly transmitted through direct contact. In some species, pseudocysts have been described that have low resistance to desiccation and might facilitate feco-oral transmission. They mostly have monoxenous life cycles, although some axostylata are transmitted by vectors.

2.3.1 *Trichomonas vaginalis*

T. vaginalis is found in the genitals of humans (vagina, urethra, or prostate), where it colonizes the surface of mucous membranes. While moving in a fluid environment, the flagellated trophozoites are pear-shaped (Figure 2.4a), however, they take on an amoeboid shape when coming into contact with an epithelial surface (Figure 2.4b). Infection is spread through direct contact, making trichomonosis (also termed trichomoniasis) a sexually transmitted disease. It is very unlikely for trichomonads to be spread via shared towels or toilet seats and elaborate experiments have revealed that indirect infection through water in swimming pools can be ruled out.

T. vaginalis multiplies by binary fission. Trichomonads do not form cysts and are therefore susceptible to desiccation. *T. vaginalis* can alternate between oval and pear shapes, measuring $10-25 \times 8-15\,\mu m$ (Figure 2.5). Pseudopodia-like extrusions in its posterior region ingest bacteria, epithelial cells, and red blood cells by phagocytosis. Of the five flagella that originate at the apical pole, four are free leading flagella, while the fifth is a trailing flagellum that runs along the cell surface and is attached by an undulating membrane. A supporting structure termed the costa runs underneath the undulating membrane. The flagellar movements set the trophozoites into a clumsy, staggering type of motion.

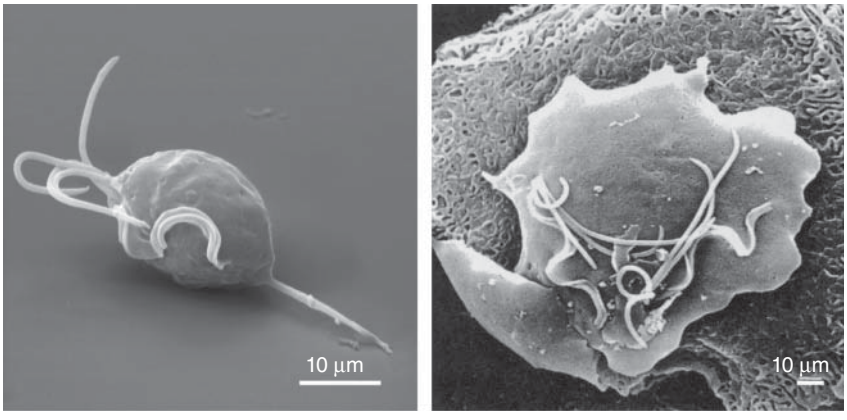


Figure 2.4 (a) Tear drop-shaped *Trichomonas vaginalis* trophozoite grown in liquid culture. (SEM Image: Courtesy of Eye of Science.) (b) Flattened *T. vaginalis* closely attached to vaginal epithelial cells. (SEM image: Rendon-Maldonaldo, JG *et al.* (1998) in Experimental Parasitology 89, 241–250. with kind permission by Elsevier.)

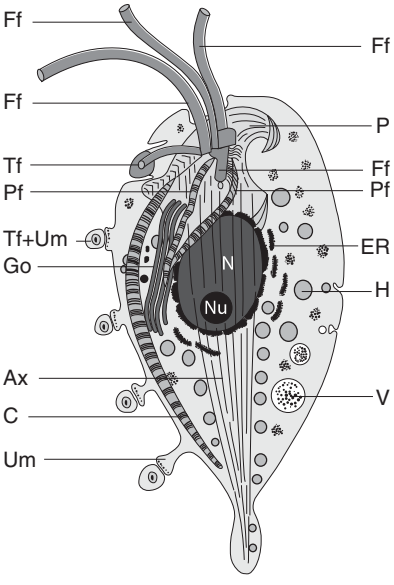


Figure 2.5 Ultrastructure of *Trichomonas vaginalis*. Ax, axostyle; C, costa; ER, endoplasmic reticulum; Ef, free flagella; Go, Golgi apparatus; H, hydrogenosomes; N, nucleus; Nu, nucleolus; P, pelta; Pf, parabasal filament; Tf, trailing flagellum; UM, undulating membrane; V, vacuole. (Adapted from Benchimol (2004) in "*Microscopy and Microanalysis*" with kind permission by Cambridge University Press.)

An axostyle runs lengthwise through the cell, forming with its apical part a collar-like pelta that partially encloses the basal bodies of the flagella without being in direct contact. The axostyle forms a barbed tail at the posterior end of the parasite and is believed to aid attachment to the epithelial cells. The parabasal apparatus consists of a modified Golgi apparatus and two sausage-shaped parabasal filaments.

T. vaginalis is a human-specific parasite. It is estimated that it is responsible for 170 million infections worldwide every year. *T. vaginalis* infection causes discomfort to 80% of all women affected. During the acute phase, trichomonads multiply in large quantities in the vagina and urethra, resulting in inflammation and erosion of the mucous membrane. Often, a yellowish green, strong-smelling vaginal discharge develops, accompanied by itching and burning sensations in the genitalia. If untreated, the infection may become chronic. *T. vaginalis* has been reported to cause temporary infertility. Women with trichomonosis are more susceptible to HIV infection. In men, trichomonads live under the foreskin, in the urethra, and in the prostate. Although 50% of men are asymptomatic, discharge from the penis, pain during micturition, and rarely prostatitis can occur. As frequent asymptomatic carriers, men play a key role in transmission of this disease. *Mycoplasma hominis* have been found to survive and multiply inside *T. vaginalis.* This possibly explains why *M. hominis* is found to co-infect 80% of people with a *T. vaginalis* infection.

With 176 Mb and 59 681 predicted genes, *T. vaginalis* has an extraordinary large number of protein encoding genes on six chromosomes. Two-thirds of the genome consist of repeat regions and transposons. The related species, *Trichomonas tenax* has a significantly smaller genome, suggesting that expansion of the *T. vaginalis* genome took place after their speciation. The expanded genome has been postulated to be associated with the transition from an ancestral intestinal environment (habitat of most trichomonads) into the genital mucosa. The possibility of a partial duplication of the genome is being discussed. Phylogeny indicates that more than 150 genes are of bacterial origin, suggesting their origin from horizontal gene transfer of intestinal bacteria. The surface of *T. vaginalis* is coated with a dense glycocalyx of lipophosphoglycans and features membrane-anchored surface proteases similar to the GP63 type found in *Leishmania*. *T. vaginalis* is the only known eukaryote unable to synthesize glycosylphosphatidylinositol (GPI) anchors. The surface proteins must therefore be inserted into the membrane by hydrophobic sequences. Trichomonads have 12 genes for pore-forming proteins that are homologous to amoebapore proteins (see Section 2.4), as well as >40 cysteine proteinases that are very likely to be pathogenicity factors. Some strains of *T. vaginalis* have been infected with one or more double-stranded RNA viruses, from the genus *Trichomonasvirus*. Their presence has the ability to alter the expression of parasite proteins including the highly antigenic p270 and cysteine proteinases that are known virulence factors.

Trichomonas hominis lives as an innocuous commensal in the colon of humans, but is sometimes associated with diarrhea. ***Trichomonas tenax*** can infect the oral cavity of humans, where it feeds on the bacterial flora. ***Trichomonas gallinae*** is a pathogen of birds responsible for chancre in pigeons. Yellow membranous lesions form between esophagus and crop (Figure 2.6). The pathogens can penetrate inner organs such as the liver, to which they cause significant damage. Juvenile pigeons are infected by being fed by latently infected older birds or through contaminated food and water containers. As the lesions

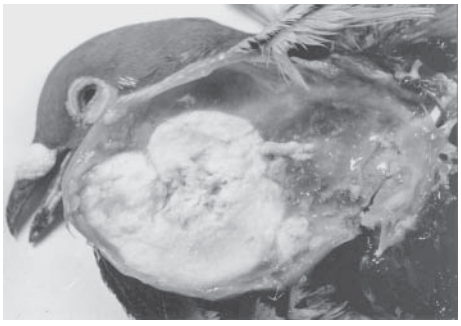


Figure 2.6 Membranous chancre caused by *Trichomonas gallinae* (yellow chancre) in the crop of a pigeon. (Image: Courtesy of T. Hiepe und R. Jungmann).

impair the ability of the young pigeons to take up food and water, they are particularly at risk during their first weeks of life. Surviving birds can become chronic carriers. The disease can be transmitted to birds of prey, particularly young hawks. *T. gallinae* is also a pathogen of chickens and turkeys.

2.3.2 *Tritrichomonas foetus*

T. foetus causes a venereal disease in cattle worldwide. It has been found in many countries. It has many similarities with other trichomonads. The pear-shaped pathogens ($14 \times 8\,\mu m$) have three free flagella and a trailing flagellum that is connected with the cell via an undulating membrane. It live as parasite in the vagina, uterus, and ovarian tubes of cows and under the foreskin and in the urethra of bulls. While tritrichonomonads are highly pathogenic in females, bulls are almost asymptomatic. Infections of cows are accompanied by mucous vaginal discharge. Infection can cause inflammation, fetal death, and abortion 6–16 weeks after mating. The infection clears spontaneously, but can leave cows infertile. The disease is now rare in Europe through the introduction of artificial insemination, whereas in South America and other parts of the world, it remains a major problem.

Further Reading

Carlton, J.M., Hirt, R.P., Silva, J.C. *et al.* (2007) Draft genome sequence of the sexually transmitted pathogen *Trichomonas vaginalis*. *Science*, **315**, 207–212.

Dessi, D., Delogu, G., Emonte, E., *et al.* (2005) Long-term survival and intracellular replication of *Mycoplasma hominis* in *Trichomonas vaginalis* cells: potential role of the protozoon in transmitting bacterial infection. *Infect. Immun.*, **73**, 1180–1186.

Goodman RP, Freret TS, Kula T, Geller AM, *et al.* (2011) Clinical isolates of *Trichomonas vaginalis* concurrently infected by strains of up to four Trichomonasvirus species (Family *Totiviridae*) *J. Virol.* **85**, 4258–4270

Leitsch, D. (2016) Recent advances in the *Trichomonas vaginalis* field. F1000Res. 5. pii: F1000 Faculty Rev-162. eCollection 2016 sequence. *Adv. Parasitol.*, **77**, 87–140.

Johnston, V.J. and Mabey, D.C. (2008) Global epidemiology and control of *Trichomonas vaginalis*. *Curr. Opin. Infect. Dis.*, **21**, 56–64.

Schwebke, J.R. and Burgess, D. (2004) Trichomoniasis. *Clin. Microbiol. Rev.*, **17**, 794–803.

Sutton, M., Sternberg, M., Koumans, L., *et al.* (2007) The prevalence of *Trichomonas vaginalis* infection among reproductive-age women in the United States, 2001–2004. *Clin. Infect. Dis.*, **45**, 1319–1326.

Test Questions

1. How is *Trichomonas vaginalis* transmitted?
2. Where does *Trichomonas vaginalis* live?
3. How does *Trichomonas vaginalis move*?
4. What type of endosymbiont organelle does *Trichomonas vaginalis* have?
5. Which is the bacterial infection mostly associated with trichomonosis?
6. Which trichomonad can cause serious decline in pigeons?
7. What is a potential long-term effect of infection with *T. foetus* in cows?

2.4 Amoebozoa

- Mostly saprophytes, some parasitic taxa
- Variable shape, locomotion through pseudopodia
- No flagella, no tubulin structures except in centrioles
- Monoxenous life cycle, propagation mostly through cysts
- Reproduction through division, sexual reproduction not known

Amoebae (Greek *amoibe* = change, modification) are a polyphyletic group defined by their way of locomotion and the lack of highly developed characteristics. They colonize all aquatic and terrestrial habitats. Amoebae can be free-living or endoparasitic endoparasites. The feeding proliferative stages (**trophozoites**) are enclosed in a membrane with an underlying layer of viscous ectoplasm. The inside contains more fluid, granular endoplasm embedding various food vacuoles, endocytic vesicles, ribosomes, glycogen granules, and the nucleus. Amoebae form **pseudopodia** for directed movement. These are ectoplasmatic protrusions at the anterior pole of the amoeba, into which endoplasm flows, while at the posterior pole, an equivalent amount of matter is retracted. The pseudopodia flow around nutrient particles such as bacteria that are then taken up into food vacuoles. According to their different shapes, pseudopodia have been described as lobopodia, filopodia, or acanthopodia (lobe, thread, or spine-shaped feet, respectively). The trophozoite stages reproduce by fission, while sexual reproduction has been not observed. Many amoebae have the ability to form environmentally resistant **cysts** in response to adverse conditions.

The best-known amoeba is probably the free-living *Amoeba proteus*, which can be easily visualized in the film residue of hay infusions. The saprophytic life in a decaying environment, where many free-living amoebae thrive, is only a small evolutionary step away from endoparasitism in the gut. The medically important *Entamoeba* species that live in an anaerobic milieu in the gut lack conventional mitochondria. However, these parasites possess an extremely reduced endosymbiotic organelle, a **mitosome,** which is the likely relict of a mitochondrion. Some nuclear-located genes encode proteins that are evolutionary related to mitochondria-derived genes. The corresponding proteins are targeted to the mitosome where they function in activities such as Fe–S cluster formation. It is likely that the predecessors of existing entamoebae exhibited an oxidative metabolism associated with the endosymbiotic organelle that was lost as they adapted to their low-oxygen environment. The cytoskeleton of entamoebae is unusual in that it does not feature microtubules apart from the centrioles and there are neither flagella nor cilia. Entamoebae were once thought to lack Golgi apparatus and rough endoplasmic reticulum, but are now known to have these in a reduced form. Thus, rather than branching from other eukaryotes prior to the acquisition of key organelles, entamoebae are believed to have lost these characteristics as they adapted to a parasitic life style. Notably, other amoebazoae, including *Acanthamoeba* species, have a full complement of eukaryotic organelles.

2.4.1 *Entamoeba histolytica*

Entamoeba histolytica (Greek: *histíon* = tissue, *lýsis* = breakdown) is the causative agent of amoebic dysentery, one of the major tropical diseases, with annual mortality between 50 000 and 100 000. In spite of this, *E. histolytica* is usually a commensal and in one study only approximately 10% of all individuals in whose stool *E. histolytica* cysts were found actually showed symptoms of the disease. Humans are the only major host, although cats and dogs can be experimentally infected. *E. histolytica* is found all over the world and was first described in St Petersburg in 1875 in a patient suffering from diarrhea. As hygiene standards have improved the importance of *E. histolytica* as a pathogen has diminished in many countries. It's sister species, *Entamoeba dispar*, which is far more common worldwide, is apathogenic, but morphologically identical and was only described as a separate species in 1992. Consequently, literature prior to this date on the distribution and pathology of dysentery-causing amoebae must be carefully interpreted.

Infection occurs through cysts containing four nuclei (Figure 2.7). Once inside the lower part of the small intestine, amoebae with four nuclei hatch from the cysts. Through further nuclear and plasmic division, each gives rise to eight small trophozoites (amoebulae). The trophozoites colonize the human colon where they may live as commensals without attacking host tissue, multiplying through fission every 12–24 h. In order to form cysts, trophozoites stop feeding, their vacuoles disintegrate and their shape becomes rounded. A chitin-containing cyst wall

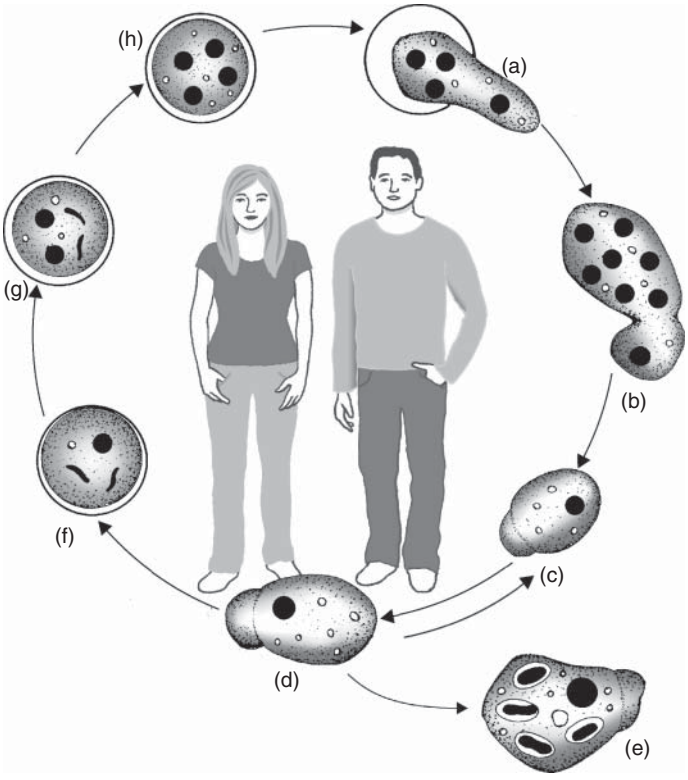


Figure 2.7 Life cycle of *Entamoeba histolytica*. (a) Hatching of tetranuclear amoeba. (b) Octanuclear amoeba with an amoebula being pinched off. (c) Amoebula. (d) Trophozoite. (e) Tissue-invading trophozoite with ingested erythrocytes. (f) Mononuclear cyst. (g) Binuclear cyst. (h) Tetranuclear cyst. (Based on several authors.)

develops. The cyst maturation process involves nuclear division and the formation of four nuclei. The very resilient, transparent cysts are excreted via feces. An infected person may excrete up to 45 million infective cysts per day.

It is not yet understood what causes commensal trophozoites to become tissue-invading amoebae that are typically larger and have a more active metabolism. These amoebae lyze the epithelial cells in the colon and penetrate the mucosa, attacking blood vessels, leading to severe blood-containing diarrhea. The amoebae can be carried in the bloodstream to internal organs, usually the liver, where they cause abscesses. Trophozoites living an abscesses do not form cysts and thus do not contribute to the parasite's transmission.

Trophozoites have a diameter between 20 and 60 µm (Figure 2.8). The ectoplasm can be clearly distinguished from the endoplasm, which contains many vacuoles (Figure 2.9). The nucleus measures 3–5 µm and – as also in other entamoebae – has a marked central nucleolus with peripheral chromatin condensation, resembling a wheel. Trophozoites show constant motion of the surface

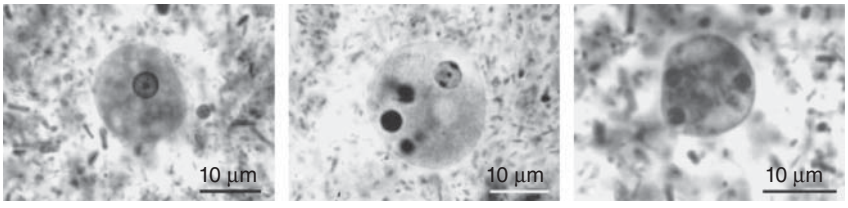


Figure 2.8 Microscopy images of *Entamoeba histolytica*. (a) Trophozoite. (b) Tissue-invading trophozoite with ingested erythrocytes. (c) Tetranuclear cyst. (Images: Courtesy of E. Tannich.)

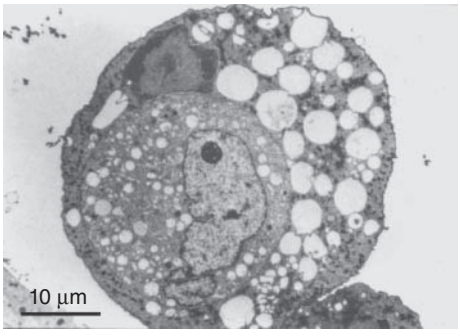


Figure 2.9 Trophozoite of *Entamoeba histolytica* with various vacuoles. (TEM image: Courtesy of E. Tannich.)

and in the cytoplasm. Fast directed movement is achieved by finger-shaped pseudopodia that are suddenly released (eruptive pseudopodia). Cysts are round or slightly oval and have a diameter of 10–15 µm (Figure 2.7). Immature cysts contain no more than a single nucleus. Initially, they have a glycogen vacuole and one or several cigar-shaped chromatoid bodies that probably consist of aggregated RNA. These are resorbed during the maturation process. Mature cysts contain four nuclei.

In the overwhelming majority of cases, amoebiosis (also termed amoebiasis) is an infection of the intestine that manifests as subclinical diarrhea and might clear spontaneously. Individuals who have recovered from the infection and are clinically healthy may remain carriers excreting cysts for up to 5 years. Amoebic dysentery is a relatively rare disease involving colitis with severe, partially bloody diarrhea, typically intermingled with jelly-like blobs in the stool. With this type of disease, trophozoites lyze the epithelial cells of the colon, penetrate the mucosa, and spread laterally in the submucosa, causing tell-tale bloody lesions. Tissue-invading trophozoites are enlarged and often contain ingested erythrocytes in their phagosomes. Clinical symptoms of intestinal amoebiosis, in addition to diarrhea, can include abdominal pain and fever, which occurs in one-third of patients. Amoebic dysentery is often self-limiting, restricted to the intestine and clears completely after some time.

If, however, trophozoites enter the bloodstream, abscesses can develop in various organs (Figure 2.10). Abscesses are most commonly found in the liver (amoebic liver abscesses), due its filter function, but the brain, lungs, skin, or other organs can also be affected. The amoebae lyze the host cells, attracting large numbers of neutrophil granulocytes in the process. The immune cells are killed by the amoebae and release cytotoxic effector proteins that lyse host tissue. Typical amoebic liver abscesses therefore consist of a fluid-filled cavity with trophozoites around their margins. The trophozoites ingest cell debris and erythrocytes. Often, there is hardly any inflammatory response, and necrotic areas are found immediately adjacent to healthy host tissue. Large abscesses may contain over a liter of matter and seriously impair liver function. Abscesses can develop very quickly and tissue invasion may lead to death within a few days, especially if the brain is affected. Amoebic abscesses often manifest several months after the intestine infection has cleared, complicating their diagnosis. It is, however, possible to treat them effectively by chemotherapy.

Amoebiosis is a feco-oral infection that occurs mainly in countries with poor hygiene, where cysts are easily transferred from feces to food. Severe amoebiosis is particularly widespread in Mexico. It can be spread through the top-dressing of vegetables and food can be contaminated by insects carrying cysts. Cysts can also be inhaled with dust and then swallowed or can contaminate drinking water. Cysts can remain infective for months and survive temperatures up to 55 °C as well as chlorine at concentrations used in urban drinking water.

E. histolytica is estimated to have approximately $n2 = 14$ chromosomes. The genome of the sequenced strain HM1 IMSS is approximately 20 Mb and contains approximately 8201 open reading frames, of which approximately 25% contain introns. About 20% of the genome consist of transposable elements that tend to be clustered at syntenic break points between this parasite and *E. dispar.* This has been suggested to contribute to speciation of the parasite. The genome of *E. histolytica* contains 96 genes, the phylogeny of which supports their horizontal transfer from prokaryotes. *E. histolytica* has an atrophic mitochondrion-like organelle, termed the mitosome. It lacks an electron transport chain and the tricaboxylate

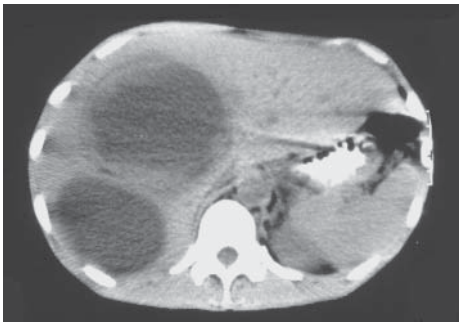


Figure 2.10 Liver abscesses as a result of an *Entamoeba histolytica* infection. The CT scan shows the two abscesses as light areas with diffuse outlines. (Image: Courtesy of E. Tannich.)

cycle and ferments glucose for energy production. Grown in culture, *E. histolytica* has a generation span of 18 h. No spindle appears during cell division, which distinguishes it from mitosis in higher eukaryotes. Although the amoeba carries the genes for sexual reproduction, there are no consistent reports on genetic recombination. It is therefore thought that reproduction is predominantly clonal. Three different types of viruses have been described in *E. histolytica,* but little is known about their actual roles.

So far, scientists have been unable to find what exactly causes *E. histolytica* to become invasive. It has been surmised that bacterial factors might trigger the invasive process and *E. histolytica* has been demonstrated to posses a G-protein-coupled receptor that recognizes Lipopolysaccharide (LPS), an immunostimulating conserved molecule, on bacteria and facilitates their engulfment. Tissue-invading pathogenic amoebae have increased levels of certain cysteine peptidases and transfection and overexpression of these can convert nonpathogenic strains to pathogenic. However, pathogenicity has also been linked to gene duplication and polyploidy. It is therefore likely that *E. histolytica* responds to changes in the environment. The development of tissue-invading forms is an evolutionary cul-de-sac, because it does not help the propagation of the pathogen and needlessly endangers the host. Thus, *E. histolytica,* in contrast to its apathogenic sister species *E. dispar*, is a rather badly adapted parasite.

When invading tissue, the amoebae attach to the mucin layer covering the intestinal epithelium with the help of a lectin on the surface of the trophozoites. This surface-bound GPI-anchored lectin is encoded by a family of 30 genes. Once a trophozoite has attached to a host cell, it will rapidly kill it in a Ca^{2+}-dependent process (Figures 2.11 and 2.12). This involves the pore-forming peptide amoebapore, the same molecule that is released into the food vacuole of apathogenic

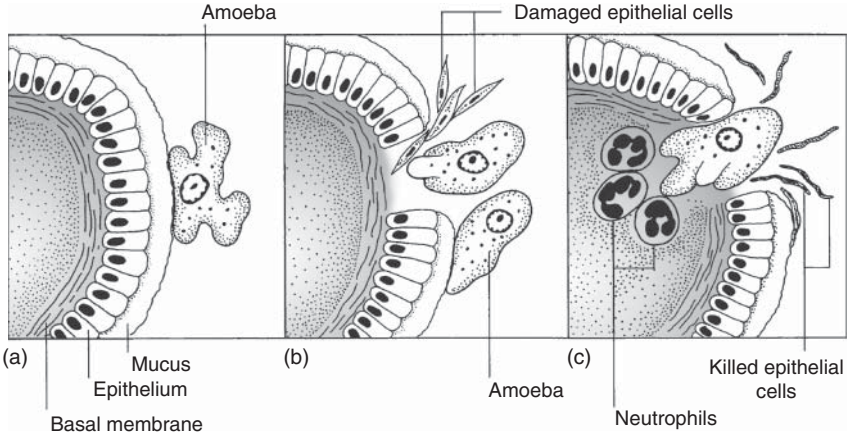


Figure 2.11 Early stage of tissue invasion by *Entamoeba histolytica*. Trophozoites kill the intestinal epithelial cells and penetrate deep into the mucosa through the resulting gaps. Neutrophils that have been attracted are also killed and the lytic products they contain contribute to dissolve the tissue. (Wyler, D.J. (1990) *Modern Parasite Biology*, W. H. Freeman & Company, New York, with kind permission by Freeman and Company.)

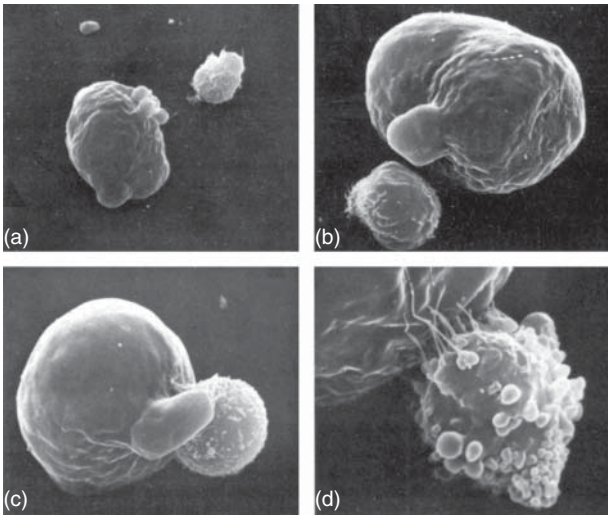


Figure 2.12 Killing of host cells by *Entamoeba histolytica* trophozoites. A trophozoite touches the target cell with its pseudopodium and kills it by secreting amoebapore. The dying target cell forms blisters. (SEM images: Courtesy of G. Kaplan, from Young, J.D., Cohn, Z.A. (1988) Sci. Am. 258, 38–44.)

amoebae to kill the ingested bacteria. This effector molecule is encoded by six different genes and is homologous to defense peptides of natural killer (NK) cells that integrate into the target cell membrane and polymerize to form pores. The integration of amoebapore leads to an uncontrolled influx of ions causing death of the host cell. In addition, *E. histolytica* produces a wide range of proteases, which are instrumental in the invasion process. Twenty different cysteine proteases have been described, some of which are GPI-anchored membrane proteins. The surface protease most relevant to the pathogenic process, CP5, is predominantly expressed in the pseudopodia, while other proteases are released into the phagosome or are involved in the formation of cysts. The mechanisms and molecules (lectin, amoebapore, and cysteine proteases) involved in tissue invasion seem to be similar to those relevant to a commensal lifestyle. However, tissue-invading trophozoites produce them in greater quantities and CP5 is not present in the apathogenic species E. dispar.

Epidemiological data and results from animal trials suggest that amoebic infections are limited by the host's immune response, and mucosal IgA production plays an important role. Looking at the host population, it is striking that 85% of amoebiois patients are male, whereas the percentage of cyst carriers is predominantly female. Amoebic abscesses develop rapidly in patients under immune suppressive treatment. However, amoebiosis has not surged as an opportunistic infection. Animal models demonstrate that vaccination with amoeba extract or purified lectin induces partial protection against a challenge infection. Malnutrition is now also known to increase susceptibility to disease.

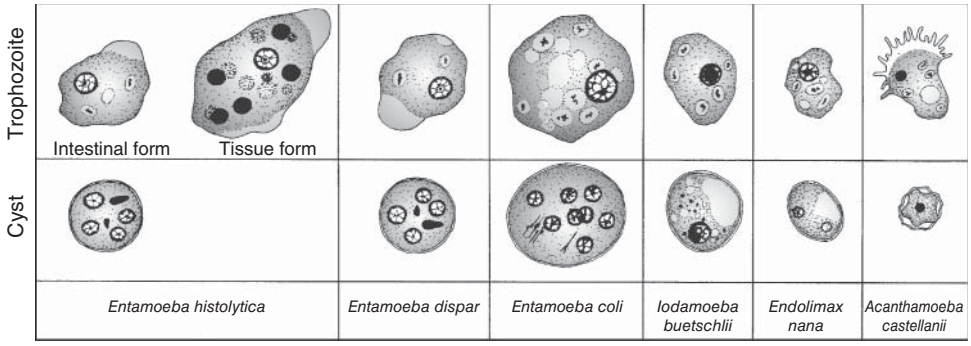


Figure 2.13 Human pathogenic amoebae. (Adapted from various sources).

2.4.2 *Entamoeba dispar*

E. dispar lives as a commensal in humans and cannot be morphologically distinguished from *E. histolytica* trophozoites (Figure 2.13). Almost everything that has been said about *E. histolytica*, apart from its tissue-invading properties, also applies to *E. dispar*. Prior to 1992, before gaining species status, *E. dispar*, based on genetic differences, had been considered to be a nonpathogenic strain of *E. histolytica*. In coding gene regions, sequences differ from *E. histolytica* by 5%, whereas introns differ up to 20%, suggesting that the species separated about 5 million years ago. One of the main genetic differences seems to be the absence of genes enabling tissue invasion. Thus, *E. dispar* does not possess a functional CP5 protease. *E. dispar* cannot be grown in culture.

It is estimated that approximately 90% of all infections involving *E. histolytica*-like amoebae (~450 million infections per year) are caused by *E. dispar*. This makes *E. dispar* the more successful amoeba. By deleting pathogenicity-associated genes, it has become less virulent and better adapted to its host.

2.4.3 Other *Entamoeba* Species

Entamoeba coli is a nonpathogenic amoeba that is found worldwide. It is a commensal in the colon of 30% of all humans. Its trophozoites can be distinguished from those of *E. histolytica* and *E. dispar,* not least by their slower movements and more granulated plasma. A mature *E. coli* cyst contains eight nuclei (Figure 2.13). ***Entamoeba hartmanni*** and ***Entamoeba moshkovskii*** are comparatively rare inhabitants of the human colon and are slightly smaller than *E. histolytica*/*E. dispar*. Cysts of the equally rare ***Entamoeba polecki*** have single nuclei. ***Entamoeba gingivalis*** inhabits the oral cavity in humans, in particular the spaces between teeth and periodontal pockets. It is a very common amoeba that thrives with poor oral hygiene and tooth decay. Since they are not known to

form cysts, it is assumed that they are directly transmitted, for example, through food and mouth-to-mouth contact. ***Entamoeba invadens*** can cause amoebic dysentery and amoebic abscesses in reptiles. The species is morphologically indistinguishable from *E. histolytica*, but thrives at a temperature of 28 °C.

2.4.4 Further Intestinal Amoebae

Endolimax nana is a fairly small apathogenic amoeba species in the human gut. Its trophozoites have a diameter of 6–15 µm and are slow-moving. They are found in 15–30% of the world population. ***Iodamoeba bütschlii*** occurs mainly in swine, but also in the intestine of apes and humans. Cysts usually have one, but sometimes two or three nuclei and a clearly defined glycogen vacuole. The species is identified by staining with iodine to reveal ingested starch as blue – hence its name.

2.4.5 *Acanthamoeba*

Acanthamoeba are normally free-living, but are also facultative pathogens capable of infecting humans. They have many spine- or thread-shaped pseudopodia (acanthopodia). These organisms have conventional mitochondria, endoplasmic reticulum (ER), and Golgi, and are capable of living in diverse environments. The recent genome project reveals that they have a genome of 45 Mb and have proteins that support the hypothesis of an aerobe with a facultative anaerobic ability.

Their trophozoites are up to 25–40 µm long, while cysts, which are formed in unfavorable conditions or in the laboratory by addition of Mg^{2+} ions to media, are approximately spherical and measure 8–30 µm (Figure 2.13). The cyst is characterized by an inner layer termed the endocyst and an outer layer termed the ectocyst. At least 25 species of *Acanthamoeba* have been named largely based on morphology. These have been classified into three groups according to the morphology of their cysts. Group I cysts are large and rounded, clearly separated from their endocysts. Group II cysts have smaller, star-shaped endocysts. Group III cysts are smallest with poorly separated endocysts and ectocysts. More recently, *Acanthamoeba* have been divided into 17 genotypes, designated T1–T17 according to 18s ribosomal RNA sequences. Only two of these genotypes, T5 and T11 relate directly to previously named species, *Acrocercops lenticulata* and *Acrocercops jacobsi*, respectively. Most infections in humans are of the T4 genotype with the T3 and T11 genotypes second and third most common, respectively.

Acanthamoeba are ubiquitous and the vast majority of humans are seropositive, but have never suffered any disease, indicating that most encounters are harmless. However, *Acanthamoeba* is an emerging pathogen and is increasingly common in contact lens wearers, where it can be acquired due to poor hygiene, contact with water while wearing lenses or ineffectual lens cleaning. Notably, contact lens solutions are not required by law to kill *Acanthamoeba* and most one

step, multipurpose contact lens solutions are at best only partially effective. This situation is currently under review.

Acanthamoeba can also be an opportunistic pathogen, which can cause cutaneous disease or granulomatous amoebic encephalitis (GAE). Pathogenic strains are thermotolerant and invade the body via mucous membranes or the skin. Similar to *Entamoeba*, they have efficient pore-forming proteins. GAE takes several months to develop, and is usually fatal.

Acanthamoebae can harbor various intracellular bacteria, including *Legionella pneumophila* (causative agent of legionnaire's disease), and are potential reservoirs of the disease. *Acanthamoeba* have been found to harbor a number of Mimiviruses, which themselves have been gaining interest as human pathogens responsible for pneumonia and arthritis. More recently, a new class of giant viruses termed Pandoravirus, with a 2.5-Mb genome, has been isolated from *Acanthamoeba.*

Further Reading

Lejeune, M., Rybicka, J.M., and Chadee, K. (2009) Recent discoveries in the pathogenesis and immune response toward *Entamoeba histolytica*. *Future Microbiol.*, **4**, 105–118.

Loftus, B., Anderson, I., Davies, R. *et al.* (2005) The genome of the protist parasite *Entamoeba histo- lytica*. *Nature*, **433**, 865–868.

Lorenzi, H.A., Puiu, D., Miller, J.R., *et al.* (2010) New assembly, reannotation and analysis of the *Entamoeba histolytica* genome reveal new genomic features and protein content information. *PLoS Negl. Trop. Dis.*, **4** (6), e716.

Matthiesen, J., Bär, A.K., Bartels, A.K., *et al.* (2013) Overexpression of specific cysteine peptidases confers pathogenicity to a non-pathogenic *Entamoeba histolytica* clone. *MBio*, **4** (2), e00072-13.

Michalek, M., Sönnichsen, F.D., Wechselberger, R. *et al.* (2013) Structure and function of a unique pore-forming protein from a pathogenic acanthamoeba. *Nat. Chem. Biol.*, **9**, 37–42.

Moona, S.N., Jiang, N.M., and Petri, W.A. (2013) Host immune response to intestinal amebiasis. *PLoS Pathog.*, **9** (8), e1003489.

Schuster, F.L. and Visvesvara, G.S. (2004) Free living amoeba as opportunistic and non-opportunistic pathogens of humans and animals. *Int. J. Parasitol.*, **34**, 1001–1027.

Skappak, C., Akierman, S., Belga, S., *et al.* (2014) Invasive amoebiasis: a review of Entamoeba infections highlighted with case reports. *Can. J. Gastroenterol. Hepatol.*, **28**, 355–359.

Tannich, E., Horstmann, R.D., Knobloch, J., and Arnold, H.H. (1989) Genomic DNA differences between pathogenic and non-pathogenic *Entamoeba histolytica*. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 5118–5122.

Teixeira, J.E. and Huston, C.D. (2008) Evidence of a continuous endoplasmic reticulum in the protozoan parasite Entamoeba histolytica. *Eukaryot. Cell*, **7**, 1222–1226.

Weedall, G.D., Clark, C.G., Koldkjaer, P., *et al.* (2012) Genomic diversity of the human intestinal parasite *Entamoeba histolytica*. *Genome Biol.*, **13** (5), R38.

Wilson, I.W., Weedall, G.D., and Hall, N. (2012) Host-Parasite interactions in *Entamoeba histolytica* and *Entamoeba dispar*: what have we learned from their genomes? *Parasite Immunol.*, **34**, 90–99.

Test Questions

1. How do amoebae move around?

2. Where does *Entamoeba histolytica* occur?

3. What makes *Entamoeba dispar* apathogenic?
4. How does *Entamoeba histolytica* cause the development of liver abscesses?
5. What is the infective stage of *Entamoeba histolytica*?
6. What is the name of the reduced mitochondria-derived organelle of *Entamoeba histolytica*?
7. Which molecules convey the ability to invade tissue?
8. Which bacterial pathogens may be harbored by *Acanthamoebae*?

2.5 Euglenozoa

- Free living or parasitic
- Parasites originally infecting arthropods
- Monoxenous cycles or heteroxenous cycles involving a vertebrate
- Locomotion through flagella
- Microtubules underlying the surface
- Reproduction by longitudinal division
- Sexual processes in the arthropod host observed in some species
- Food ingestion by pinocytosis in the flagellar pocket

Euglenozoa are a phylum of basal, flagellate protozoa, comprising organisms with photosynthetic and heterotrophic metabolism. The cell is typically long and slender. The **flagellum** contains a network of protein filaments running parallel to the microtubules ($9 \times 2 + 2$ pattern), the **paraxial rod**. Microtubules stretch under the plasmalemma from the anterior to the posterior cell pole giving the cell a firm structure, while also allowing some flexibility. Euglenozoa are diploid. They have a well-formed mitochondrion with cristae, a Golgi apparatus and an endoplasmic reticulum.

The **Kinetoplastea** class**,** also known as kinetoplastids, which includes some of the major pathogens of tropical diseases, branched off a common ancestor around a billion years ago. The name is derived from the term **kinetoplast**, a highly organized agglomeration of mitochondrial DNA near the origin of the flagellum. The flagella (either one or two) are **leading flagella** that originate from a **flagellar pocket**. The basal taxa of *Kinetoplastea* comprise ectoparasites and endoparasites of fish, which are either monoxenous or are transmitted by leeches as intermediate hosts, for example, *Trypanoplasma borreli*, the sleeping sickness pathogen in carp.

The economically and medically most important *Kinetoplastea* are members of the *Trypanosomatida* order and, more precisely the *Trypanosomatidae* family. The most striking feature of this parasite group is that nearly all of them alternate between arthropod and vertebrate hosts. Some, not yet well researched, sexual behavior has been observed in some species during development in their arthropod host. Only highly derived forms have reduced secondary life cycles that do not

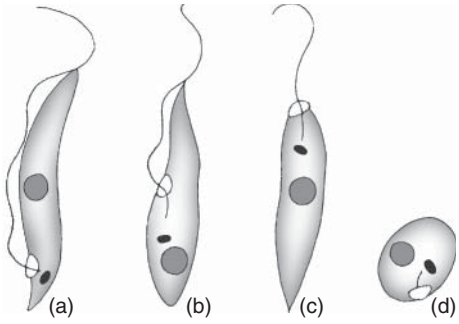


Figure 2.14 Schematic representation of the position of flagellum and the kinetoplast in *Trypanosomatidae*. (a) Trypomastigote. (b) Epimastigote. (c) Promastigote. (d) Amastigote. (Adapted from various authors.)

involve arthropod hosts, while other taxa spend their entire life cycle in the intestine of insects, for example, *Crithidia* and *Leptomonas*. The *Phytomonas* genus uses insects, often heteroptera, as final hosts and plants as intermediate hosts where they live in the sap-carrying vessels. These protozoa are phytopathogens found all over the world, infesting plants as diverse as *Euphorbiaceae*, tomatoes, beans, apple trees, coffee shrubs, and palm trees. Other taxa, such as *Trypanosoma* and *Leishmania*, have vertebrates as intermediate hosts. Some of them cause tropical diseases such as sleeping sickness, Chagas disease, and leishmaniosis or livestock diseases in livestock such as Nagana or covering sickness (dourine).

During their life cycle, many *Trypanosomatids* undergo distinct morphological changes, which include changes of the position, where the flagellum originates in relation to the nucleus (Figure 2.14). At the **trypomastigote stage**, the flagellum originates behind the nucleus and runs forward along the cell surface. It is attached to the surface, where its wave-like movements make the surface appear like an **undulating membrane** (Latin: *unda* = wave). At the **epimastigote stage**, the flagellum originates just anterior to the nucleus, resulting in a short undulating membrane. At the **promastigote stage**, the flagellum originates at the anterior cell pole. At the **amastigote stage**, an adaptation to an intracellular lifestyle, the flagellum shortens to such an extent that it can only be identified by electron microscopy. The pro and epimastigote stages inhabit arthropod hosts, whereas the trypomastigote and amastigote stages are found in vertebrate hosts.

2.5.1 Cell Biology and Genome

The surface of Trypanosomatids is covered by glycolipids and/or glycoproteins that are anchored to the membrane through GPI anchors. This thick surface coating is also known as a glycocalyx. The exchange of metabolic products takes place only in the flagellar pocket (Figure 2.15). In the mammalian bloodstream, trypanosomatids generate energy from glycolysis in itself an energy-intensive process that requires a daily supply of sugar amounting to up to ten times their

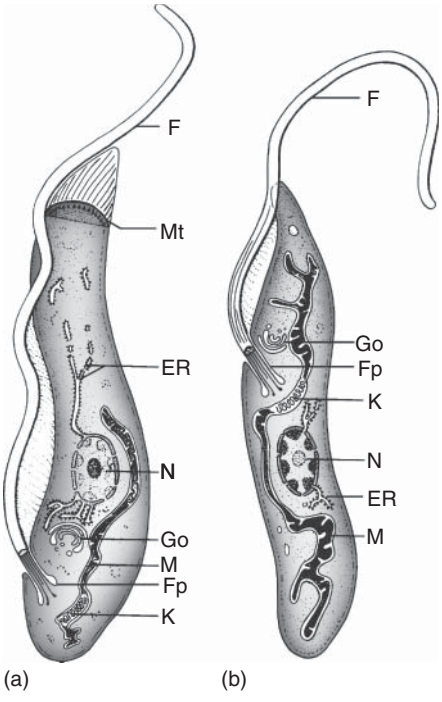


Figure 2.15 Ultrastructure of the trypomastigote (a) and epimastigote forms (b) of *Trypanosoma brucei*. ER, endoplasmic reticulum; F, flagellum; Go, Golgi apparatus; Fp, flagellar pocket; K, kinetoplast; M, mitochondrion; Mt, microtubules; N, nucleus. In order to better visualize the microtubules running below the surface, the anterior end of the trypomastigote form has been cut perpendicularly to the longitudinal section. (From Dönges (1988). Parasitology. Georg Thieme-Verlag, Stuttgart.)

own weight. Glycolysis takes place in glycosomes (peroxisome-like organelles) with little mitochondrial involvement. Insect stages, by contrast, have an oxidative metabolism taking place in the mitochondrion, which is highly active and considerably enlarged. They rely on proline as their main energy source. Although these metabolic peculiarities are well known, drugs with highly specific activity are still scarce and chemotherapy of diseases caused by trypanosomatids still relies partly on agents with numerous side effects.

The genomes of three major pathogens, *Trypanosoma brucei*, *Trypanosoma cruzi, and Leishmania major*, have been sequenced in parallel, leading to a considerable increase of knowledge and an insight into the functioning of basal protozoa. All three parasites have a highly segmented genome, consisting of megabase, intermediate, and mini chromosomes (Table 2.1). The relatively low number of chromosomes in *T. brucei* is probably the result of secondary chromosomal fusion. Among their roughly 10 000 genes, a relative large proportion of 6000 genes is shared among the *Trypanosomatida*, whereas relatively few genes are group-specific. These are mostly located near the telomeres and encode

Table 2.1 Information on the genomes of Trypanosomatidae.

	Trypanosoma brucei	*Trypanosoma cruzi*	*Leishmania major*
Size of the haploid genome (Mb)	36	55	33
Number of chromosomes (haploid set)	11[a)]	28	36
Number of protein-coding genes (haploid set)	9 068 including 904 pseudogenes	Approximately 12 000 including 2 271 pseudogenes	8 311 including 34 pseudogenes

a) Plus 100 mini and small chromosomes (in total approximately 10 Mb).

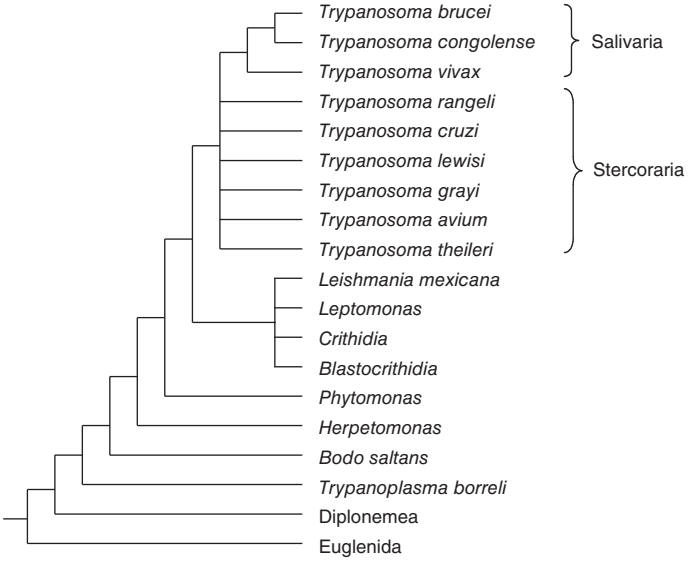


Figure 2.16 Phylogeny of Kinetoplastea and representatives of outgroups, composed based on the phylogenetic analysis of *Trypanosomatidae* (based on Hamilton P.B. *et al.* (2004) Int. J. Parasitol. 34, 1393–1404) *Kinetoplastida* (based on Simpson, A.G.B., *et al.* (2004). *Protist*, **155**, 407–422). Please note that *Stercoraria* (including *Trypanosoma cruzi*), *Salivaria* (including *T. brucei*), and *Leishmania* occupy different branches of the phylogenetic tree.

surface proteins. **Transcription** is largely **polycistronic**, that is, several genes (which are almost all intron-free) are controlled by a common promoter sequence. The resulting transcripts are processed by cutting individual RNAs out of the polycistronic transcript, then adding a 39-bp sequence with a function of a cap (spliced leader) to the 5′ terminus by trans-splicing and finally by polyadenylation (see Box 2.1). Gene expression is largely controlled posttranscriptionally. It is thought that specific sequences in the 3′-untranslated regions play a major role in the regulation of mRNA stability.

The mitochondrial genome of *Trypanosomatids* has several peculiar features. It consists of thousands of closely interwoven DNA rings of varying size, which form the kinetoplast. The kinetoplast takes up DNA staining easily. Approximately 30 **maxicircles** (21–38 kb) mainly encode mitochondrial genes, whereas 5000–10 000 **minicircles** (0.9–2.5 kb) encode **guide RNAs**, which are key elements in RNA-editing. RNA-editing is a very complex process in which the transcripts for the mitochondrial proteins are substantially modified by deleting or inserting uridines before they are translated.

2.5.2 Phylogeny

Trypanosomatidae are monophyletic and separated from other kinetoplastids about 400–600 million years ago. They began their parasitic life style in the intestine of arthropods. The ability to parasitize vertebrate hosts is thought to have evolved twice independently as *Leishmania* and *Trypanosoma* occupy completely separate branches of the phylogenetic tree (Figure 2.16). *Leishmania* belong to a group of parasites that use plants as well as animals as intermediate hosts. All *Leishmania* are intracellular in their vertebrate hosts. The genus *Trypanosoma* is also highly diverse, comprising several genera that use different transmission routes. The more primitive **Stercoraria** are transmitted through the arthropod host's feces. Their surface consists of mucin-like, highly glycosylated proteins. **Salivaria** are transmitted to the vertebrate host in saliva of the arthropod. *Trypanosoma equiperdum* has evolutionary lost its ability and requirement to inhabit insect intestines and is now a sexually transmitted parasite of horses.

2.5.3 *Trypanosoma brucei*

T. brucei (Greek: *tr$\bar{y}$ panon* = borer, *soma* = body) causes sleeping sickness in humans and is one of the trypanosome species that causes Nagana, a devastating disease of cattle. A variety of mammals can act as vertebrate hosts, while tsetse flies, that is, blood-feeding muscids of the *Glossinidae* family, act as insect hosts. *T. brucei* comprises a species complex of three subspecies (Table 2.2). The subspecies *Trypanosoma brucei gambiense*, *Trypanosoma brucei rhodesiense*, and *Trypanosoma brucei brucei* differ in terms of host range and pathogenicity, but are not clearly distinguishable by morphology or biochemistry. *T. b. brucei* and *T. b. rhodesiense* are more closely related to each other than to *T. b. gambiense*.

When a vertebrate host becomes infected, up to 20 000 metacyclic, trypomastigote-stage organisms are transmitted to the skin via saliva of the tsetse fly (Figure 2.17). The infective stages remain in the intercellular spaces around the bite site for 2 weeks, where they reproduce by binary fission, before entering the blood circulation. The trypomastigote bloodstream forms are initially long and slender, very active, and motile (Figure 2.18). They have a doubling time of approximately 6 h and consequently within a very short time, they can build up

Table 2.2 Overview of major *Trypanosoma* species and the diseases they cause.

Species/subspecies	Distribution	Vertebrate host	Pathogenicity	Insect host	Development within insect
Salivaria					
Trypanosoma brucei brucei (Nagana)	East Africa	Wild ungulates, Carnivores, cattle, equidae Cattle, Equidae, dogs	+ +++ + +++	*Glossina morsitans*	Cyclical (midgut and salivary gland)
Trypanosoma brucei rhodesiense (sleeping sickness/Nagana)	East Africa	Wild ungulates, cattle, sheep, goats, humans (rarely affected)	+ + +++ +++	*G. morsitans*	Cyclical (midgut and salivary gland)
Trypanosoma brucei gambiense (sleeping sickness)	West Africa	Humans, pigs, dogs	+++ +	*G. palpalis*	Cyclical (midgut and salivary gland)
Trypanosoma evansi (Surra)	North Africa, Asia, South America	Horses, camels, water buffaloes, dogs	+++ ++	Tabanidae, *Stomoxys*	Mechanically (mouth parts)
Trypanosoma equinum – dyskinetoplastic variant of *Trypanosoma evansi*				–	
Trypanosoma equiperdum (covering disease)	Worldwide	Horses	++	–	–
Trypanosoma congolense (Nagana)	Africa	Wild ungulates, cattle, sheep, goats, horses	+ +++	*Glossina* (all species)	Cyclical (foregut)
Trypanosoma vivax (Nagana)	Africa South/Central America	Wild ungulates, cattle, sheep, goats, horses	+ +++	*Glossina* (all species)	Cyclical or mechanical
Stercocaria					
Trypanosoma cruzi (Chagas)	South/Central America	150 mammalian species	+++	Reduviid bugs	Cyclical (mid and hindgut)
Trypanosoma rangeli	South/Central America	Mammals	–	Reduviid bugs	Cyclical, mid and hind gut, salivary glands
Trypanosoma theileri	Worldwide	Cattle	–	Tabanidae, ticks (?)	Cyclical, mid and hindgut
Trypanosoma melophagium	Worldwide	Sheep	+	*Melophagus ovinus*	Cyclical, mid and hindgut
Trypanosoma lewisii	Worldwide	Rats	+	Flees	Cyclical, mid and hindgut

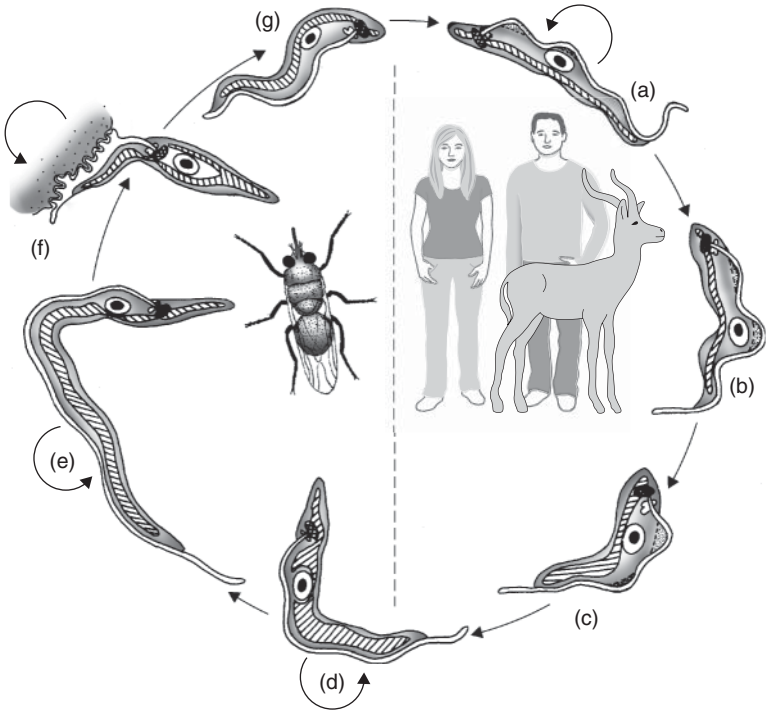


Figure 2.17 Life cycle of *Trypanosoma brucei* including mitochondrial function. (a) Slender trypomastigote blood form. (b) Trypomastigote intermediary form. (c) Stumpy trypomastigote blood form. (d) Procyclic trypomastigote form in intestinal lumen. (e) Mesocyclic trypomastigote form in the ectoperitrophic space. (f) Epimastigote form, with flagellipodia attached to microvilli of salivary gland cells. (g) Trypomastigote metacyclic form in the saliva of *Glossina*. Striped structure: Mitochondrion. (Adapted from Vickerman, K. (1985). *Br. Med. Bull*., **41**, 105–114.)

Figure 2.18 Long slender trypomastigote blood form of *Trypanosoma brucei*, its anterior end lying on top of an erythrocyte. (SEM image: Courtesy of Eye of Science.)

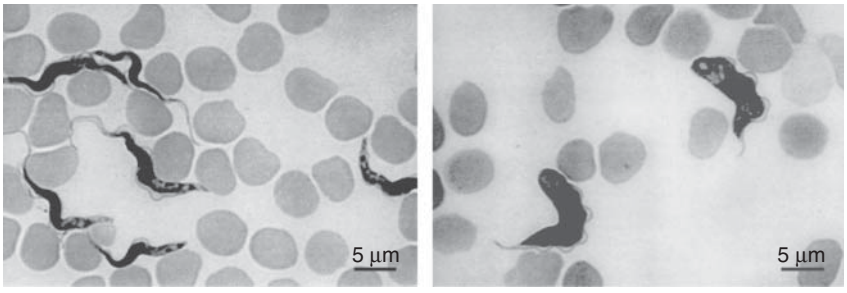


Figure 2.19 Slender trypomastigote (a) and stumpy blood form (b) of *Trypanosoma brucei*. (Images: Courtesy of E. Vassella and M. Boshard.)

a high population density (up to 100 000 parasites/ml of blood in humans and up to 100 million parasites/ml of blood in mice or rats). Antigenic variation provides long-term protection from the immune response (see below and Box 2.1). Every time parasite density has reached a threshold, the slender forms transform into intermediate and finally short stumpy trypomastigote forms. These no longer actively divide, but are infective to tsetse flies (Figure 2.19).

In tsetse flies, *Trypanosoma* first enter the crop where they adapt to their insect habitat, and subsequently enter the gut. In the tsetse's midgut, long trypomastigotes develop and reproduce. After approximately 4 days, they break through the peritrophic membrane, a chitin-containing structure separating the ingested blood from the intestinal epithelium. They colonize the space between peritrophic membrane and the midgut epithelium, lengthen further, and reproduce by division. The offspring of these stages migrate to the lumen of the salivary glands, where they develop into epimastigotes. These stages adhere to glandular cells by extensions to their flagella. Epimastigotes multiply substantially through division and finally develop into the trypomastigote metacyclic stage, which infects vertebrate hosts. The whole process of development inside the fly into the metacyclic stage takes 3–5 weeks. The infection persists throughout the life of the arthropod host. There is genetic exchange between *Trypanosoma* involving meiosis-like processes that probably occurs within the salivary gland of the tsetse fly.

The slender, actively dividing trypomastigotes in the bloodstream measure $1-2 \times 20-30\,\mu m$, with the flagellum protruding over the anterior end and a centrally located oval nucleus, whereas the stumpy trypomastigote bloodstream form has a size of $3-5 \times 15-25\,\mu m$. The flagellum hardly exceeds the length of the cell. Promastigotes in the midgut of tsetse flies are approximately 40 µm long, whereas the metacyclic forms resemble small stumpy bloodstream forms.

As *T. brucei* is biologically tied to the tsetse fly host (*Glossina* spp.), this fly species occurs only in Africa's "tsetse belt," a broad stretch between the Sahara desert and southern Africa. The prevalence of *T. brucei* in tsetse flies is extremely low. In general, only 0.1% of the flies are infected, which significantly reduces the

likelihood of tourists becoming infected. *T. b. gambiense* is transmitted by tsetse flies of the *palpalis* group, which inhabit wet biotopes, such as the rain forests of West Africa. Humans are their main hosts, while dogs and pigs are reservoir hosts. *T. b. rhodesiense* is transmitted by tsetse flies of the *morsitans* group, whose preferred habitats are savannahs. The main hosts of *T. b. rhodesiense* are wild animals, such as bushbucks and hartebeests, but infection is also found in sheep, goats, and cattle. The infection is mostly latent in animals, and humans are only rarely infected, because the main vector, *Glossina morsitans*, rarely targets them. *T. b. brucei* is also transmitted by tsetse flies of the *morsitans* group. Their hosts include all ungulates as well as certain carnivore species (e.g., hyenas, lions, and dogs). Apart from rare exceptions, humans cannot be infected by *T. b. brucei*, because they carry two trypanolytic serum factors that lyse the parasites. Both factors are high-density lipoproteins that contain haptoglobin-related protein and apolipoprotein L1 (apoL1). The latter is the catalytic subunit of the complex, and is thought to exert its trypanocidal activity by inserting into the single *T. brucei* lysosome and causing its rupture. Interestingly, in endemic regions, high prevalence of the organism correlates with the apparent selection in the population of apoL1 variants that are more aggressively lytic for the parasite, therefore also more protective for the population. However, in a situation reminiscent to that of malaria and sickle cell disease, these trypanosome-protective variants are also associated with high rates of kidney disease. In contrast to humans, apoL1 in rats and mice is not protective against *T. b. brucei*, and this makes *T. b. brucei* a good laboratory model for mammalian disease. Infection in cattle, sheep, and goats is mostly asymptomatic, whereas it is often fatal in dogs, horses, and donkeys. The success of zebras in Africa, on the contrary, is due to their relative resistance to Nagana and the fact that their striped pattern confuses tsetse flies.

The ability of trypanosomes from blood to infect tsetse flies varies according to the age of the insect, with an 8% success rate on the first day of life dropping to 2% on the second day of life. From the third day, tsetse flies are refractive to infection. This low efficiency of infection is due to *T. brucei* taking about 1 h to switch from anaerobic energy generation used in the vertebrate bloodstream to aerobic energy generation required in the insect intestine. The change of metabolism happens in the crop of the fly, where ingested blood is initially stored in blood-sucking arthropods. The flies have a Type II peritrophic membrane (see 4.11 and 4.4), which is just a short pocket in newly hatched flies and only forms after the first blood meal. Its grow rate is only 1 mm/h. Accordingly, young flies can pass on blood from the crop to the midgut at a very slow speed, allowing ample time for adaption of the trypanosomes. In older flies, the peritrophic membrane has grown longer or is already perfectly formed, and the ingested blood is passed to the midgut more quickly, leaving less time for the trypanosomes to adapt their metabolism.

The WHO has estimated that currently approximately 10 000 humans are newly infected with *T. brucei*, most of them in the Democratic Republic of Congo, whereby 98% of cases are due to infection with *T. b. gambiense* (http://www.who.int/mediacentre/factsheets/fs259/en/). This is a record low, but it is not excluded that the numbers rise again. The incidence of this disease has

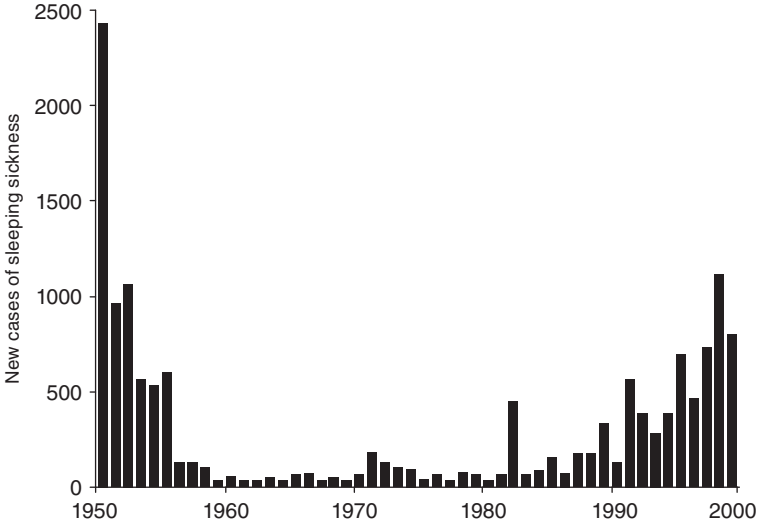


Figure 2.20 Changes in the prevalence of sleeping disease in the Central African Republic between 1950 and 2000. (Adapted from Cattand, P., Jannin, J., and Lucas, P. (2001). *Trop. Med. Int. Health*, **6**, 348–361.)

significantly increased in some African regions affected by unrest and civil war, having been nearly extinct by the middle of the 1970s (Figure 2.20).

After an infective bite, trypanosomes multiply first locally in intercellular spaces of the skin, which can lead to inflammation and swelling (trypanosomal chancre). After 1–3 weeks, the parasites enter the bloodstream and lymphatic system, which results in fever, headache, aching joints, fatigue, and swollen lymph nodes, particularly around the neck. Infected individuals develop enlarged liver and spleen, edema, and diarrhea. One of the key factors in the development of the disease is generalized vasculitis, which may lead to myocarditis and encephalitis. Death is often caused by heart failure. Some days or weeks after the first observation of symptoms, the typical characteristics of sleeping sickness develop. Initially, central nervous disorders affecting speech and coordination appear, developing into convulsions, epileptic seizures, and somnolence. Exaggerated sensations and aggressive behavior have also been observed. While the infection progresses, patients become apathetic, refuse to eat, and suffer dramatic weight loss before finally falling into a coma (Figure 2.21). When left untreated, the infection usually becomes fatal. A *T. b. gambiense* infection may take its course over 4–6 years, whereas the fulminant *T. b. rhodesiense* infection becomes fatal within 3–7 months. The central nervous disorders are mainly caused by perivascular inflammation around the blood vessels supplying the brain. It is thought that changes in the kinin system are also involved.

Epidemics of sleeping sickness in central and eastern Africa in the early years of 1900 resulted in approximately 250 000 deaths and one-third of the population in certain areas died. These outbreaks are now thought to have been caused by

Figure 2.21 Historic photograph of an African woman suffering from an advanced stage of sleeping sickness. (Image: Courtesy of G. Stich.)

massive disruptions in the ecological balance due to introduction of European cattle carrying *Rinderpest* (cattle plague), a viral disease. This resulted in the death of masses of indigenous ungulates allowing large areas of savannah to revert to woodland. This provided better breeding conditions for tsetse flies, which, combined with a lack of ungulates for them to feed from, resulted in more humans being fed upon and infected.

In spite of some progress made in the past years, safe and easily applicable chemotherapy of sleeping sickness remains unsatisfactory. The development of new drugs is not commercially viable and little progress has been made. The fight against the disease therefore focuses on controlling tsetse fly populations. Initially, this meant destroying biotopes by cutting shrubs and trees and culling the wild animal population to eliminate reservoir hosts. Subsequently, widespread use of insecticides over vast stretches of land was used to control tsetse populations. These ecologically dubious methods have now been successfully replaced by the use of decoys and traps enhanced by pheromones and impregnated with insecticides.

With the exception of the flagellar pocket, the surface of *T. brucei* bloodstream stage is covered in a 12- to 15-nm-thick layer of glycoprotein, termed variant surface glycoprotein (VSG), as its composition varies throughout the infection site (Figure 2.22). This is achieved through the successive expression of antigenically distinct VSG genes present in the *T. brucei* genome. Early estimates of approximately 1000 VSG genes have been revised based on new genome data. We now know that the genome contains approximately 2000 VSG gene or gene fragments, of which approximately 400 are complete. The rest are pseudogenes or gene fragments, which are thought to contribute raw material for the generation of new genes.

VSGs comprise a conserved C-terminus and a highly variable N-terminus with specific B cell epitopes. These proteins insert into the plasmalemma via a membrane anchor consisting of GPI (Figure 2.23). GPI anchor and protein are synthesized separately and are combined when they reach the cell surface. The VSGs form a tight coat with a uniform repetitive pattern of B cell epitopes.

VSGs mainly induce strong T cell-independent IgM responses targeting the surface epitopes. If these antibodies bind to the trypanosome surface, they activate

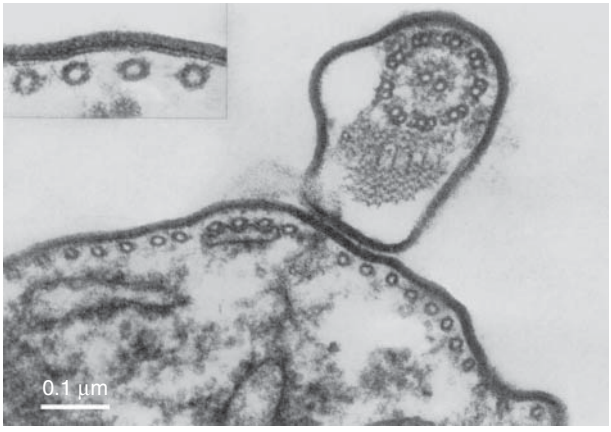


Figure 2.22 Electron micrograph of the surface coat of *Trypanosoma brucei*. Please note the electron-dense, blurred layer of variable glycoproteins on cell body and flagellum. Inset: Detail of the surface with clearly recognizable elementary membrane and underlying microtubules. (From Wyler, D.J. (1990) *Modern Parasite Biology*, W. H. Freeman & Company, New York, with kind permission by Freeman and Company.)

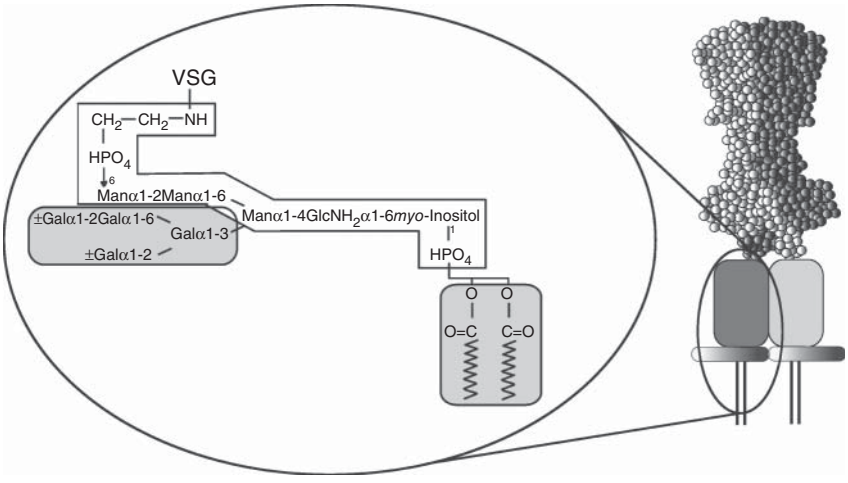


Figure 2.23 Schematic representation of the glycosylphosphatidylinositol membrane anchor of a variable surface antigen of *Trypanosoma brucei*. (Based on Ferguson, M.A., Homans, S.W., Dwek, R.A., Rademacher, T. (1988) Biochem. Soc. Trans. 16, 265–268.)

complement, which leads to recognition and killing by phagocytic effector cells, mainly by Kupffer cells (also known as stellate cells) in the liver. However, **antigenic variation** (Figure 2.24) generates successive waves of clones with different surface antigens, facilitating the repeated evasion of the developing immune response. The consequence of this is that the number of trypanosomes fluctuates in a wave pattern (Figure 2.24, inset graph). A new peak is reached every 6–10 days. Peaks occur when trypanosome clones with specific surface antigens

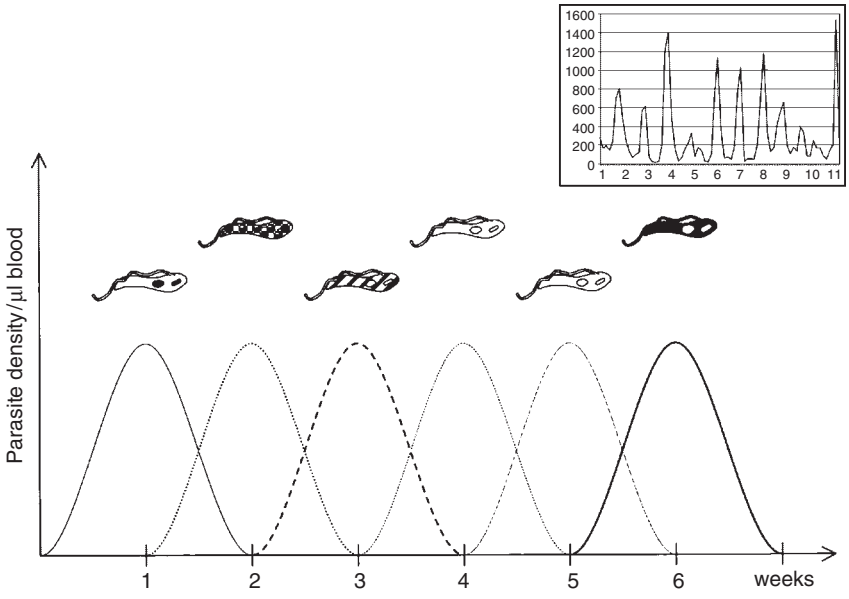


Figure 2.24 Schematic view of antigen variation in *Trypanosoma brucei*. Ever-new clones of *T. brucei* blood forms develop, each of which has its specific variable surface antigens. By the time the host's antibody response has eliminated the predominant clone, a new variant has developed and continues to multiply until antibody responses to the new surface antigen develop. Although a small number of clones dominate each peak, there is a surprising amount of diversity as well, such that peaks are never homogeneous. (Top right: original patient data.)

thrive that are not yet recognized by the immune system. In contrast to earlier descriptions the populations are not homogenous, but consist of several cloes at the same time. Once specific antibody responses have developed, the clone carrying the surface antigen recognized by the antibodies is eliminated. Meanwhile, however, other clones with different surface antigens thrive. In other words, *T. brucei* both stimulates and evades the immune system, thus ensuring the long-term survival of the parasite in the host. This makes the development of vaccines extremely difficult (see also Box 2.1).

Box 2.1 Antigen Variation in T. brucei
by Nina Papavasiliou, Rockefeller University, New York

In the genome of *T. brucei,* approximately 2000 genes, including pseudogenes and gene fragments, with homology to variant surface glycoprotein (VSG) were found. Less than 20% of these genes were considered intact. Two different VSG gene populations exist. While the overwhelming majority of VSG genes forms clusters of 3–250 (pseudo)genes in various chromosomal regions as basic copies, which cannot be directly transcribed, there are

15–20 expression-linked copies in proximity to most telomeres. These can be expressed. It has been hypothesized that these expression-linked copies are derived from the clustered (pseudo)genes by a process of duplication and relocation.

Only one VSG is expressed in an individual trypanosome. As a prerequisite, the gene must be located near a telomere of a chromosome, near eight other genes known as expression site-associated genes (ESAGs), which probably support the expression process (Figure B2.1). The function of many of these ESAGs remains unclear, but some encode proteins relevant to particular metabolic activities, such as those of an iron-binding protein. The exact mechanism resulting in just one VSG being expressed in a trypanosome is not completely understood. It has been surmised that each nucleus contains limiting amounts of proteins required for VSG transcription and thus only one telomeric VSG is transcribed (though the mechanism that chooses which one remains unclear).

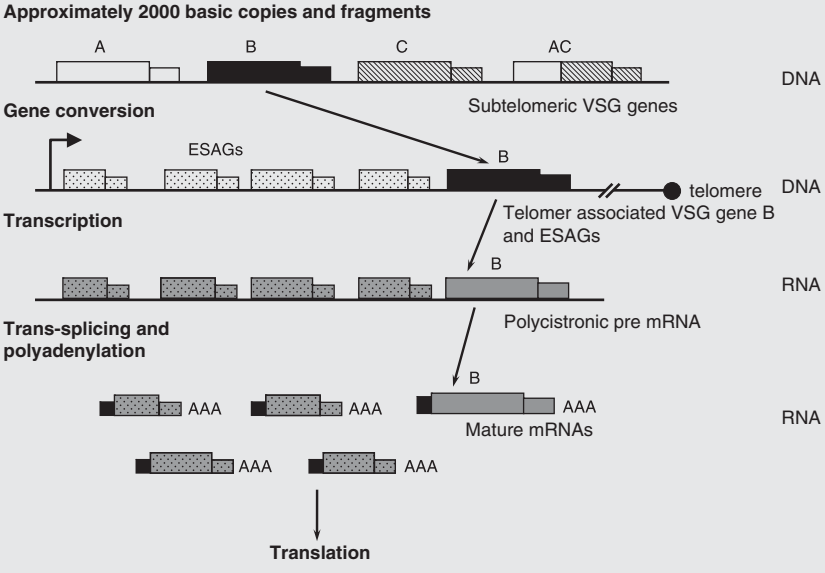


Figure B2.1 Polycystronic transcription in *Trypanosoma brucei*. (Graphic: J. Donelson.)

It has been observed that *in vitro* a new VSG gene is switched on after about 10^6 cell divisions. Therefore, given the huge number of blood stages, different VSG variants are constantly produced (though a handful dominates each peak). The VSG variants at the peak are then eliminated by antibody responses and are replaced by the new VSGs. Early in infection, this replacement process is considered "semi-predictable": each expressed VSG has a preference to switch to a small set of other VSGs. Later, the choice of the new VSG appears random.

A complex of transcription proteins is responsible for the expression of only one VSG gene per trypanosome. Whereas it was thought that each peak of parasitemia represents a homogenous parasite population this has been revised, and current understanding is that each peak consists of a handful of dominant VSG clones and a large number of additional VSGs at any given time. This VSG coat is eventually replaced with an invariant coat composed of repeats of the procyclin protein, once the parasite enters the intestine of the tsetse fly.

As well as a *T. brucei*-specific B cell response, an increase in nonspecific B cell responses is observed during infection. The activation of nonspecific B cells is thought to have evolved as it facilitates immune evasion and dilutes specific antibody responses. A degree of immunosuppression and immune deviation also occurs, further facilitating parasite survival. A Th1-dominated response present in the early stages of infection is replaced with a Th2-dominated response as infection progresses. This change would appear to be caused by trypanosome-mediated secretion of prostaglandin E2 from macrophages. The resulting less aggressive immune response increases the likelihood of survival for the parasites, and at the same time protects the host from excessive immune responses and immunopathology.

T. brucei is not only good at evading immune responses, but also has, to a certain extent, evolved to be dependent on at least one of its products. Thus, interferon-γ (IFN-γ) acts as growth factor for the bloodstream stages, and a factor secreted by the parasite has been demonstrated to selectively stimulate the production of IFN-γ from $CD8^+$ T cells. Consequently, parasite growth is reduced in IFN-γ knockout mice compared with immunocompetent wild-type mice

2.5.4 *Trypanosoma congolense*

Trypanosoma congolense is transmitted by tsetse flies and infects almost all species of wild ruminants. While these show few signs of disease, ruminant livestock is usually badly affected. *T. congolense* is therefore the most prominent pathogen responsible for Nagana. The bloodstream trypomastigote stages measure 9–18 μm and their flagellum hardly protrudes over the posterior end of the body, making their movement relatively slow. There is evidence of antigen variation similar to that observed for *T. brucei*. Upon entering the insect host, they convert into slender forms that multiply in the midgut. These migrate into the upper mouthparts, where they differentiate into rapidly dividing epimastigotes. The infective trypomastigote form also develops in the mouthparts. Development inside a tsetse fly takes 2–3 weeks. The infection rate is typically 10–15%. *T. congolense* uses a wide range of *Glossina* species, including the *palpalis*, *morsitans,* and *fusca* groups (see Arthropods, Family *Glossinidae*) as intermediate hosts, which is why the Nagana pathogen is found throughout all tsetse fly habitats.

The word **Nagana** is taken from the Zulu, which means a depressed state of mind. The disease is found in all tsetse fly habitats, making 11 million square

kilometers, that is, about half of all arable land south of the Sahara, making it unsuitable for intensive cattle farming. Nagana is the economically most significant cattle disease in Africa. The disease is characterized by a constant decline, leading to weight loss, weakness, and finally death. Bleeding into the endocardium occurs and gelatinous edema infiltrations are found. Although central nervous system problems have been observed, these do not dominate the clinical picture. Susceptibility varies considerably between different breeds of livestock. West African dwarf shorthorn, Baoulé and N'Dama cattle (Figure 2.25), and guinea sheep, which are all fairly small and are largely trypanotolerant, due to their adapted immune system. Under normal conditions, these breeds of animals are able to control infection, but succumb to disease when malnourished or due to underlying disease or other stress factors. By contrast, Zebus and European high-yielding varieties of cattle are highly susceptible to infection.

2.5.5 *Trypanosoma vivax*

T. vivax (Latin: *vivax* = long-lived) attacks a wide range of wild ruminants and livestock, using tsetse flies as insect hosts. Biologically, they are very similar to *T. brucei*. Their trypomastigote bloodstream forms are 18–27 µm long and are very motile. They have a long free lead flagellum at the front and a pear-shaped posterior end. Their entire development takes place in the fly's mouthparts where epimastigote forms attach their flagellum to the labrum and hypopharynx and reproduce. They then differentiate into metacyclic trypomastigote forms. In any tsetse population, up to 40% may be infected. Under favorable conditions, development

Figure 2.25 West African dwarf shorthorn cattle. This dwarf variety has efficient immune responses against trypanosomes, as long as the animals are healthy. (Image: P. Agbadje with kind permission by W. Bernt.)

within *Glossina* takes no longer than 5 days. *T. vivax* may also be mechanically transmitted by horseflies, and is therefore the only Nagana pathogen found outside the tsetse belt. It is thought that infected livestock brought *T. vivax* to South America, where it has now spread to some regions. However, major Nagana epidemics are rare in South America. Symptoms in cattle and small ruminants are similar to those of *T. congolense* infection.

2.5.6 *Trypanosoma evansi*

Trypanosoma evansi is a parasite of many ungulate species, where it causes a disease called Surra. *T. evansi* is also found in rodents and carnivores. *T. evansi* has a geographical range spanning from Africa north of the tsetse belt over the Middle East to East Asia. It has also been introduced to the New World. This trypanosome species is mechanically transmitted, through infected blood remaining in the mouthparts of blood-feeding insects when an animal is bitten. They do not develop within the insect. In most areas, the vectors are *Tabanidae*, but tsetse flies, stable flies, and similar insects are also likely vectors. In South America, the infection can also be carried by vampire bats, which can also develop disease. Some authors consider *T. evansi* to be a subspecies of *T. brucei* that adapted to a mechanical transmission mode. Some strains have lost their kinetoplast, indicating a loss of function of the mitochondrion, with an ensuing inability to thrive in an insect host.

Surra is a Nagana-like disease, which causes fever, central nervous system disorders, and extreme emaciation in horses, camels, water buffalos, and dogs. In horses, the central nervous system may be affected as soon as 2 weeks post infection with *T. evansi*. The horses suffer from progressive paresis of their hind legs, making it difficult for them to stand. Horses are very susceptible and almost all succumb to the disease, whereas cattle and pigs can be carriers, but do not suffer from the disease. Surface antigen variation has been described for *T. evansi* in a similar manner as in other *Trypanosoma* species.

2.5.7 *Trypanosoma equiperdum*

The species is found in horses worldwide, and is transmitted during the covering act. The disease is therefore known as covering sickness or dourine. Due to strict hygiene, it has been eradicated in most countries in Europe and North America.

Trypanosoma equiperdum (Latin: *equus* = horse, *perdere* = to lose) is morphologically similar to *T. evansi* and often does not have a kinetoplast. After transmission, they begin to multiply in mucous tissues of genitals, causing thickening of tissue and local lesions. Infected mares have vaginal discharge and may suffer miscarriage. At a later stage, the parasites enter the bloodstream, and swellings containing trypanosomes develop in the subcutaneous tissue. These manifest as

typical wheals. The disease leads to nervous disorders and paralyses. It is usually of a chronic nature with few fatalities. The covering disease clinically manifests in three different stages – initial local inflammation, followed by systemic manifestations and finally neurological deficits.

2.5.8 *Trypanosoma cruzi*

T. cruzi affects more than 150 mammalian species, including humans, causing Chagas disease, an infection limited to Central and South America, where approximately 8 million people suffer from the disease, with an annual death toll of 12 500 (see http://www.who.int/topics/chagas_disease/en/). Chagas disease also occurs as an imported disease in countries outside Latin America, for example, in the United States, where an estimated 300 000 persons are infected. Blood-sucking reduviid bugs, including *Triatoma, Panstrongylus*, and *Rhodnius*, act as insect hosts. *T. infestans* is the main arthropod host. All developmental stages of the bug are susceptible, and the parasite is transmitted via their excrement. There is a wide variety of *T. cruzi* strains with different geographical variants and biological properties. These strains are grouped into *T. cruzi* I lineage (occurring mainly in the northern part of Latin America) and *T. cruzi* II lineage (distributed mainly in the Southern Horn).

Trypomastigote infective stages from the excrement of blood-sucking reduviid bugs enter the mammalian host via a wound cauaed by the insect's bite, facilitated by host scratching, or through a mucosal membrane (Figure 2.26). Some authors report on another effective transmission route, where orally ingested metacyclic trypomastigotes penetrate the buccal mucosa. Therefore, food contaminated with bug excrement is a possible infection route. The parasites invade a number of cell types, including mononuclear phagocytes, muscle cells, and neuroglial cells, where they transform into amastigote stages (~2 µm) and reproduce by fission. Initially, they are contained in a parasitophorous vacuole that is acidified by lysomes, but ultimately escape into the cytoplasm after 2–3 h. Host cells may contain large numbers of amastigotes and are sometimes called pseudocysts (Figure 2.27b). The amastigotes develop into trypomastigotes within the host cell. When the host cell bursts, these are released into the bloodstream as 20 µm long, often C-shaped trypanosomes with a free flagellum at their end and very prominent kinetoplast (Figure 2.27a). Such trypomastigotes may infect further mammalian cells or may be taken up by bugs. In cases where cells filled with amastigotes rupture, these are also able to invade a new host cell.

Once a reduviid bug has ingested them, they develop in the midgut into actively dividing epimastigote forms. In the hindgut, the epimastigotes differentiate into metacyclic epimastigote forms that are excreted via the feces directly after a blood meal (Figure 2.28). Not all reduviid bugs are competent vectors as only species that defecate while ingesting blood will allow infective stages to enter the bite wound.

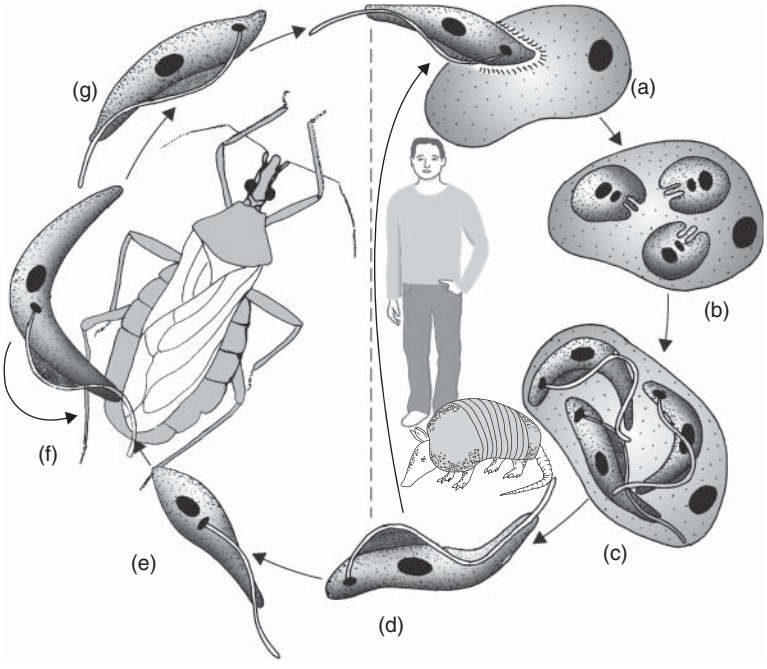
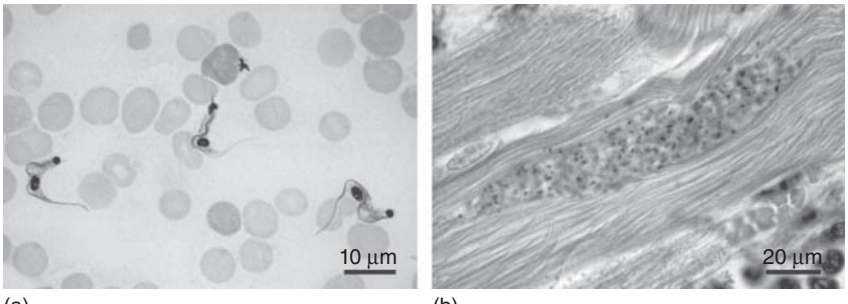


Figure 2.26 Life cycle of *Trypanosoma cruzi*. (a) Invasion of a cell by a trypomastigote metacyclic infective form from excrement of a reduviid bug. (b) Intracellular amastigotes. (c) Transformation into intracellular trypomastigotes. (d) Trypomastigote form in the bloodstream. (e, f) Epimastigote insect form. (g) Trypomastigote, metacyclic form. (Adapted from various authors.)



(a) (b)

Figure 2.27 Stages of *Trypanosoma cruzi*. (a) Trypomastigote blood form, with distinct kinetoplast and a curved cell body. (b) Amastigote forms in a murine muscle cell. (Images: Courtesy of P. Kimmig.)

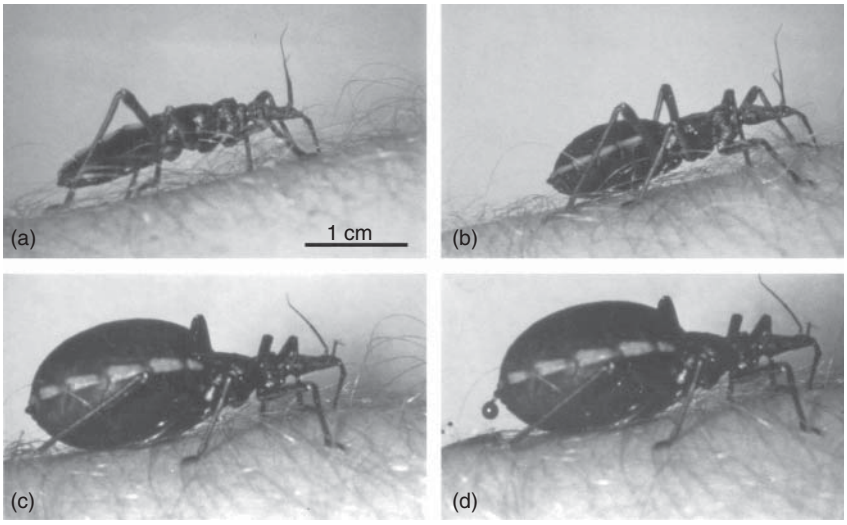


Figure 2.28 Reduviid bug *Dipetalogaster maximus*, an arthropod host of *Trypanosoma cruzi*, during a blood meal. Only reduviid bug species that defecate immediately after a blood meal can transmit *T. cruzi*. (Image: Courtesy of G. Schaub.)

The chance of infection increases if the bug drags a drop of excrement over the wound after feeding or when the host scratches the wound. Bedbugs are not suitable as *T. cruzi* vectors, because they do not defecate before they approach their hiding place.

T. cruzi can easily be confused with ***Trypanosoma rangeli***, an apathogenic *trypanosome* with a similar distribution area that is also transmitted via reduviid bugs.

The disease was named after Carlos Chagas (Figure 2.29), who discovered *T. cruzi*. The first clinical sign after the painless bite is an initial local swelling with signs of inflammation (Figure 2.30a). This swelling around the bite site is known as a chagoma and persists for 2–3 weeks. Since the reduviid bugs have a preference for thin skin with a good blood supply, chagomas often form in the face around the eyes and mouth. For this reason, the reduviid bugs are sometimes referred to as "kissing bugs." The typical swelling near the eye is also known as Romana's sign. In the acute phase of the infection, the bloodstream contains many trypomastigotes. Mononuclear phagocytes, striated muscle cells, and cardiac muscle cells in particular are frequently infected. Fever, respiratory distress, swollen lymph nodes, and inflammation of the brain (encephalomyelitis) and heart muscle (myocarditis) may develop.

This proliferative phase is limited by the host's immune response. In approximately 60% of all infected individuals, no further symptoms are found and they are considered as cured The remaining approximately 40% of patients enter the chronic phase, where trypanosomes persist over years and mainly cause diseases

Figure 2.29 Brazilian bank note with the image of Carlos Chagas, the discoverer of *Trypanosoma cruzi* and its life cycle.

of the heart. Chronic inflammation can damage the heart muscle tissue to such an extent that the heart is enlarged and the heart wall at the apex becomes paper-thin (Figure 2.30b) and eventually tears. Chagas patients can thus literally die of a broken heart. The destruction of parasympathetic ganglia in smooth muscle may, in rare cases, lead to enlargement of organs (ectasia) of the gastrointestinal tract, referred to as "megacolon," "megaesophagus" and so on (Figure 2.30c). This enlargement probably develops due to peristaltic disorders. It was long thought that autoimmune responses were the cause of such pathological deformations, since in both heart disease and intestinal ectasia, autoimmune antibodies have been detected. However, using sensitive methods, trypanosomes can now be detected in these patients, and the prevailing opinion therefore is that the chronic inflammatory responses are caused by antigenic stimuli of the trypanosomes. The available drugs are only effective during the acute phase and cannot eliminate the parasite during the chronic phase.

In chronically infected individuals, *T. cruzi* are often not detectable in blood smears due to their scarcity. Consequently, xenodiagnoses have been used for diagnosis. This involves letting lab-reared, *T. cruzi*-free reduviid bugs feed on the patient and examining the gut of the reduviid bugs sometime after for *T. cruzi* epimastigotes to indicate infection. State-of-the-art detection of the infection uses antibody tests. As *T. cruzi* can be transmitted through blood donors, blood donations from regions where the infection is endemic must be tested.

Opossums, dogs, and other animals play an important role as reservoir hosts. Since the reduviid bugs inhabit crevices, typically in thatched roof and depend on frequent blood meals, they thrive in rural areas where transmission conditions are ideal. The parasite thrives where humans live in close proximity with reservoir hosts such as dogs or guinea pigs (e.g., in Central and South America). This indicates that the disease could be controlled by improving housing conditions and

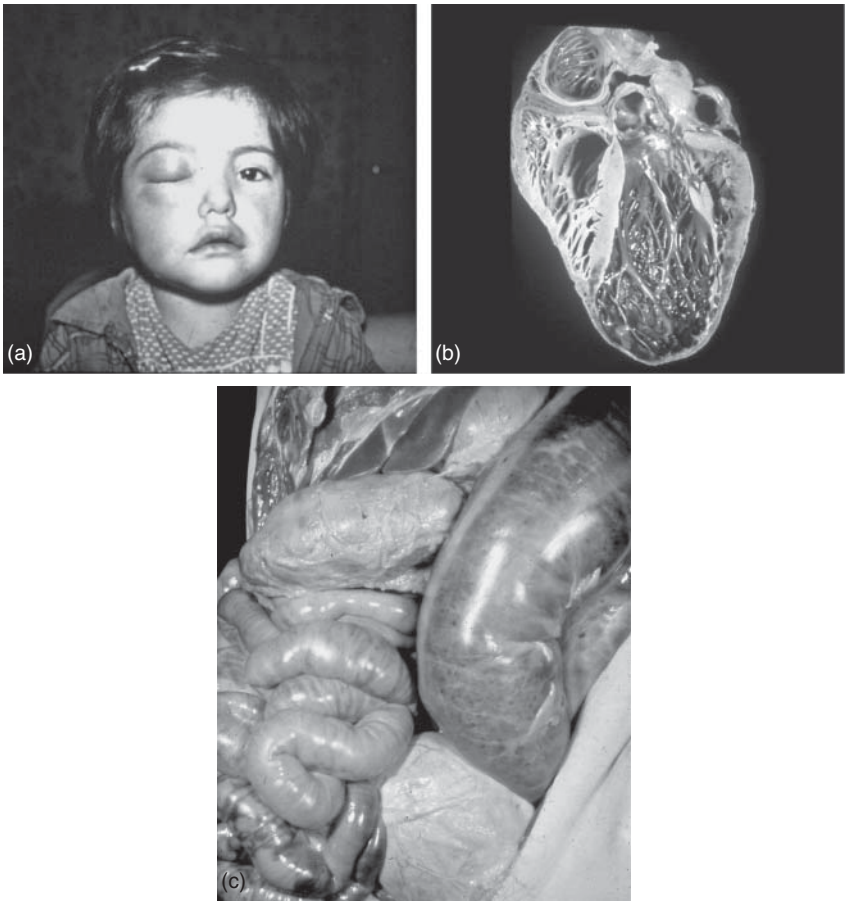


Figure 2.30 Chagas disease. (a) Swollen eye lid (Chagoma or Romana's sign) after transmission of *Trypanosoma cruzi* by reduviid bugs. (b) Enlargement of the heart with thinned heart wall at the apex. (c) Intestinal ectasia (Images (a) and (b) archive of the Department of Parasitology, University of Hohenheim, Image: Courtesy of (c) J. S. Olivera, received from MA Miles.)

reducing contact with reservoir hosts. *T. cruzi* is also found in animals but not humans in some southern states of the United States.

Approximately 18% of the *T. cruzi* genes encode surface proteins highlighting the importance of the parasite–host interface. A large proportion of these proteins are members of two superfamilies, mucins, and mucin-associated surface proteins. Mucins are highly glycosylated proteins that are essential constituents of mucus. Insect and vertebrate forms of *T. cruzi* are coated in their specific sets of mucins that are inserted into their membrane with GPI anchors. They are protease-resistant, forming a protective coat against the host's enzymes. The immense variability of mucins and the large proportion of pseudogenes and gene

fragments are reminiscent of the wide range of VSGs in African trypanosomes, which probably evolved from such molecules.

Most of the surface proteins are highly glycosylated and some of them include sialin residues. The trypanosomes, however, are unable to synthesize sialic acid and must rely on their host. It is extraordinary that no less than 737 functional *T. cruzi* genes encode trans-sialidases. Many members of this GPI-anchored enzyme family have the ability to cleave sialic residues from host glycoproteins and transfer them onto the surface of the parasite (Figure 2.31), while others are enzymatically inactive. Transfer of sialic acid completes the formation of glycoproteins in *T. cruzi* and enables them to invade host cells, while other proteins belonging to the trans-sialidase family that have no transfer function are probably involved in binding to host cells. GPI-anchored surface proteases (of the GP63-protease type, which is prominent in the *Leishmania* invasion process) form a very strong contingent of 174 proteins. Their function, however, has not yet been elucidated in detail.

T. cruzi trypomastigote can infect a large variety of cell types, including non-phagocytes, such as muscle cells. When establishing contact with the host cell,

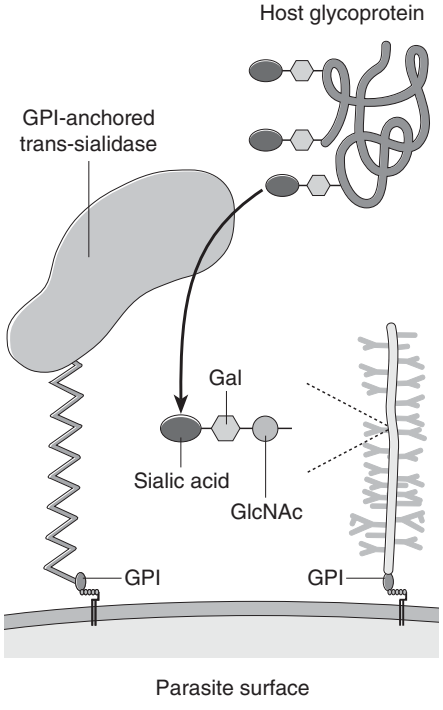


Figure 2.31 Transfer of sialic acid groups from the host cell to glycoproteins of *Trypanosoma cruzi* by virtue of trans-sialidases. (Adapted from Buscaglia, C.A., Campo, V.A., Frasch, A.C.C., and Di Noia, J.M. (2006) *Nat. Rev. Microbiol.*, **4**, 229–236, with kind permission by the publisher.)

fibronectin, and laminin have an important part to play, as they facilitate cell-to-cell interaction. Mucins and proteins of the trans-sialidase family may facilitate the attachment of the parasite to the host cell.

Invasion is initiated by factors released by trypomastigotes that increase calcium levels in the host cell. The enzyme endopeptidase was described to play a key role by processing a hitherto unknown factor, which, in turn, stimulates the host cell. Another mechanism potentially involved in calcium increase is the release of pore-forming proteins that damage the host cell membrane. These processes induce a repair mechanism of the host cell that triggers recruitment of lysosomes and autophagosomes to the attachment site. In this process, vesicles within the host cell are transported along tubulin fibers to reach the adhesion site. Moreover, exocytosis of lysosomes results in the extracellular release of host cell repair components. Together, these and other mechanisms envelop the trypanosome to form a parasitophorous vacuole (Figure 2.35). Approximately 2 h following invasion, the trypanosomes destroy the vacuole membrane with the help of a pore-forming protease, cruzipain, and trans-sialidases, and are free in the cytoplasm. It is unusual that cells that normally have no phagocytizing ability (e.g., muscle cells) actively participate in the internalization of a pathogen. The process has therefore been termed "induced phagocytosis." It is however not unique and some intracellular bacteria and viruses have similar mechanisms that enable nonphagocytizing host cells to ingest them by stimulating certain host cell receptors.

During chronic *T. cruzi* infection, the majority of parasites are intracellular. The most important mechanism limiting infection then involves killing the intracellular parasites by activating the host cell through externally produced IFN-γ. This cytokine stimulates the host cells to produce NO and reactive oxygen products that kill the parasites. GPI anchors and *T. cruzi* DNA that stimulate TLR2 and TLR9 are important triggers for the production of IFN-γ. These immune responses result in inflammation that can permanently damage host tissue. In other words, *T. cruzi* has the ability to elicit an immunopathogenic response in the host (see Figure 1.42 and Figure 2.30). The few circulating trypomastigotes are attacked by complement-activating antibodies. Sensitivity to complement proteins varies considerably between parasite strains. Trypanosomes that have been opsonized by antibodies may be attacked and phagocytosed by various effector cells. In contrast to African trypanosomes, *T. cruzi* is unable to evade antibody responses through variation of its surface antigens.

T. cruzi does however have other means of immune evasion. Infection is associated with polyclonal B cell activation and results in nonspecific IgM antibody production and hyperimmunoglobulinemia. Proliferation of B cells is triggered by an enzyme called proline racemase that is secreted by trypomastigotes and has a mitogenic effect on B cells, resulting in a 250–1000% increase in their numbers. Trypomastigotes have the ability to cleave antibodies binding to their surface with a secreted protease. In addition, host proteins have been found associated with the surface of trypomastigotes potentially further masking the parasite from the

immune system. Three different complement inhibitors have been described in *T. cruzi*, highlighting the evasion of the complement system by *T. cruzi.*

2.5.9
Leishmania

Parasites of the *Leishmania* genus require, an insect host and a vertebrate host for completion of their life cycles (Figure 2.32). Sand flies (family *Psychodidae*), that is, very small nematocera that are adapted to dry habitats serve as insect hosts. *Leishmania* are often zoonotic as their specificity for mammalian hosts is low. *Leishmania* infections in humans have been found in 88 countries and all inhabited continents. The number of new cases was estimated at 1.2 million per year and 20 000–30 000 deaths occur annually (see http://www.who.int/topics/leishmaniasis/en/). In the Old World, sand flies of the *Phlebotomus* genus are responsible for transmission, while in the New World, species of the *Lutzomyia* genus are responsible. These are tiny blood and sap-sucking insects (length 1.5–2.5 mm) that breed in rotting substrate of small wet biotopes, such as rodent dens, termite mounds, and crevices, in the ground or in walls, which are also

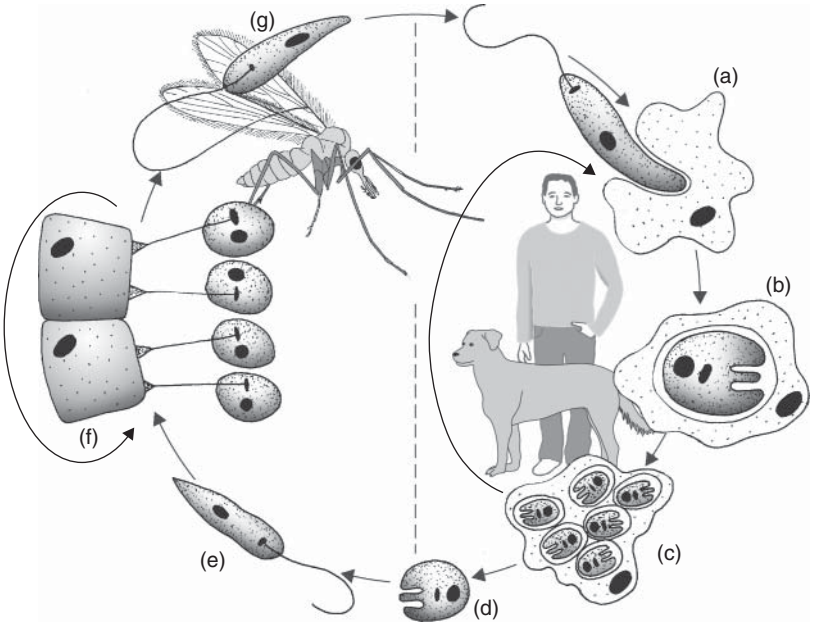


Figure 2.32 Life cycle of *Leishmania* sp. (a) Invasion of a macrophage by a metacyclic promastigote infective form. (b) Change into amastigote form. (c) Multiplication during the amastigote stage. (d) Released amastigote form. (e) Promastigote form from the insect foregut. (f) Promastigotes firmly attached to foregut cells. (g) Metacyclic promastigote form. (Adapted from various authors.)

found in dry areas. Only the female sand flies feed on humans and are thus responsible for disease transmission.

2.5.9.1 **Development**

A sand fly can transmit several hundred promastigotes via its saliva. These stages are ingested by phagocytes after the insect takes a blood meal from a human. During the early stage of the infection, neutrophilic granulocytes and dendritic cells have a temporary role to play (see below), whereas later, macrophages become the predominant host cells. The parasites convert into the amastigote form in tissue macrophages and Langerhans cells of the skin. The amastigotes reside in the harsh environment of the phagolysome where they reproduce by binary fission until the host cell bursts. At this stage – in some species even in the promastigote stage – the lysosome is considerably enlarged into what is known as multivesicular tubule or megasome, which could be a storage vesicle for metabolic end products (Figure 2.33b). The released amastigotes infect neighboring macrophages or are swept through lymph vessels into the bloodstream. The systemic spread may result in the infection of macrophage populations of the inner organs (liver, lungs, spleen, and bone marrow).

When free amastigotes or infected host cells are ingested by a sand fly, the amastigotes convert into actively dividing promastigotes in the foregut. These initially stay freely in the gut before attaching to the gut epithelium with their flagellum. They continue to divide and finally differentiate to free-floating

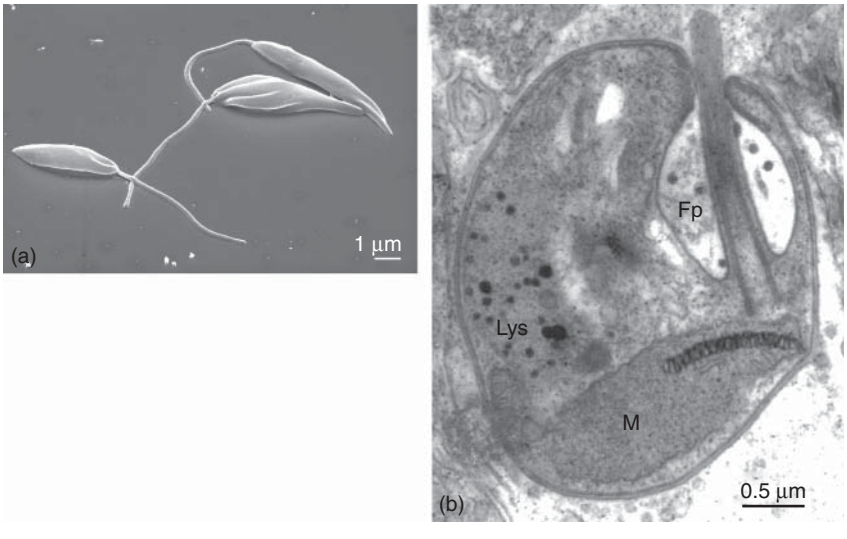


Figure 2.33 Stages of *Leishmania*. (a) Promastigotes of *L. major* (image: Department of Molecular Parasitology, Humboldt Universität). (b) Amastigote stage of *L. mexicana*. Section through the flagellar pocket with longitudinal section of the flagellar stump. Lys, lysosome; Fp, flagellar pocket; M, mitochondrion. (From Waller and McConville (2002) Int. J. Parasitol. 32, 1435–1445, with kind permission by Elsevier Publishers.)

metacyclic promastigotes that migrate to the mouthparts of the phlebotomines. There are indications that sexual processes exist, but details are not known yet. Development within the insect host takes approximately 1 week. Infected sand flies display dysfunctional feeding behavior, due to the parasites living in their foregut and in their mouthparts. This, in turn, facilitates transmission (see Section 1.7.3).

2.5.9.2 Morphology

Promastigotes are 15–25 µm long and have a fairly sturdy flagellum (Figure 2.33a), whereas amastigotes are round or oval and 2–4 µm long and have no external flagellum (Figure 2.33b).

2.5.9.3 Leishmaniosis

There are 18 species and subspecies of human-pathogenic *Leishmania* that vary in their geographic distribution and their tropism for particular organs in the human host (Table 2.3), causing different forms of leishmaniosis (also termed leishmaniasis). In the early stages of an infection, a transient inflammatory swelling at the site of the bite develops, termed a leishmanioma. From there, the *Leishmania* spread to invade their preferred organs, such as the viscera, skin, or ear cartilage. Depending on their preferences, a variety of diseases may develop, from the usually spontaneously healing cutaneous to the often fatal visceral leishmaniosis. The outcome of leishmaniosis largely depends on the intensity and quality of the T helper cell response (see below). This explains why *Leishmania* is a common opportunistic pathogen in AIDS patients and other immune-suppressed individuals. Some of the most relevant *Leishmania* species and associated diseases will be discussed below.

2.5.9.4 Cell and Immune Biology

The surface of *Leishmania* is coated by a glycocalyx consisting of GPI-anchored glycoproteins with high sugar content (Figure 2.34). In infective promastigotes, but not amastigotes, lipophosphoglycan (LPG), a highly glycosylated molecule with a molecular weight of approximately 9 kDa, is the most important surface component. Proteophosphoglycans (PPGs) that are also lipid-anchored feature unusual phosphate groups in their sugar side chains. Many such molecules are secreted. In promastigotes in particular, GPI-anchored surface molecules include GP63, a small family of highly glycosylated metalloproteases (63 kDa), whereas a large part of the amastigote surface is covered in small glycophosphoinositol (GPI) phospolipid. (GIPLs). Apart from a GPI anchor, these have sugar side chains that have been assumed to exhibit immunomodulatory properties. In amongst these GPI-anchored molecules, there are integral membrane proteins involved in nutrient intake, transport, and communication with the host cell. In contrast to *T. brucei*, these functions are not limited to the flagellar pocket.

Contact between *Leishmania* and host cells is made through receptor–ligand interaction, involving a wide range of molecules. After opsonization by complement or antibodies, the parasites are recognized by the Complement receptor

Table 2.3 Overview of major *Leishmania* species.

Species/subspecies	Distribution	Disease	Reservoir host
Old World			
Leishmania donovani	Indian Subcontinent, East Africa, China	Visceral leishmaniosis *Kala Azar*	Not known
Leishmania infantum	Mediterranean countries, Iraq, Iran, China	Visceral l.	Dogs, foxes, jackals
Leishmania major	Middle East, Africa, Central Asia, Iran/rural areas	Cutaneous l, "Oriental sore"	Rodents inhabiting steppes
Leishmania tropica	Middle East, North Africa, Namibia/cities	Cutaneous l, "Oriental sore"	Indicators point to rock hyraxes and dogs
Leishmania Aethiopica	Ethiopia, Kenya	Cutaneous l.	Rock hyraxes
New World			
Leishmania infantum (chagasi)	Latin America	Visceral l.	Dogs, foxes, opossums
Leishmania mexicana	Central America	Cutaneous l., especially of the ear, chicleros' disease	Rodents inhabiting woodland
Leishmania amazonensis	Amazonian region	cutaneous l.	Rodents inhabiting woodland
Leishmania braziliensis	Central and South America	Cutaneous l., mucocutaneous l. *espundia*	Rodents inhabiting woodland, dogs Equidae
Leishmania braziliensis guyanensis	Guyana, Amazonian region	Cutaneous l., mucocutaneous l.	Sloths
Leishmania braziliensis panamensis	Central America	Cutaneous l.	Sloths
Leishmania braziliensis peruviana	Peruvian Highlands	Cutaneous l.	Dogs

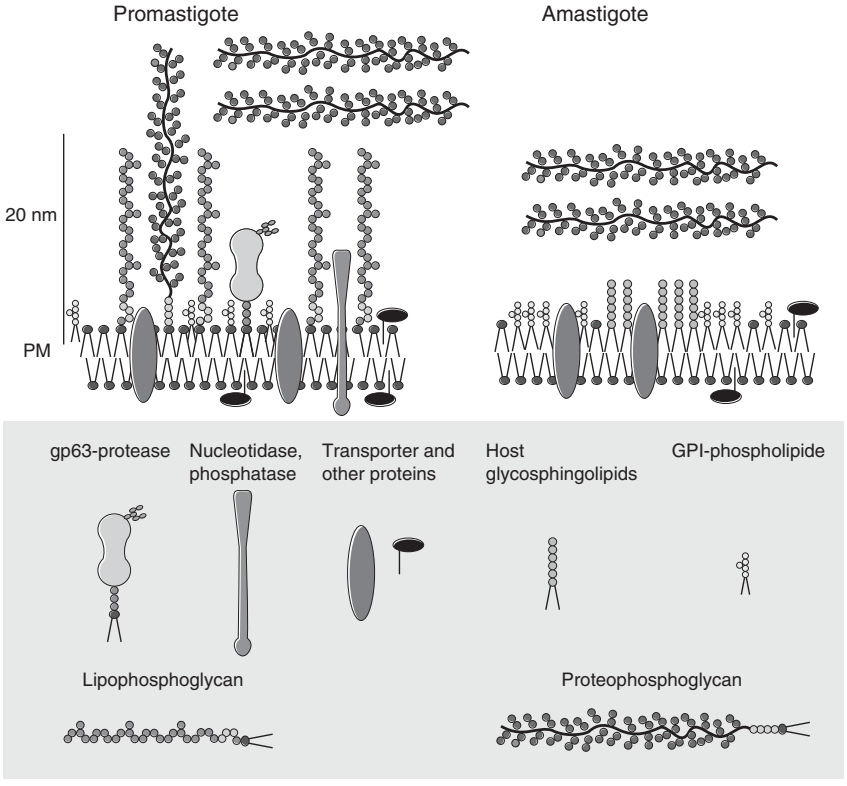


Figure 2.34 Surface components of the promastigote and amastigote stages of *Leishmania*. (From Naderer, T., Vince, J.E., and McConville, M.J. (2004) *Curr. Mol. Med.*, **4**, 649–665, with kind permission by Bentham Science Publisher Ltd.)

CR1 or Fc receptors of host macrophages. Independent of opsonization, the macrophages can also bind to *Leishmania* via their mannose/fucose and fibronectin receptors. On the *Leishmania* side, LPG and GP63 are involved in the binding process.

Leishmania provide an impressive example of usage of host immune responses for the parasite's own advantage, as they invade their preferred host cell, the macrophage, by exploiting its phagocytotic mechanism (Figure 2.35). The surface-bound metalloprotease GP63 of infective promastigote forms was described to cleave the complement component C3 in an untypical manner into inactive C3bi. Through their complement receptor CR1 and the integrin CD11, macrophages recognize the C3bi fragment and phagocytose *Leishmania*. This process elicits only weak activity in the host cell, possibly due to influence of the surface component LPG. The parasites are enclosed in a parasitophorous vacuole formed by the phagolysosome of the macrophage, where they manage to thrive due to various immune escape mechanisms (see Box 2.2).

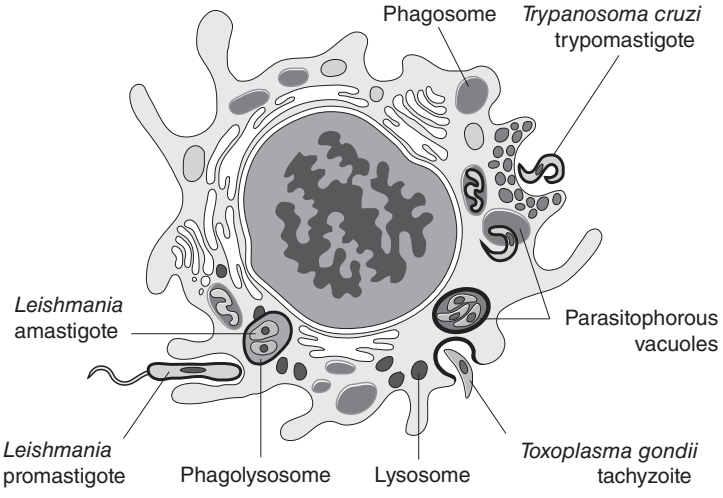


Figure 2.35 Invasion of protozoan parasites into a host cell. *Leishmania* is taken up by phagocytosis. *Trypanosoma cruzi* invades by induced phagocytosis. *Toxoplasma gondii* invades by gliding motility. All parasites lie in a parasitophorous vacuole of different origin, from which *T. cruzi* escapes shortly after invasion. (From Sacks and Sher (2002) Nature Immunology 3, 1041–1047, with kind permission by the publisher.)

Box 2.2 Survival of *Leishmania* Inside a Macrophage

Following phagocytosis, the promastigotes of *Leishmania* are held in phagosomes, which then fuse with lysosomes to form phagolysosomes. Within the phagolysosome, the parasites are exposed to multiple stresses by acidic pH, lytic enzymes, and oxidative effector molecules, which all threaten survival and reproduction. They respond to the situation using several interlinked mechanisms (Figure B2.2). LPG, a complex GPI-anchored surface protein (~9 kDa), is partially released into the parasitophorous vacuole, where it inhibits fusion with the lysosome. LPG and small GPI-anchored phospholipids (GIPLs) on the amastigote surface have an inhibitive effect on the production of reactive oxygen and NO. The effect of whatever aggressive molecules still produced is mitigated by detoxifying enzymes secreted by *Leishmania* (e.g., catalase or superoxide dismutase) and LPG. In addition, *Leishmania* expresses proteins that regulate proton levels of the parasite cell and integrate into the membrane of the parasitophorous vacuole to act as proton pumps and increase pH levels within the compartment. This reduces the activity of lysosomal enzymes released by the host cell. The host enzymes themselves come under attack by the surface-bound protease GP63. The infection induces the production of the anti-inflammatory cytokines IL-10 and TGF-β in the host macrophage, as well as prostaglandin E2, which reduce T cell responses in its surroundings and interfere with the activation of the macrophage by IFN-γ.

LPG also specifically reduces the production of the pro-inflammatory cytokines IL-1β and IL-12. Amastigotes reduce the expression of MHC II molecules on the surface of host macrophages, thus reducing antigen presentation and inhibiting the activation of T helper cells. The intracellular parasites in such a modified cell can only be killed if the host cell is activated by external IFN-γ.

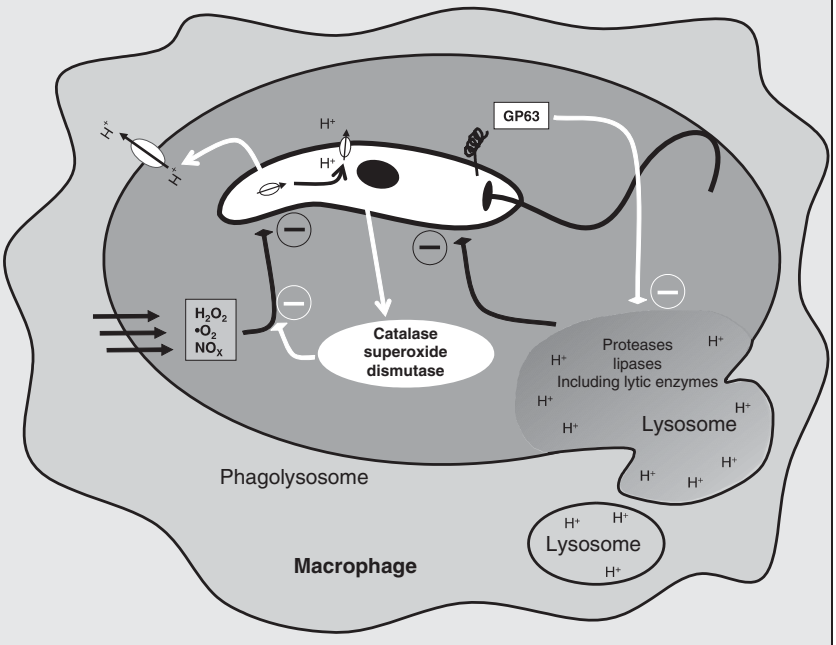


Figure B2.2 *Leishmania*–host interactions inside a macrophage.

In the initial stage of the infection, promastigotes are also phagocytized by neutrophilic granulocytes that are attracted to the infection site by chemotaxis. At this stage, they are the most common cells on site. In this type of primary cell, *Leishmania* can survive without further differentiation and delay apoptosis of the host cell. When the cells finally undergo apoptosis, the resulting vesicles (containg leishmania) and debris are ingested by macrophages via scavenger receptors and digested, allowing the parasites to enter the cell. The ingestion of apoptotic cells and vesicles by scavenger receptors triggers strong deactivating signals within the macrophages and the parasites are not attacked. Thus, the neutrophilic cells act as Trojan horses during the early stage of the infection. In addition, the saliva of sand flies has an immunosuppressive effect, which also aids parasite establishment.

Similarly to humans, inbred mouse strains infected with *L. major* show very different responses to a *Leishmania* infection. At one end of the spectrum, highly

susceptible mouse strains have been identified (e.g., BALB/c) that develop a serious, usually lethal form of the disease, while at the other end, there are mouse strains (e.g., C57BL6) in which the infection heals within a short period. A significant difference between these mouse strains seems to be the cytokine milieu that develops in the lymph nodes draining the infection site, which ultimately affects T cell differentiation. The cytokine IL-12 and IL4, respectively, seem to play a key part. IL-12 causes NK cells to produce IFN-γ, resulting in the formation of T helper 1 (Th1) cells (see Figure 1.37). If, however, during the early stage, very little IFN-γ is available at the site and IL-4 dominates, such as in susceptible animals, Th2 cells develop, which produce IL-4 and IL-5. IFN-γ crucial for recovery from the infection, as it activates the production of reactive oxygen and nitrogen metabolites that kill *Leishmania* (see Figure 1.38). Nitric oxide (NO) is thought to be the major reactive product involved in the killing process. However, the mouse model also demonstrates that even after complete recovery, small quantities of *Leishmania* are still detectable. This is possibly also the case in humans. The differentiation of T cell responses into Th1 and Th2 subtypes and their regulation was first described in *Leishmania* infections in mice and has wide implications for the understanding of other parasitic and infectious diseases as well as allergies.

Another factor determining susceptibility that has been identified in mouse strain studies is a glycoprotein with 12 transmembrane domains that carries activation factors into the nucleus of the macrophage. A polymorphism in Solute carrier family 11 member a1 (Slc11a1), formerly Nramp1 (natural resistance associated macrophage protein 1), determines resistance to several intracellular pathogens (*Leishmania*, *Salmonella, Mycobacteria*) of macrophages in mammals, including mice, humans, and dogs. The gene encodes a divalent cation transporter expressed in the late endosome/lysosome that regulates iron homeostasis in macrophages and has been hypothesized to control pathogens by removing iron from the phagosome. However, the gene product has multiple pleiotropic effects on the immune response, including regulation of MHC II expression as well as IL-12 and IL-10 production. Ultimately, the presence of wild-type gene product preferentially induces a Th1 response rather than a Th2 response, which favors resistance to *Leishmania* infection.

Allelic replacement through homologous recombination has been established in *Leishmania* and such methods have elucidated the function of a wide range of virulence factors. Attenuated (weakened) mutants that lack vital genes, such as the gene for dihydrofolate reductase/thymidylate synthetase, have also been generated. This renders these *Leishmania* avirulent without interfering with their ability to induce protective immune responses in the host. Such attenuated *Leishmania* are currently being studied in view of their potential as vaccines.

2.5.10 *Leishmania tropica*

Leishmania tropica is a causative agent for cutaneous leishmaniosis (Oriental boil, Aleppo boil) in humans, which is mostly found in cities of the Middle East.

Humans are the major and possibly the only hosts, as no epidemiologically significant reservoir hosts have so far been described. The infection is transmitted by *Phlebotomus sergenti*. Typically, local swelling (Leishmanioma) develops around the bite site and then an itching papule that ulcerates and leaves a connective tissue scar in the center, while the inflammation progresses along the thickened edge. A ring-shaped lesion develops that can extend to several centimeters in diameter. It heals after 6–12 months, leaving behind a characteristic flat scar (Figure 2.36a). The infection induces permanent immunity, which is why a simple form of vaccination became known as leishmanization. Infective material was collected from the wound edge of an infected person and inoculated at an inconspicuous place in a healthy person, where an oriental boil developed and healed. This prevented the later development of disfiguring scars in the face. This form of vaccination is now no longer acceptable, as disease can reappear

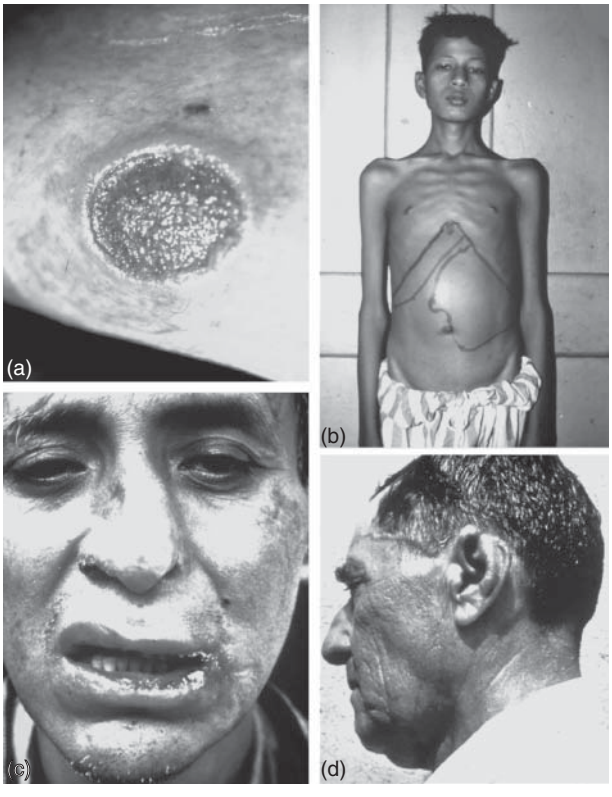


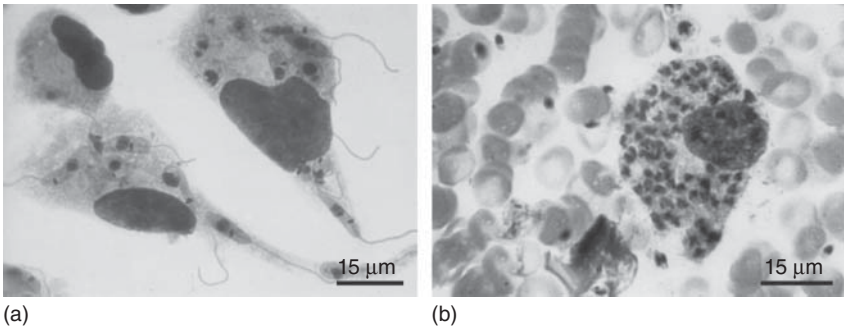
Figure 2.36 Manifestations of leishmaniosis. (a) Oriental boil after *Leishmania tropica* infection. (b) Child with hepatosplenomegaly following *Leishmania donovani* infestation. The outlines of the organs have been marked. (Image: Courtesy of W. Solbach.) (c) Mucocutaneous leishmaniosis in the corner of the mouth after *Leishmania braziliensis* infection. (d) Deformation of ear cartilage in chicleros' disease caused by *Leishmania mexicana*. (a, c, and d: Archive of the Department of Parasitology; University of Hohenheim.)

in immunosuppressed individuals. In a rarer form of the disease, termed diffuse cutaneous leishmaniosis, no ulcers develop, but macrophages laden with masses of parasites are found in thickened skin nodules. In these cases, the host does not develop a proper inflammatory response.

2.5.11 *Leishmania donovani*

Leishmania donovani causes visceral leishmaniosis or Kala Azar (Hindi for black disease, black fever, because of the discoloration of the skin). This parasite prefers macrophages in the visceral organs, especially the spleen, liver, and bone marrow. Most human infections are unnoticed and only in a fraction of infected individuals (<10%) does the disease actually manifest. After 3–6 months of incubation, fever, anemia, platelet deficiency, and a substantially enlarged spleen develop, due to the large quantities of *Leishmania*-laden macrophages (Figure 2.37b). Emaciation, apathy, and general immune deficiency follow. Death mostly occurs because of secondary infections such as tuberculosis, measles, or pneumonia due to immune deficiency caused by erythropoietic disorders in the affected bone marrow. Infection is lethal in approximately 90% of diseased individuals if untreated. In a small number of patients, the infection manifests not as the visceral form, but as dermal leishmanoid with symptoms resembling the low-reactive form of diffuse cutaneous leishmaniosis in *L. tropica* infection. The golden hamster is a suitable animal model for this parasite (Figure 2.34).

Whereas no significant reservoir hosts are known for *L. donovani,* the biologically similar species *Leishmania infantum* infects dogs, and is transmitted as a zoonotic infection. Interestingly, the dogs suffer from pronounced cutaneous leishmaniosis (not the intestinal form), with alopecia and ulcers, in particular around the head. *L. donovani* can be cultured in golden hamsters where they can be easily detected in the enlarged spleen



(a) (b)

Figure 2.37 Stages of *Leishmania donovani*. (a) *In vitro* invasion by promastigotes into macrophages of a golden hamster. (b) Blood smear of a golden hamster with a macrophage containing amastigotes and some free amastigotes. (Images: archive of the Department of Parasitology, University of Hohenheim.)

2.5.12

Leishmania braziliensis and *Leishmania mexicana*

Leishmania braziliensis is a prime example of the large number of New World *Leishmania* species and the causative agent of most cutaneous leishmanioses in Central and South America. The lesions heal badly and may persist over many years. From the primary skin lesions, secondary infections of the skin and of mucous membranes of the nasopharyngeal cavity develop, leading to the destruction of underlying connective tissue and cartilage (Figure 2.36c). This is known as mucocutaneous leishmaniosis. It only develops in a fraction of infected patients with erosion of the nose septum, palate, and lips, resulting in severe disfigurement and inevitable secondary infections. The syndrome is called Espundia. Another New World species, *Leishmania mexicana*, has a tropism for macrophages of the outer ear, where inflammation leads to the deformation of ear cartilage. This is known as chicleros' ulcer, because it mainly affects caoutchouk collectors in humid woodlands (Figure 2.36d).

Further Reading

Bafica, A., Santiago, H.C., Goldszmid, R., *et al.* (2006) TLR9 and TLR2 signaling together account for MyD88-dependent control of parasitemia in *Trypanosoma cruzi* infection. *J. Immunol.*, **177**, 3515–3519.

Bartholomeu, D.C., de Paiva, R.M., Mendes, T.A., DaRocha, W.D., Teixeira, S.M. (2014) Unveiling the intracellular survival gene kit of trypanomatid parasites. *PLoS Pathog.*, 10, e1004399.

Cecflio, P., Pérez-Cabezas, B., Santarém, N., *et al.* (2014) Deception and manipulation: the arms of Leishmania, a successful parasite. *Front. Immunol.*, 5, Art. 480, 1–16.

Cross, G.A.M., Kim, H.-S., and Wickstead, B. (2014) Capturing the variant surface glycoprotein repertoire (the VSGnome) of *Trypanosoma brucei* Lister 427. *Mol. Biochem. Parasitol.*, **195**, 59–73.

Dc-Rubin, S.S. and Schenkman, S. (2012) *Trypanosoma cruzi* trans-sialidase as a multifunctional enzyme in Chagas' disease. *Cell. Microbiol.*, **14**, 1522–1530.

El-Sayed, N.M., Myler, P.J., Blandin, G. *et al.* (2005) Comparative genomics of *Trypanosoma*tid parasitic protozoa. *Science*, **309**, 404–409.

Fernandes, M.C. and Andrews, N.W. (2012) Host cell invasion by *Trypanosoma cruzi*: a unique strategy that promotes persistence. *FEMS Microbiol. Rev.*, **36**, 734–747.

Horn, D. (2014) Antigenic variation in African trypanosomes. *Mol. Biochem. Parasitol.*, **195** (2), 123–129.

Hughe, A.L. and Piontkivska, H. (2003) Phylogeny of *Trypanosoma*tidae and Bodonidae (Kinetoplasti- da) based on 18SrNA: evidence for paraphyly of *Trypanosoma* and six other genera. *Mol. Biol. Evol.*, **20**, 644–652.

Kennedy, P.G. (2013) Clinical features, diagnosis, and treatment of human African trypanosomiasis (sleeping sickness). *Lancet Neurol.*, **12**, 186–194.

Maudlin, I., Holmes, P.H., and Miles, M.A. (eds) (2004) *The Trypanosomiases*, CABI Publishing, Wallingford and Cambridge, 614 pp.

Mugnier, M.R., Cross, G.A., Papavasiliou, F.N. (2015) The in vivo dynamics of antigen variation in Trypanosoma brucei. *Science*, **347**, 1470–473.

Nagajyothi, F., Machado, F.S., Burleigh, B.A., *et al.* (2012) Mechanisms of trypanosoma cruzi persistence in Chagas disease. *Cell. Microbiol.*, **14**, 634–643.

Nunes, M.C., Dones, W., Morillo, C.A., *et al.* (2013) Chagas disease: an overview of clinical and epidemiological aspects. *J. Am. Coll. Cardiol.*, **62**, 767–776.
Rassi, A., Rassi, A., Marin-Neto, J.A. (2010) Chagas Disease. *Lancet*, **375**, 1388–1402.
VanZandbergen, G., Bollinger, A., Wenzel, A. *et al.* (2006) *Leishmania* disease development depends on the presence of apoptotic promastigotes in the virulent inoculum. *Proc. Natl. Acad. Sci. U.S.A.*, **103**, 13837–13842.
Vicco, M.H., Pujato, N., Bontempi, I., Rodeles, L., Marcipar, I., and Bottasso, O.A. (2014) ß1-selective andrenoceptor antagonists increase plasma levels of anti-p2ß antibodies and decrease cardiac involvement in chronic progressive Chagas heart disease. *Can. J. Cardiol.*, **30**, 332–337.

Test Questions

1. Which are the different morphological forms in *Kinetoplastea* parasites?
2. How is the genome of *Trypanosoma brucei* organized, what is its size and for how many proteins does it encode?
3. What is a kinetoplast?
4. What is special during the processing of *Trypanosomatidae* transcripts?
5. What energy sources do *Trypanosomatidae* rely on respectively in their vertebrate and insect hosts?
6. Which cattle disease and which disease in humans is caused by *Trypanosoma brucei*?
7. What different forms can *Trypanosoma brucei* take in the bloodstream?
8. What is the cause of central nervous disorders in *Trypanosoma brucei* infections?
9. What is the structure of the surface coat in *Trypanosoma brucei*?
10. What mechanism does *Trypanosoma brucei* use to evade an immune response?
11. Why is *Trypanosoma vivax* also found outside Africa?
12. Which disease is caused by *Trypanosoma evansi*?
13. How is *Trypanosoma equiperdum* transmitted?
14. Which insect host transmits *Trypanosoma cruzi*?
15. Why are bedbugs unable to transmit *Trypanosoma cruzi*?
16. How is the heart muscle damaged during *Trypanosoma cruzi* infections?
17. Which GPI-anchored enzymes are found on the surface of *Trypanosoma cruzi* trypomastigotes?
18. How does *Trypanosoma cruzi* invade a host cell?
19. Which are the arthropod hosts of *Leishmania*?
20. What stages of *Leishmania* are transmitted in the saliva of arthropod hosts?
21. How do *Leishmania* invade their host cell?
22. Which is the principal host cell of *Leishmania* parasites?
23. What mechanisms do *Leishmania* use to survive in the phagolysosome of its host cell?
24. Which kind of leishmaniosis is caused by *Leishmania tropica*?
25. Of what diseases do patients infected with *Leishmania donovani* die?

2.6
Alveolata

- Free-living or parasitic
- Surface membrane underlain by vesicles (alveoli) and microtubules, creating a complex pellicle
- Food intake by pinocytosis-like mechansims

Phylogenetic analyses suggest the three major subphyla of Alveolata (dinoflagellates, Apicomplexa, and Ciliophora) originate from a common extinct ancestor (Figure 2.38). The hypothetical ancestors of the Alveolata are likely to have possessed a chloroplast and been capable of photosynthesis. They probably had a complex surface with pinocytosis organelles, extrusomes (organelles capable of expelling their contents), a basket-like brace in the top of the cell, and two flagella. They were likely to have been haploid up to the zygote stage. They likely had a predatory lifestyle such as that seen in early Alveolata, attached themselves to protozoa via their rostrum, and sucked out their contents. This mode of feeding can be observed in some extant dinoflagellates and primitive gregarines.

In order to understand the morphology of the Alveolata, in particular their surface structure, it is helpful to envision the recent dinoflagellates. These are photosynthetically active, but often mixotrophic predatory marine and freshwater protozoans with two flagella. Many species live as endosymbionts of animals, the best known of which are the zooxanthellae of corals. Flattened vesicles termed alveolae underlie the surface of typical dinoflagellates. Dinoflagellates can be athecate (naked, lacking a wall) or thecate (possessing a wall). The vesicles in athecate examples are empty or contain amorphous material, but are filled with cellulose in thecate examples. These alveolae are empty in Apicomplexa and Ciliophora. Their membranes form the "inner membrane complex" and together with the outer unit membrane form the characteristic triple membrane (Figure 2.39) underlain by microtubules, the whole structure being termed the pellicle.

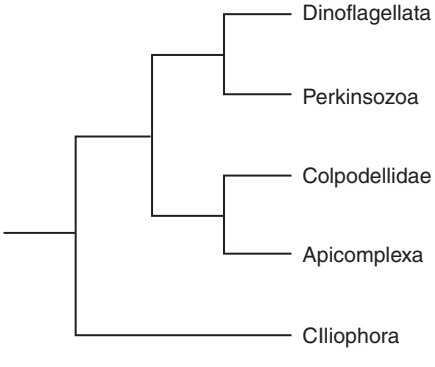


Figure 2.38 Phylogeny of Alveolata. (According to Leander, B.S. and Keeling, P.J. (2003) *Trends Ecol. Evol*., **8**, 395–402.)

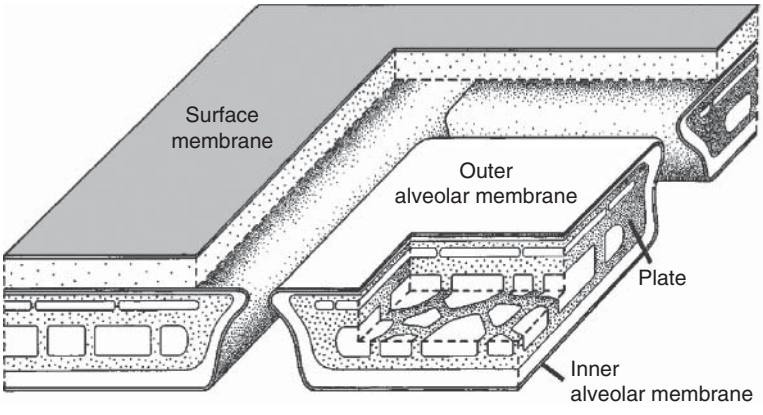


Figure 2.39 Schematic representation of the surface of Alveolata. The plasma membrane is underlain by alveoli (=vesicles), which are filled in the thecate dinoflagellates, but empty in the Apicomplexa and Ciliophora and this results in a triple membrane. (From Hausmann, Hülsmann, Radeck (2003) Protistology, with permission from Scheizerbart'sche Verlagshandlung, Stuttgart, www.schweizerbart.de.)

The **Apicomplexa** comprise a diverse collection of exclusively parasitic organisms capable of infecting a wide range of host taxa. It is thought that this wide variation resulted from an early precursor that converted to parasitism. A defining characteristic of the Apicomplexa is the presence of an apical complex comprising a **conoid**, **polar rings,** and specialized secretory organelles, termed rhoptries and micronemes. Further secretory organelles are the **dense granules**. Proteins found in the rhoptries, micronemes or dense granules of apicomplaxans have been designated as ROP, MIC or GRA followed by a number that normally reflects the order it was discovered and characterised. The cell contains a mitochondrion and usually a plastid organelle, the apicoplast. Nutrients in some organisms are taken up by a pinocytosis organelle, the micropore, and processed within a food vacuole. Secondary loss of some of these organelles has occurred in some members. Apicomplexans lack flagella (except the microgamete stage of some taxa) and their surface is underlaid by microtubules that provide a rigid shape and allow for the typical locomotion, termed gliding motility.

Most **Ciliophora** are free-living and often predatory, while some have converted to ectoparasitism or endoparasitism on mucous membranes. Some Ciliophora are commensals or endosymbionts especially in the rumen of ruminants. Parasitic ciliates have monoxenous life cycles and are generally well adapted and nonpathogenic. Their ciliated surface, nuclear dualism, and ability to conjugate demonstrate that Ciliophora have developed very individual characteristics. They lack plastid organelles, which have been lost during the course of their evolution.

2.6.1 Apicomplexa

- Obligate endoparasites of invertebrates and vertebrates, mostly intracellular
- Monoxenous or heteroxenous
- Apical complex of conoid, rhoptries, micronemes
- Rudimentary plastid organelle (apicoplast) usually present
- Alternation of generations through schizogony, gamogony, and sporogony
- Pathogens of major diseases and animal diseases

Apicomplexans are obligate intracellular parasites, named after their characteristic apical organelle complex that consists of a spirally arranged microtubule structure termed the conoid, secretory club-shaped organelles termed rhoptries and small secretory organelles termed micronemes. Dense granule secretory organelles are present throughout the cytoplasm in some members of the Apicomplexa. The most primitive forms of Apicomplexa are found in marine polychaetes, from where they radiated and acquired more sophisticated host, including arthropods, where heteroxenous life cycles formed. Other groups are likely to have developed direct monoxenous life cycles in vertebrates (e.g., *Cryptosporidium*). Secondary loss of heteroxenous life cycles might also have resulted in monoxenous groups. In many groups, the very long coevolution of the host and parasite has generally resulted in low pathogenicity. The Apicomplexa include important human pathogens, including *Plasmodium* sp. and *Toxoplasma gondii*, that cause malaria and toxoplasmosis, respectively. Apicomplexans such as *Eimeria* sp. and *Babesia* sp. are also important pathogens of domestic animals responsible for coccidiosis and piroplasmosis, respectively, and some like *T. gondii* and *T. cryptosporidium* are zoonotic. Table 2.4 shows the classification of Apicomplexa, but only the taxa discussed in this book are listed.

2.6.1.1 Development

In their **life cycles**, Apicomplexa exhibit an alternation of generations, with asexual **schizogony**, sexual **gamogony,** and asexual **sporogony** (Figure 2.40). During their development, the Apicomplexa undergo many changes of form. The Apicomplexa are haplonts as they are haploid with the exception of the zygote, which is diploid. Sex determination is being dependent on environmental factors and thus either female or male individuals may develop from one parasite clone.

The vermiform (with a worm-like shape) **sporozoite** is the first invasive stage of Apicomplexa. It penetrates the host cell and transforms into a growth stage, the **trophozoite**. The trophozoite undergoes asexual reproduction termed schizogony (or sometimes merogony). During this process, numerous offspring can be produced simultaneously, ranging between 2 ("Endodyogeny," e.g., in *T. gondii*) and >100 000 (e.g., in *Sarcocystis*). During the schizogony phase, the DNA and organelles are replicated between one and several times. The newly formed daughter nuclei migrate to the periphery of the organism. This stage of the parasite is

Table 2.4 Classification of the Apicomplexa dealt with in this book.

Subphylum Apicomplexa	
Class Coccidea	
Incertae sedis[a)]	
Fam. Cryptosporidiidae	*Cryptosporidium*
Order Eimeriida	
Fam. Eimeriidae	*Eimeria*
	Isospora
Fam. Sarcocystidae	*Sarcocystis Toxoplasma Neospora*
Class Haematozoa	
Order Haemosporida	
Fam. Plasmodiidae	*Plasmodium*
Order Piroplasmida	
Fam. Babesiidae	*Babesia*
Fam. Theileriidae	*Theileria*

a) The taxonomic position of Cryptosporidiidae is unclear; they resemble the members of the Coccidea, yet they evince features of the more basic Gregarinea.
Source: Tenter, A (2006) Evolution des Parasitismus. In: *Allgemeine Parasitologie*. Eds. T. Hiepe, R. Lucius, B. Gottstein. Parey Publishers, Stuttgart.

described as a **schizont**. Through the process of cytokinesis, the daughter nuclei are separated into identical **merozoites,** each containing a single mitochondrion and apicoplast organelle, where they have been evolutionarily retained. There are several variations of schizogony (see below). Often the merozoites released from a host cell set more schizogony cycles in motion and more merozoites emerge as a result, and hence this stage can cause multiplicative reproduction. Acute disease is caused by sucessive rounds of such lytic cycles in host cells, which consits of host cell invasion, intracelular replication from the dying host cell and re-invasion of

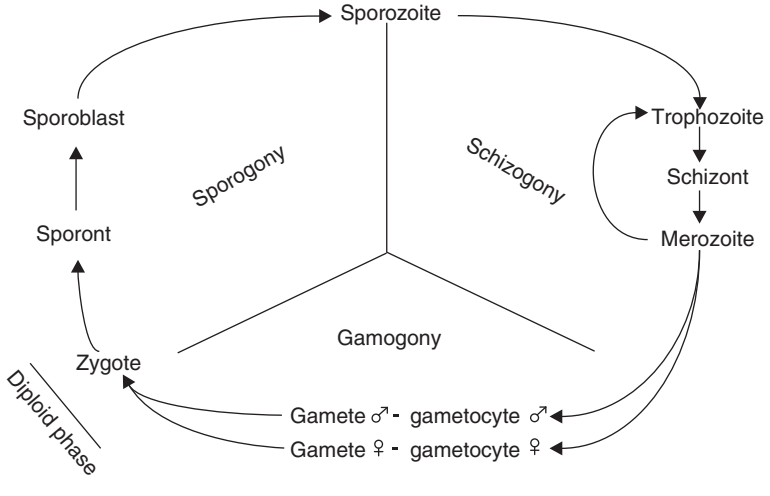


Figure 2.40 Schematic life cycle of Apicomplexa.

fresh neighboring cells. The merozoites can also continue to develop in a process known as **gamogony**, whereby they then become sexually determined sexual precursor, the **gametocytes**. The gametocytes differentiate into female macrogamete and male microgamete forms. In most other Apicomplexa, the female macrogametes are relatively large and the male counterparts are small and often flagellated. The fusion of a microgamete with a macrogamete produces the diploid **zygote**. A reduction division occurs here. Mitoses usually follow after a short time and this process of **sporogony** creates the **sporont** which often secretes cyst wall material. It differentiates further to **sporoblasts** which also secrete cyst wall material. The sporoblasts divide and form sporozoites. The variety of asexual division forms in the Apicomplexa can be traced back to a few basic processes (Figure 2.41).

In the **schizogony phase,** DNA is first replicated and then distributed to daughter organisms. The simplest case of schizogony is **endodyogeny**, in which two daughter cells are produced within the mother cell with complete consumption of the material. In typical **schizogony**, multiple replication of the DNA takes place, after which daughter nuclei organize themselves at the periphery of the cell. There they divide again, surround themselves with plasma, and differentiate into merozoites. In **endopolygeny,** a strong strand-like or lobulated polyploid nucleus is first produced by multiple replication of the genome. Inside the cell, daughter nuclei differentiate, migrate to the periphery, and merozoites organize around them. Instead of immediate merozoite formation, a multidivision of the marginal DNA can first take place, so that daughter regions (**cytomeres**) are formed, on the edge of which numerous merozoites ultimately organize themselves. This allows widely differing numbers of offspring to be created in one generation during the schizogony phase. In the case of asexually reproducing *Toxoplasm*a, stages growing in the intermediate host, only two daughter cells are formed by endodyogeny in one subdivision step. By contrast, Sarcocystis produces up to several tenthousand offspring in a synchronized manner. In the **sporogony phase**, diploid DNA is reduced and the haploid DNA is replicated and mitotically distributed to individual daughter cells. Here again, the formation of actively dividing daughter regions (cytomeres) within a cell can occur, resulting in the simultaneous creation of very large numbers of sporozoites.

2.6.1.2 Morphology

The sporozoites of the Apicomplexa are worm-shaped and measure 2–20 µm. The intracellular stages in which asexual reproduction occurs by schizogony vary from just a few microns to approximately 1 cm (e.g., *Sarcocystis gigantea*); they can produce individual merozoites that number between a few thousand and hundreds of thousands. These merozoites are usually oval or oblong and measure 2–7 µm. The size of the round or oblong gametocytes varies greatly. The male gametes can be aflagellated or flagellated. In the case of forms dealt with in this book, male gametes are significantly smaller ("microgametes") than the female counterparts ("macrogametes"). In the case of Apicomplexa transmitted by arthropods, the zygotes are formed as a worm-shaped stage that can pass tissue barriers; sporozoites without surrounding shell structures are then formed and transmitted with

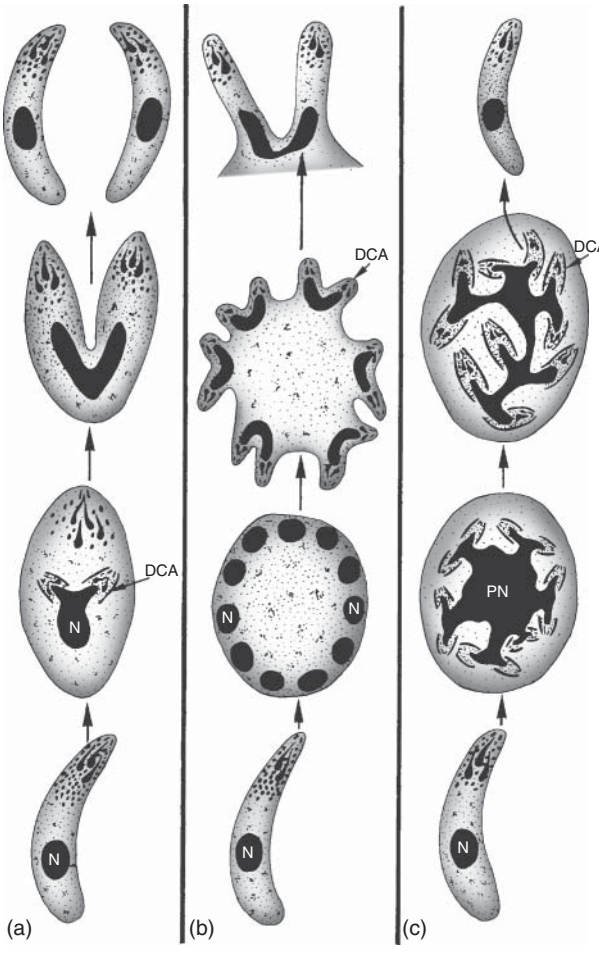


Figure 2.41 Schematic representation of the different forms of Apicomplexan daughter cell formation. (a) Endodyogeny. Two daughter cells are formed inside the mother cell. (b) Typical schizogony. Two daughter cells are formed during the last nuclear division of the multipart schizonts. (c) Endopolygeniy. Many daughter cells are created simultaneously at the periphery of a polyploid nucleus. DCA, daughter cell anlage (= primordium); N, nucleus; PN, polyploid giant nucleus. (From Mehlhorn and Piekarski: Grundriß der Parasitenkunde, (2002) with kind permission of Spektrum Akademischer Verlag, Heidelberg.)

the saliva. In the case of Apicomplexa, which disseminate their progeny in the environment, the zygote produces cyst wall material, which encloses the sporozoites in a resistant cyst, which is then released from the host.

The furnishing of the different stages of the Apicomplexa with organelles varies widely. Many of the typical organelles are found in the invasion stages (sporozoites and merozoites). Merozoites are very well suited as an example to illustrate the structures of the Apicomplexa (Figure 2.42). As is standard in eukaryotes,

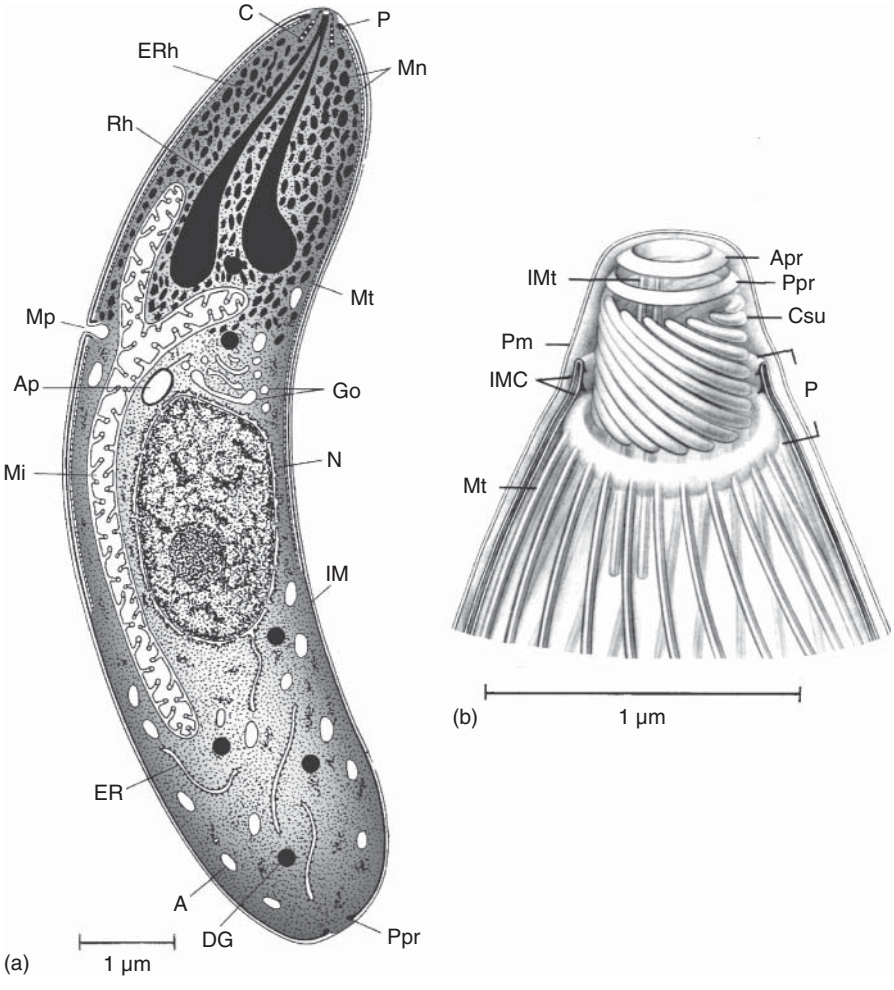


Figure 2.42 Ultrastructure of an Apicomplexa merozoite. (a) Longitudinal section. A, amylopectin; Ap, apicoplast; C, conoid; DG, dense granules; ER, endoplasmic reticulum; ERh, efferent duct of the rhoptries; Go, Golgi apparatus; IM, inner membrane complex (flat vesicles); Mi, mitochondrion; Mn, micronemes; Mp, micropore; Mt, pellicular microtubules; N, nucleus; P, pole ring; Ppr, posterior pole ring; Rh, rhoptries. (Adapted from Scholtyseck und Mehlhorn (1973), Naturwissenschaftl. Rundsch. 26, 421–427.) (b) Detailed representation of the microtubular apical structures of a tachyzoite of *Toxoplasma gondii*. Apr, anterior preconoidal ring; Csu, conoid subunit; IMC, inner membrane complex; IMt, internal microtubules; Mt, microtubules; P, pole ring complex; Pm, plasma membrane; Ppr, posterior preconoidal ring. Nichols, B.A., Chiappino, M.L. (1987) J. Protozool. 34, 217–226.

Apicomplexa contain a nucleus, Golgi apparatus, and rough endoplasmic reticulum. The cell boundary of the merozoite is formed from a multilayered **pellicle**.

The outer plasmalemma covers the entire cell and surrounds an inner membrane complex derived from the flattened vesicles, which are characteristic of the Alveolata. Microtubules running under the surface as part of the pellicle give the cell rigidity and are actively involved in its characteristic gliding motility. The microtubules originate from the apical polar ring and spiral down the cytosolic face of the pellicle, just past the nucleus. A **micropore** (previously named "ultracytostome") is laterally incorporated in the pellicle and enables the parasite to ingest host material into its food vacuole by a pinocytosis-like process. The eponymous feature of the Apicomplexa, the **apical complex** (Latin: *apex* = tip), is located in the front region of the cell and is composed of several organelles. In most (but not all) Apicomplexa, the **conoid**, a cone-shaped hollow structure, lies in the tip. The organelle is composed of spirally arranged microtubules and two rings; it can move forward (and backwards) during invasion of the host cell. The efferent ducts or necks of the club-shaped **rhoptries** (Greek: *rhopali-kós* = club-shaped) run through the tip of the conoids. The rhoptries contain electron-dense material, which includes lipids and a number of proteins that are secreted during cell invasion. Some of these exhibit immunomodulatory properties and others, located within the rhoptry necks, are incorporated into the membrane of the host cell. Numerous **micronemes** are also located in the apical end of the parasite. The contents of the micronemes are essential for host cell invasion and are released before penetration into host cells. Other organelles are **dense granules**, the products of which contribute to the modification of the membrane of the parasitophorous vacuole, where the parasite resides and multiplies. Dense granule proteins have also been reported to enter the host cell, where they can also affect key host processes. Certain life cycle stages, including merozoites and bradyzoites (in *T. gondii*), often contain nonorganellar bound amylopectin granules as a reserve energy source. Most Apicomplexa have a functional mitochondrion with a much reduced genome, even to the extreme of total loss in *Cryptosporidium.* Near the mitochondrion lies the **apicoplast,** a greatly reduced and no longer photosynthetically active plastid organelle with a genome of approximately 35 Kb. This organelle has been shown to perform essential biochemical pathways such as Type II fatty acid biosynthesis in many apicomplexans, but would appear to be absent in others including *Cryptosporidium.*

2.6.1.3 Cell Biology

The size of the haploid genome of Apicomplexa is between 8.4 Mb (in *Theileria*) and ~65 Mb in *Toxoplasma* (see Table 2.5). The genome is organized in 4–14 chromosomes, but these are not condensed during mitosis. The mitochondrion is generally active in Apicomplexa. However, the mitochondrion of *Cryptosporidium* has lost its genome and has a much reduced function. *Cryptosporidium* has also lost its apicoplast. The transcription of nuclear genes is gene-specifically regulated, and does not occur polycistronically. The expression of at least some proteins is posttranscriptionally regulated significantly, that is, it is determined through

Table 2.5 Genomic data of selected Apicomplexan parasites.

Organism	Genome size (Mb)	No. of chromosomes	No. of protein coding nuclear genes
Cryptosporidium parvum	9.1	8	3887
Plasmodium falciparum	22.9	14	5268 (including 561 apicoplast-derived genes)
Plasmodium yoelii	23.1	14	5878
Toxoplasma gondii	63.5	14	7793
Theileria annulata	8.4	4	3792
Theileria parva	8.3	4	4035
Babesia bovis	8.2	4	3671

control of the mRNA stability and/or translation. Many metabolic pathways of Apicomplexa are specifically adapted to their preferred host and even their respective host cells. Parasites such as *Plasmodium* or *Cryptosporidium* have reduced their metabolic pathways. The metabolism of other species such as *T. gondii* is relatively complex, to facilitate survival in a broad spectrum of hosts and host cells. The unprecedented metabolic plasticity in T. gondii at least in part explains the prmoiscuous survival in very different nutritional milieus, likely encountered in different host cells and species.

The apicoplast contains its own, significantly reduced, circular genome of approximately 35 kB of cyanobacterial origin and contains genes mainly associated with DNA replication, transcription, and translation. The organelle is derived from a red alga-like ancestor, which had previously endocytosed a cyanobacterium ("secondary endosymbiosis," Figure 2.43).

This secondary endosymbiotic origin of the apicoplast explains why it is surrounded by four membranes. The vast majority of the genes formerly encoded by the plastid genome have been lost or transferred to the nucleus of the parasites during the course of evolution. In the case of *Plasmodium*, about 10% of the nuclear-encoded genes appear to be derived from the apicoplast. A number of these proteins are synthesized in the cytoplasm and actively transported back into the apicoplast using a characteristic bipartite transit sequence consisting of a cleavable von Heijne secretory signal and a chloroplast-like transit sequence. In the apicoplast, the synthesis of isoprenoids, fatty acids (by the type II pathway), and some steps of heme occurs. The apicoplast is in fact necessary for most of the apicomplexans; in the case of *Plasmodium falciparum*, this essentiality is due to the need for isoprenoid biosynthesis within the organelle. Since the metabolic pathways of this organelle are of prokaryotic origin and are thus very different from the metabolism of the host, they represent attractive targets for the development of new drugs.

The development of facile techniques for transfection of some Apicomplexa has facilitated the production of gene-deficient parasites, transgenic parasites, and

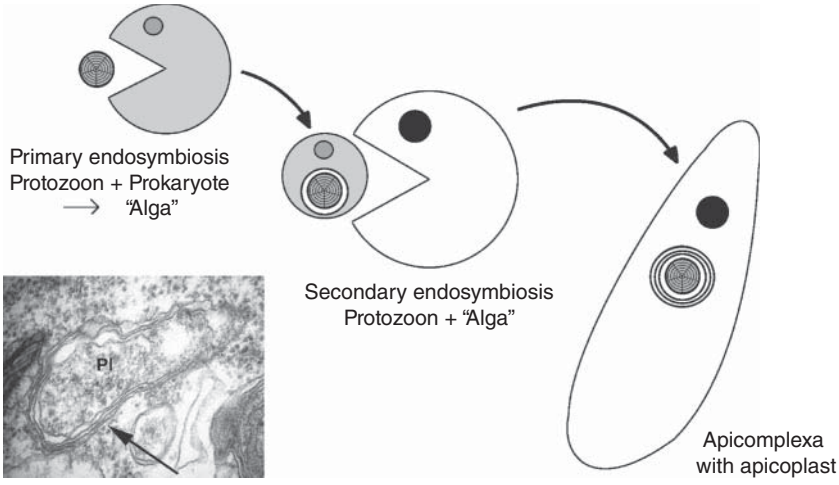


Figure 2.43 Probable development of the apicoplast by secondary endosymbiosis. In the first step, uptake of a cyanobacterium resulted in a red alga with a plastid organelle. This alga was taken up by a single-celled eukaryote and became an endosymbiont. The respective membranes were retained. The result is an apicoplast with a quadruple membrane. Detail: TEM image of the apicoplast of *Plasmodium falciparum*. Pl, Plastid organelle; Arrow, quadruple membrane. (Photo from Ralph, S.A. *et al.* (2004) Nat. Rev. Microbiol. 2, 203–216, with kind permission of Macmillan Publishers Ltd.)

conditional mutants. This progress has greatly aided understanding of protein functions and the cell biology of many apicomplexans.

Several apicomplexan parasites, like Toxoplasma, Plasmodium and Cryptosporidium, are now genetically tractable and the CRISPR/cas technology is expected to ease the experimental access to even unconventional parasites. The production of deletion mutants has greatly eased the study of protein functions. The expression of fluorescent hybrid proteins allows the localization of proteins in the living cell, so that the processes of cell invasion and communication between organelles and cell division can be observed. Fluorescent-labeled parasites and parasite expressing firefly luciferase can now be used to observe the parasite in the host cell and even in animals by intravital microscopy or bioluminescence imaging using an *in vivo* imaging system.

Apicomplexa have developed a unique form of gliding locomotion, known as "**gliding motility**." Much of what is known about Apicomplexan movement and cell invasion is known through studies of *T. gondii.* Gliding motility is dependent on proteins secreted from the micronemes that insert themselves into the parasite's surface as a transmembrane protein and get linked through a hinge protein to a subsurface actin–myosin motor (Figure 2.44). The extracellular domain of the micronemal protein protruding from the surface binds to ligands of the host cell. When the head domains of the myosin proteins move along the subsurface actin fibers, they set the micronemal protein into motion. Detachment from the substrate is mediated by intramembrane proteases of the parasite that

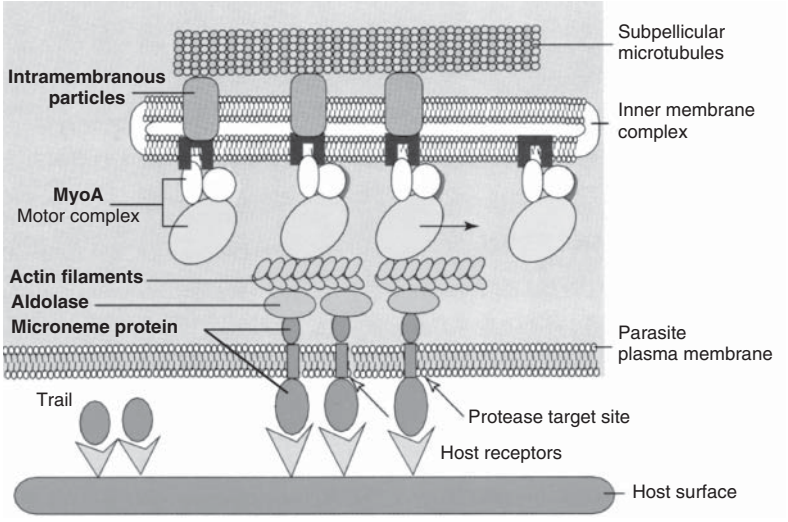


Figure 2.44 Schematic representation of the "gliding motility" of Apicomplexa. Details are in the text. (From: Soldati, D., and Meissner, M. (2004). *Curr. Opin. Cell Biol*., **16**, 32–40, by kind permission of Elsevier.)

cut the micronemal protein, leaving a trace on the substrate that can be detected with antibodies. Due to the slightly twisted shape of *T. gondii* motile stages, the parasite rotates around their longitudinal axis and therefore leaves circular traces. This movement advances the cell with a speed of up to 20 µm/s.

The **invasion of the host cell** as well as the egress from it is require calcium-dependent gliding motility. Perturbation of ion homoeostasis in the dying host cell and signaling pathways in the parasite are among other factors governing the gliding motility and subsequent egress and invasion events. Before invasion, parasites attach to the host cell and reorient, so that their apical ends are in contact with the host cell (Figure 2.45). The conoid extends and rhoptries introduce their contents into the host cells by an unknown mechanism. A ring-shaped region of contact forms between the parasite and host cell, known as the moving junction. The parasite moves this junction to its posterior pole, using its actin/myosin motors, thereby pushing itself into the cell until it is entirely internalized. At the same time, material continues to be secreted by the rhoptries and dense granules, and incorporated into the growing invagination. The parasite is thus surrounded by a membrane of host origin, but containing a large proportion of material of parasite origin. This compartment is termed the parasitophorous vacuole and is known to allow molecules of less than 1300 Da to pass freely. The parasitophorous vacuole does not fuse with lysosomes, so the parasite is protected from the digestive enzymes and acidification of the vacuole.

Glucose and glutamine are two major host-derived nutrients that provide carbon sources for the biosynthetic activities of proliferating parasites. Apicomplexan parasites are auxotrophic for host-derived purines. By contrast, most parasites

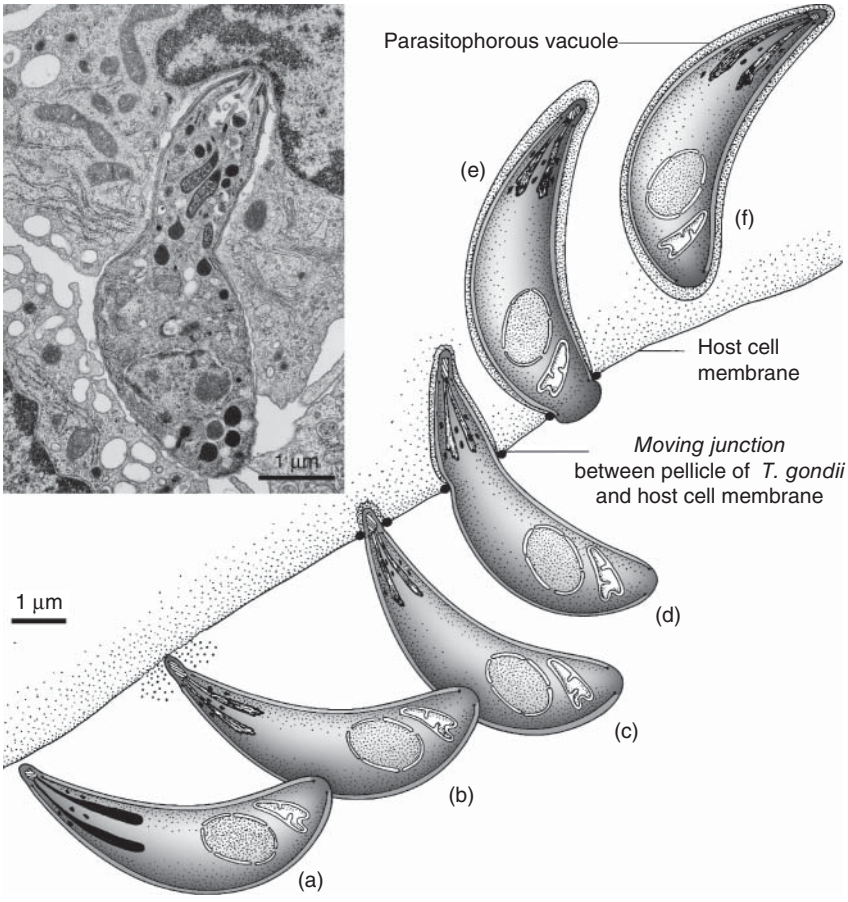


Figure 2.45 Model of the host cell invasion of a *Toxoplasma gondii* tachyzoite. See text for details. (a) Binding. (b) Protrusion of the conoid and secretion of components of the micronemes and rhoptries. (c–e) Formation of a *moving junction* between parasite pellicula and host cell surface, plus formation of the parasitophorous vacuole. (f) The membrane closes and the formation of the parasitophorous vacuole is completed. (From Wyler, D.J. (1990) *Modern Parasite Biology*, W. H. Freeman & Company, New York. By kind permission of W.H. Freeman and Company. Top left: Penetration of a host cell by a Toxoplasma tachyzoite. EM image: Courtesy of B. A. Nichols.)

can synthesize pyrimidines except for *Cryptosporidium*, which depends entirely on the salvage pathway. Their metabolic potential to make various fatty acids also differs substantially. For instance, *T. gondii* harbors the mammalian-type FAS I and bacterial-type FAS II pathways in the ER and apicoplast, respectively, whereas *Plasmodium* lacks FAS I, and *Cryptosporidium* is devoid of FAS II. These parasites also appear to salvage fatty acids from host cells to varying degrees. Phospolipid synthesis in apicomplexan parasites is an evolutionary patchwork of eukaryotic and procaryotic-type pathways. Selected enzymes and transport routes for such

essential molecules show a considerable divergence from the mammalian hosts, and thus offer attractive chemotherapeutic targets.

2.6.2 Coccidea

The class Coccidea (Greek: *kókkos* = kernel) consists of intracellular parasites of invertebrates and vertebrates. The development typically follows the pattern of the Apicomplexa (schizogony, gamogony, sporogony). The zygotes of the Coccidea arise from fusion of a macrogamete and a triflagellar microgamete. This zygote differentiates into a sporont that builds a cyst wall, forming an **oocyst**. Within the oocyst, the sporont divides and gives rise to sporoblasts, which in turn form cyst walls, such that **sporocysts** arise within the oocyst. The sporoblasts then divide into **sporozoites**. Thus, the typical resistant transmission stage of the Coccidea resembles a Russian doll, with an outer oocyst that contains one or several sporocysts, each containing several sporozoites (Figure 2.46).

The basic type of life cycle of Coccidea proceeds in one host (e.g., *Cryptosporidium* and *Eimeria*) and includes cells of the intestine or intestine-associated organs. Host specificity of these intestinal parasites is usually high. The "**cyst-forming coccidia**" of the Sarcocystidae family, however, use intermediate hosts, in which the schizogony phase occurs in tissue cysts. These cysts consist of a modified host cell, in which several hundred or even thousands of long-lived waiting stages (cystozoites) are present. Here the intermediate host is typically the prey of a predator, which acts as the final host, after having infected itself with tissue cysts. While *Sarcocystis* as a specialist exploits a very narrow spectrum of intermediate host species and develops only in certain cell types, the asexually replicating stage of

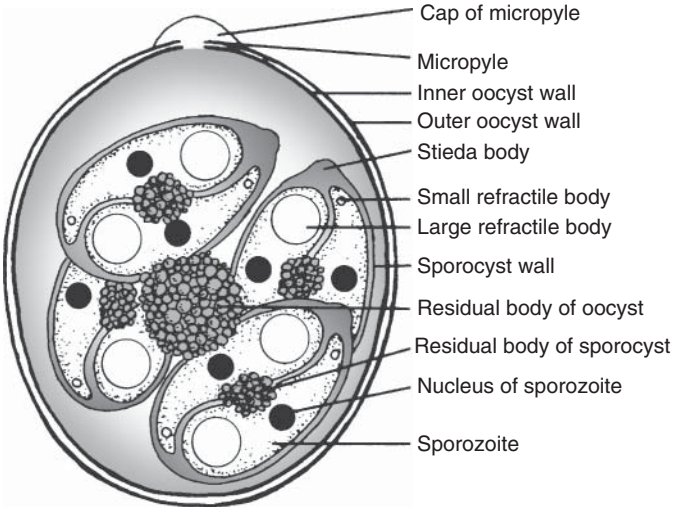


Figure 2.46 Sporulated oocyst of *Eimeria*. (Image: R. Lucius/J. Gelnar.)

Toxoplasma is a generalist and infects not only a broad spectrum of vertebrates, but also invades many different types of nucleated cells.

2.6.2.1 *Cryptosporidium parvum*

Cryptosporidium parvum belongs to the Cryptosporidiidae family. The classification of this family is unclear, because the morphological criteria have so far shown that this pathogen is a member of the Coccidea, but DNA sequences also indicate a relationship with the more basal Gregarinea, most of which are parasites of vertebrates. Species circumscription is not clearly defined within the *Cryptosporidium* genus. *C. parvum* is distributed worldwide and is known as a major cause of severe diarrhea in calves, children, and immunocompromised adults. Host specificity is low, therefore *C. parvum* is found in more than 40 species of vertebrates. There are several genotypes that differ with respect to the harmful effects, and the host range.

Development The infection is caused by sporozoites that are released from oocysts in the small intestine – they then adhere to the surface of the microvilli of intestinal epithelial cells (Figure 2.47). They push the villi apart and are enveloped by them in a bell-shaped manner. Basal forms an adhesion zone, in which the pellicle and the host cell membrane fuse. This situation is referred to as "intracellular but extra-cytoplasmic." Two generations of schizonts develop – in an electron-microscope image, these appear like balloons lying on the surface of the host cells (Figure 2.48).

While the first schizogony produces merozoites, the second-generation merozoites differentiate first into gametocytes and then into macrogametes or aflagellate microgametes, which fuse to form a zygote. The sporogony leads to the formation of oocysts, which are atypical for Coccidea in that the oocyst wall merges with that of the sporocyst. Sporulation occurs while still within the host and leads to the formation of four sporozoites/oocysts. About 80% thick-walled and 20% thin-walled oocysts are produced. Thick-walled oocysts are excreted via the feces and these are extremely resistant to environmental influences and chemicals, remaining infectious for up to 2 years. Thin-walled oocysts release the sporozoites to the intestine, and this can result in massive autoinfections if the immune system does not exert any limiting effect.

Morphology The spherical oocysts measure about $5 \times 4.5\,\mu m$, while the sporozoites lying in them are very small, measuring only about $5 \times 1\,\mu m$. In the case of thick-walled oocysts, the wall consists of three layers of membrane and two of chitin. They have a "seam", through which the sporozoites are released. Thin-walled oocysts are surrounded by only one single membrane.

Cell Biology The genome of the "Iowa Type II" strain of *C. parvum* (published 2004) is organized into eight chromosomes, with a size of 9.1 Mb (haploid) and 3807 protein coding genes. Only 5% of the genes have introns. *C. parvum* has no apicoplast; it has only a rudimentary mitochondrion, the genome of which has been lost. *C. parvum* also evinces no variable antigen such as those found in

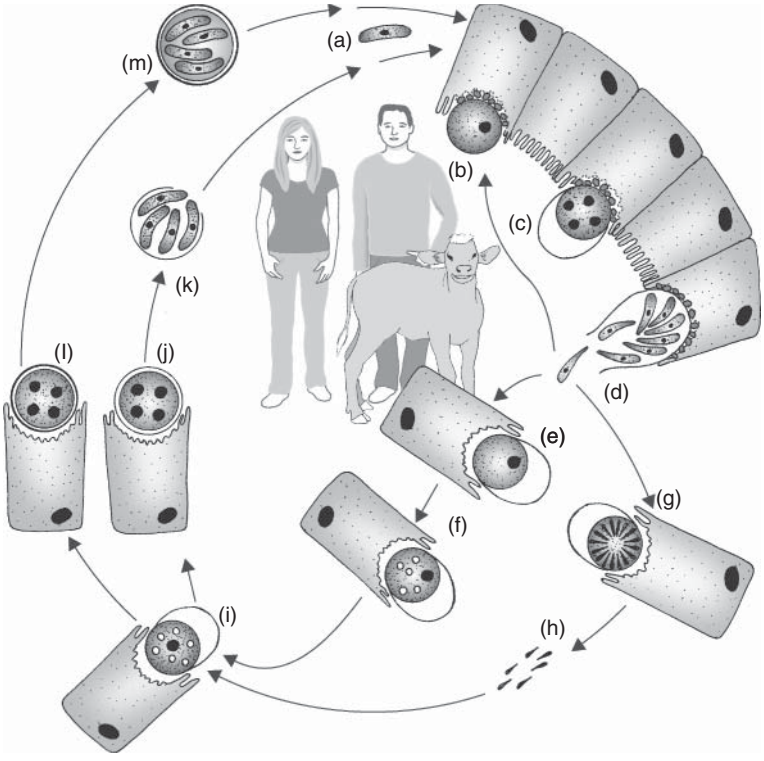


Figure 2.47 Lifecycle of *Cryptosporidium parvum*. (a) Sporozoite. (b) Trophozoite at the surface of an intestinal epithelial cell. (c) Schizont. (d) Merozoites. (e) Macrogametocyte. (f) Macrogamete. (g) Microgametocyte. (h) Microgametes. (i) Zygote. (j) Sporont with thin cyst wall. (k) Thin-walled oocyst. (l) Sporont with thick cyst wall. (m) Thick-walled oocyst. (Adapted from Mehlhorn H. (1998) Parasitology in Focus, with permission by Springer-Verlag, Heidelberg.)

Plasmodium for example – and this species has much fewer of its own metabolic pathways than the malaria parasite. The metabolic pathway for oxidative phosphorylation is missing, for example, so *C. parvum* probably obtains its energy through glycolysis. Amino acids and nucleotide precursors are largely taken from the host via specific transport molecules, the number of which is greatly increased in comparison to other Apicomplexa. *Cryptosporidium* are eliminated by T cell responses of the Th1 type; here the production of IFN-γ plays an essential role. Recent work has succeeded in genetic transformation of the parasite, which will ease studies on the function of proteins, and the development of vaccines and drugs.

Cryptosporidiosis in Calves In cattle-breeding units (infestation in Germany: 14–100%), neonatal calf diarrhea can lead to weight loss, which often takes on important economic dimensions. Infected when just a few days old, calves fall sick for 2–14 days after an incubation period of approximately 4 days. The infection causes severe yellowish and watery diarrhea, the origin of which is unclear and

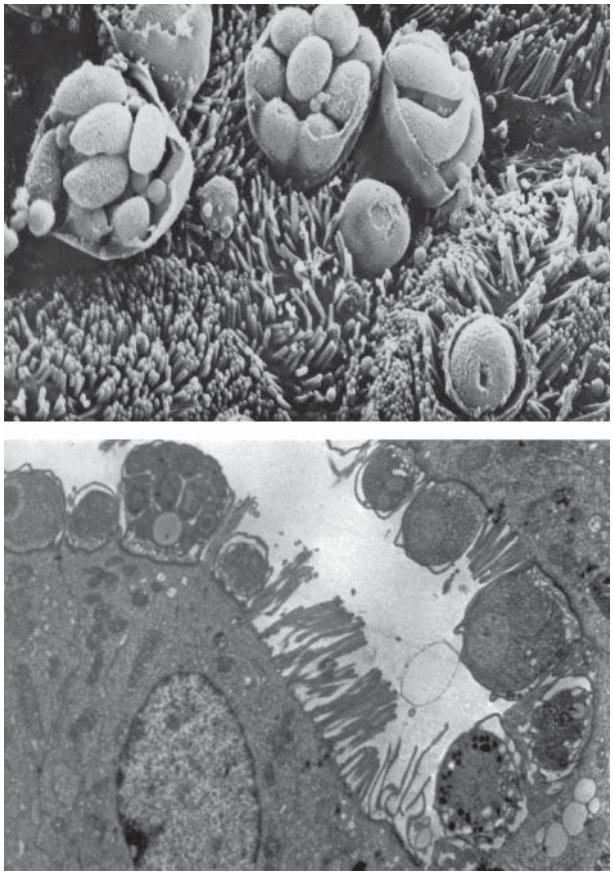


Figure 2.48 *Cryptosporidium parvum*. (a) Stages on the surface of a villus, 6 days after experimental infection of a pig (SEM). Note that the parasitophorous vacuoles of the major stages are broken open and the individual pathogens are lying free. (Image: Courtesy of Pohlenz.) (b) TEM image with different stages embedded by the intestinal microvilli of a calf. (Image: Courtesy of E. Goebel.)

leads to atrophy of the microvilli, to fusions of the villi and to inflammatory symptoms in the submucosa. Several million oocysts may be present in 1 g of feces of infected calves, and hence these animals can excrete billions of oocysts. The oocysts are highly resistant to different environmental conditions. They are often present in large numbers in surface water – and their small size even enables them to get into the groundwater, where they can travel long distances. They can also survive the treatment (filtration, chlorination) during the processing of drinking water. Since standard drinking water hygiene criteria (e.g., number of coliform bacteria and nitrates) provide no information on the content of infective *Cryptosporidium* oocysts, contamination is difficult to detect. The detection of oocysts often only succeeds through the examination of larger water samples using filtration or sedimentation procedures.

Cryptosporidiosis in Humans In Europe, an average of 1.5% of the population excretes Cryptosporidium oocysts, the largest proportion of these being children below 6 years of age. In a study in Ghana, an average of 12.9% of the healthy population was infected. In immunocompetent individuals, the result is serious diarrhea, which, however, usually heals spontaneously. Contamination of drinking water, for example, surface water contamination by bovine manure, or failure of filtering systems, can result in diarrheal epidemics of the first magnitude. More than 400 000 diarrheal patients and 104 deaths were documented during a 1993 drinking water-related epidemic in Milwaukee, USA. In addition to diarrhea, symptoms such as dizziness, slight fever, abdominal cramps, and weight loss occur. In contrast to opinions that have been expressed in the past, these data show that *C. parvum* is a significant diarrheal pathogen – and one that is very difficult to control.

Cryptosporidiosis is especially significant as an opportunistic pathogen in connection with the AIDS pandemic. In the United States, two studies showed that 16% of AIDS patients with diarrhea were infected, while in Haiti, the rate of *Cryptosporidium*-infected AIDS patients is 41%. In persons with defective immune system, *C. parvum*, as opportunistic pathogen, can multiply massively through autoinfections in the intestinal tract and in the epithelia of the respiratory tract. The infection leads to severe, watery diarrhea, which is difficult to cure. Some statistics state that *C. parvum* is the direct cause of death in approximately 3% of all AIDS-related deaths.

Besides *C. parvum*, many other *Cryptosporidium* species, currently up to 24, have been recorded. *Cryptosporidium muris* is found in the stomach of rodents and also in other mammals. This species is only slightly pathogenic. *Cryptosporidium baileyi* occurs in the microvilli seam of the respiratory and gastro-intestinal tract of chickens. Turkeys host the *C. meleagridis* species; snakes harbor *Cryptosporidium serpentis* and *Cryptosporidium nasorum* inhabits fish. The biology of these species essentially corresponds to that of *C. parvum*.

2.6.2.2 *Eimeria*

Parasites of the genus *Eimeria* have monoxenous life cycles almost exclusively in epithelial cells of the intestinal tract of vertebrates and some invertebrates. The genus contains more than 1200 species, including most important parasites of poultry and other farm animals, but never of humans. The genus has been described as a melting pot of biologically diverse coccidians, suggesting that future molecular analyzes will change the taxonomy. Under natural conditions, young animals tend to be infected with small numbers of oocysts, and after a self-limiting illness develop immunity. However, young animals reared in farms or even factory farming can be infected with large doses of oocysts before developing immunity, which may cause severe diarrhea and bleeding. Under conditions like these, significant losses can occur. The parasites are typically strictly host-specific, and many are in addition adapted to a very specific habitat within their hosts.

Development Infection occurs following oral ingestion of oocysts, which release sporozoites in response to pepsin and other stimuli in the gut (Figure 2.49).

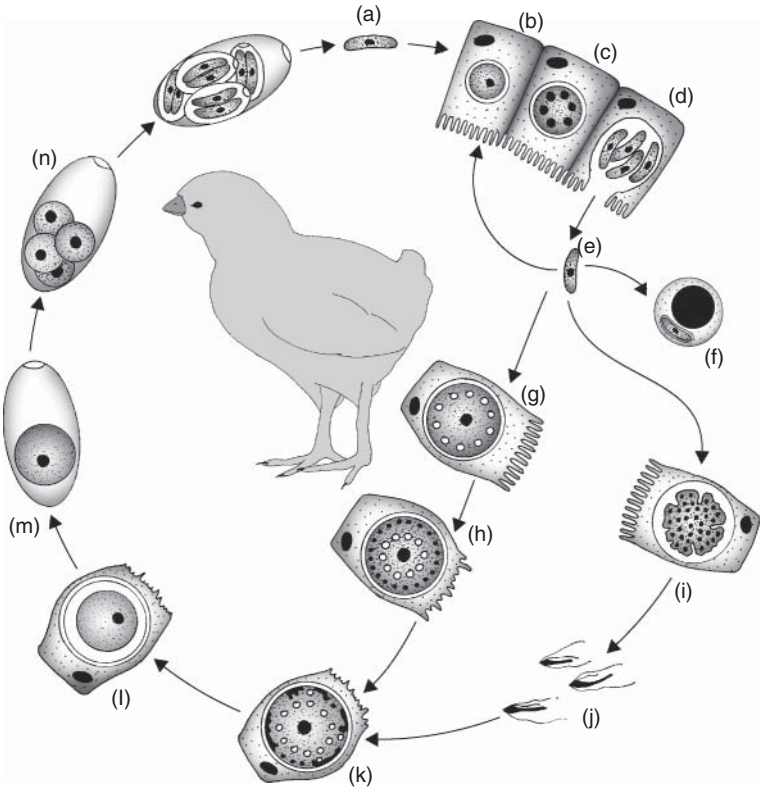


Figure 2.49 Life cycle of *Eimeria tenella*. (a) Sporozoite. (b) Trophozoite in an intestinal epithelial cell. (c) Schizont. (d) Merozoites. (e) Free merozoite. (f) Dormant stage in intraepithelial lymphocytes. (g) Macrogametocyte. (h) Macrogamete. (i) Microgametocyte. (j) Microgametes. (k) Zygote. (l) Intracellular sporont. (m) Excreted sporont within the oocyst. (n) Excreted sporoblasts within the oocyst. (o) Oocyst with four sporocysts, each containing two sporozoites. (Adapted from Mehlhorn, H. (ed.) (1988) Parasitology in Focus, Springer-Verlag, Heidelberg.)

These sporozoites invade epithelial cells, where they form a parasitophorous vacuole and divide by schizogony. The number of merozoites formed is species-dependent. After a number of schizont generations, characteristic of each species, gametocytes develop and ultimately give rise to macro- and micro-gametes. Microgametocytes of the Eimeriida usually produce large numbers of flagellated microgametes. Fertilization produces a zygote, which develops into a sporont, which is in turn surrounded by a cyst sheath. Sporulation occurs after release into an environment with an appropriate temperature, humidity and oxygen levels. Sporulated oocysts are extremely resistant and can survive for several months.

Morphology The sporozoites of *Eimeria* are worm-shaped and measure approximately 15 µm. The size of the schizonts varies in most species between 10 and 50 µm, but they can also reach 240 µm. Merozoites have the typical Apicomplexa

structure, but they exhibit very large refractile bodies, which probably contain reserve material (Figures 2.50 and 2.51). The oocysts have a two-layer wall. In the case of *Eimeria tenella*, the outer layer consists mainly of long-chain alcohols, while the inner layer is composed of a highly networked glycoprotein. A preformed orifice of the cyst wall, the micropyle, is closed by a micropyle cap.

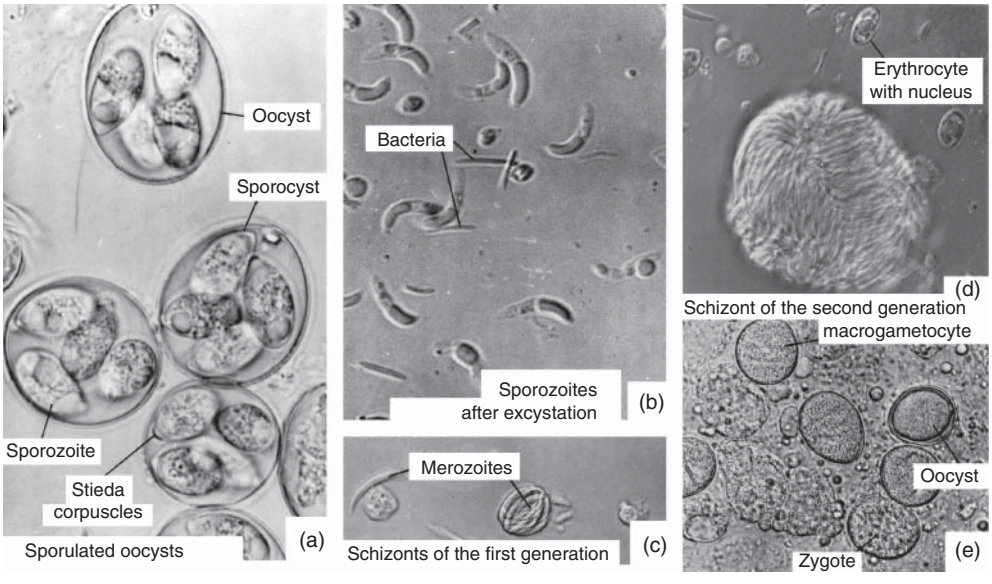


Figure 2.50 Stages of *Eimeria*. (a–e) Light microscope images of *E. tenella* stages. (Images: Courtesy of R. Entzeroth.)

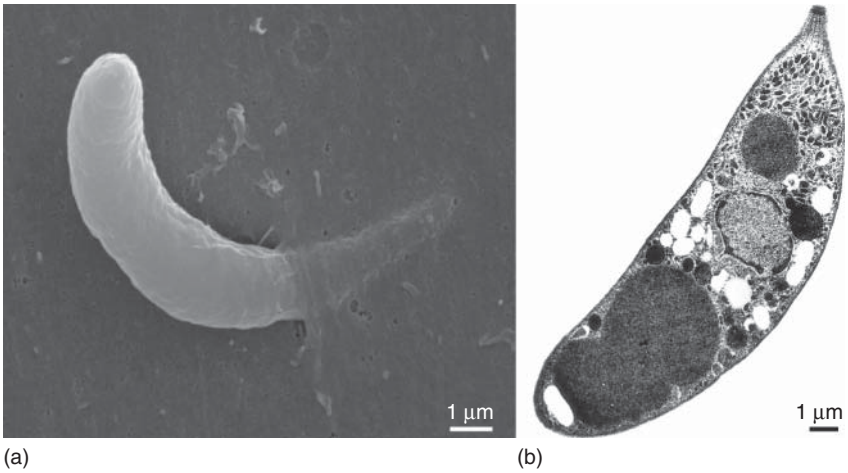


Figure 2.51 Sporozoites of *Eimeria*. (a) A sporozoite of *E. falciformis* invades a host cell. (EM image: Department of Molecular Parasitology, Humboldt Universität.) (b) Sporozoite of *E. tenella*. (EM image by Institute for Animal Health, Compton, UK, with kind permission.)

Inside there are four thin-walled sporocysts, each containing two sporozoites. The sporocysts each contain one preformed opening, which is initially sealed with a plug, the Stieda corpuscle. The sporozoites are characterized by refractile bodies that probably contain storage substances. Residual bodies lie in the oocyst and sporocyst. The morphology of the oocysts is an important determining feature, where shape, size, color, and texture are used to differentiate between species.

Genome The genomes of the seven Eimeria species of the chicken have been sequenced. The nuclear genome of the economically most important species, *E. tenella*, has a size of 51.8 Mb, with 8603 protein encoding genes organized on $2n = 14$ chromosomes. The mitochondrial genome comprises about 6200 bp and contains three protein-encoding genes, while the apicoplast genome has a size of approximately 35 kb. Only very limited synteny has been reported between genomes of the *Eimeria* species infecting chicken and the genome of *Eimeria falciformis*, a model parasite of the mouse. A conserved feature of all *Eimeria* genomes, however, is their richness in repeat sequences, dispersed in low complexity in bands over all chromosomes. This includes repeats in protein-coding exons conserved at the nucleotide level, which translate into homolpolymeric amino acid repeats, mainly stretches of alanine, glutamine, or serine. Other analyzes revealed a broad repertoire of probably GPI-anchored surface proteins, a part of which is expressed in a stage-specific fashion. The parasites stages seem to coexpress several of these proteins, thus presenting a complex set of surface antigens at a given time point to the host.

Coccidiosis Infection with *Eimeria* species can cause serious illnesses in young animals. The degree of pathogenicity of a "coccidiosis" is species-specific, but depends largely on the infectious dose, and hence the parasitic disease may present a severe problem under conditions of factory farming. Several species of *Eimeria* occur in most animal species (see Table 2.6). For example, seven species of Eimeria infect the chicken. The development of *Eimeria* is not only bound to one specific host species, but often to a particular segment of the intestine. This is particularly evident in the *Eimeria* species, which infect poultry (Figure 2.52).

Massive infections with pathogenic species cause severe diarrhea, and extensive damage to the epithelial cells. Lesions in deeper tissue layers may occur depending on the location of the parasite. Infection may result in severe bleeding ("Red Ruhr chicks," Red Ruhr = red dysentery/diarrhea), loss of appetite, malabsorption, weight loss, and possibly death. Entire poultry industry stocks can perish in a short time. Stress, change in diet, rehousing, and similar factors enhance the pathogenicity of the infection. Low infectious doses often trigger only subclinical infection, but leave behind a substantial, species-specific immunity. In this way, epidemiological stability is often achieved in wild animal populations.

Table 2.6 Overview of important Eimeriidae.

Type	Host	Organ	No. of schizont generations	Pathogenicity	Average oocyst size in microns
Eimeria bovis	Cattle	Hindgut, cecum, colon	2	++	23 × 20
Eimeria zuernii	Cattle	Hindgut, cecum, large intestine	2	++	20 × 15
Eimeria bakuensis	Sheep	Small intestine	2	++	29 × 20
Eimeria arloingi	Goat	Small intestine	2	+	29 × 21
Eimeria debliecki	Pig	Small intestine	2	+	25 × 17
Eimeria stiedai	Rabbit	Bile ducts	6	+++	37 × 20
Eimeria tenella	Chicken	Ceca	3(2)	+++	25 × 19
Eimeria necatrix	Chicken	Midgut	3(4)	+++	20 × 17
Eimeria acervulina	Chicken	Midgut	4	++	17 × 14
Eimeria mivati	Chicken	Small intestine, large intestine	4	+	16 × 13
Eimeria truncata	Goose	Kidneys	?	+++	22 × 17
Isospora suis	Pig	Small intestine	3	+	21 × 19
Isospora belli	Humans	Small intestine	2	+	30 × 20

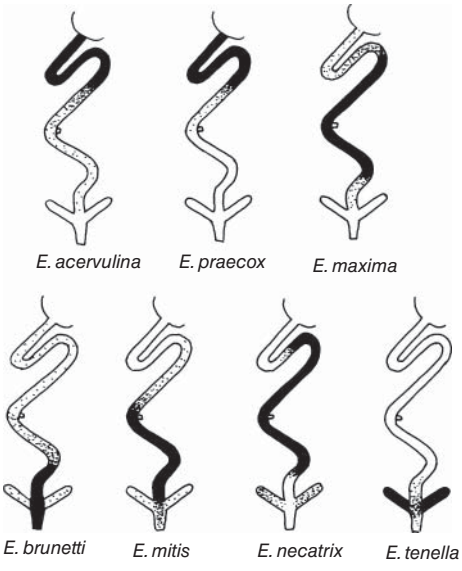


Figure 2.52 Preferred colonization locations of *Eimeria* species in the intestine of the chicken. (Adapted from Ball, Pitilo and Long (1989) Adv. Parasitol. 28, 1–54.)

Cell and Immunobiology So far, attempts to grow *Eimeria* in cell cultures have only been partially successful with 1 million sporozoites used for an initial infection of cell cultures only yielding one oocyst, while about 400 000 oocysts develop from one sporozoite in natural infections. This deficiency is a major impediment to studying this economically important infection. Although it has been possible to transfect *Eimeria* with reporter genes, production of gene-deficient mutants has not been successful so far.

$CD4^+$ T cells play an important role in limiting the parasites in primary infections of rodents and poultry. Cytokines produced by T cells, especially IFN-γ, contribute significantly to the killing of parasites. $CD8^+$ T cells are of particular importance regarding immunity against subsequent infection. Antibodies have no protective effect.

Besides their function as effector cells, T cells possibly play another important role in *Eimeria*, because according to some studies, they serve as transport vehicles for the sporozoites. Experiments have shown that in infections caused by *E. tenella* and other Eimerian species, sporozoites attack intraepithelial lymphocytes at the site of invasion. These lymphocytes then migrate, spreading the parasite. Vaccines based on attenuated parasites are commercially available for the control of *Eimeria.* The basic idea here is to cause weak infections that do not cause real illness, but which induce protective immunity. This is why selected "precocious" strains are used as a life vaccine, in which a (genetically determined) generation of schizonts is missing. These strains are therefore less pathogenic, but induce protection. Such treatment is only viable for breeding animals or laying hens, and are unprofitable for fattening animals. Vaccines based on recombinant antigens are still at the experimental stage.

2.6.2.3 *Eimeria tenella*

E. tenella is a pathogenic Eimerian species, which affects chickens. It can lead to large losses in factory farming ("Red Ruhr chicks") and reduced growth performance (Figure 2.49). The first and second schizont generation infects epithelial cells of the central part of the ceca. Up to several hundred merozoites are formed in parasitized cells. The merozoites resulting from the second generation migrate to subepithelial tissue of the villi and form a third schizont generation, each of which has 4–32 merozoites. These differentiate into gametocytes in the next host cell. However, there are also *E. tenella* strains with a different number of schizont generations. The maximum oocyst excretion level is reached approximately 7 days after infection. In warmer temperatures, the excreted cysts sporulate within 2 days. Under experimental conditions, a maximum of 400 000 oocysts can develop from one single introduced oocyst.

The damage to the epithelium of the cecum caused by the first schizont generation can usually be compensated for by the rapid replacement of the epithelial cells, which occurs under natural conditions every 2–5 days. The second and third schizogonies, which take place in lower intestinal tissues, result in the destruction of capillaries, which leads to excessive bleeding and malabsorption. Severe

diarrhea, blood loss, and secondary bacterial infections lead to the weakening of animals ("mourning attitude") and high losses are the result.

A total of seven Eimerian species that cause diarrhea of varying degrees of severity have been found to infect chickens. Specific localization in the intestine and pathogenicity are species-specific (Figure 2.52, Table 2.6). *Eimeria* are present in almost every chicken flock, but it is important not to expose young animals to a major infectious dose. In order to prevent this, chicks and young hens are treated with anticoccidials that are routinely added to the feed. In addition to the costs incurred for the treatment of the world's annually produced 39 billion broilers and the loss of production (~3 billion Euros), the residual exposure to drugs must also be considered. The ongoing development of resistance to many drugs makes continuous further drug development necessary.

2.6.2.4 *Eimeria bovis*

Eimeria bovis is one of the 21 different *Eimeria* species that infect cattle. This parasite has a global distribution and causes the "Red Ruhr" (red diarrhea), particularly in calves. The sporozoites penetrate the lymphatic endothelial cells in a section of the hindgut. Within 12–14 days, they develop into macroschizonts measuring up to 300 µm each producing up to 120 000 merozoites. The merozoites develop into a second schizont generation in the epithelial cells of the cecum and large intestine. Each schizont generation has 30–36 merozoites. The merozoites of this generation become gametocytes. It is the second schizont generation and the gametocytes that cause most of the damage, with symptoms ranging from severe diarrhea and large-scale loss of epithelial cells to blood loss and malabsorption. Anemia and exsiccosis (dehydration) resulting from the diarrhea can cause death.

2.6.2.5 *Isospora* and *Cyclospora*

The biology of the genus *Isospora* is broadly similar to that of the *Eimeria*. Each oocyst of this genus, however, contains two sporocysts, each of which in turn has four sporozoites. ***Isospora belli***, the pathogen of coccidiosis in humans is especially prevalent in warmer areas and can cause diarrhea. A prevalence of 3–4% has been observed in stool samples. While most cases are subclinical, severe illnesses with accompanying fever, persistent diarrhea, and fatigue, particularly in immunosuppressed patients, may eventually lead to death. ***Isospora suis*** in piglets can cause severe diarrhea. Here the schizogony phase occurs in epithelial cells of the small intestine. After three generations of schizonts, the formation of gametocytes takes place. Oocysts are excreted in unsporulated form, but these sporulate within several days. Piglets that have undergone an infection are protected against subsequent *I. suis* infection.

Cyclospora cayetanensis is a human-specific diarrheal pathogen that is widespread in tropical and subtropical countries and is especially infamous in South America. The life cycle of *C. cayetanensis* is monoxenous. While the infection is self-limiting in healthy individuals, it can last a long time in immunocompromised individuals. Agricultural products contaminated with oocysts can also cause occasional infections in industrialized countries.

2.6.2.6 *Toxoplasma gondii*

T. gondii (Greek: *toxon* = arc, bow, *plasma* = figure, body: owing to the arcuate shape; *gondii*: from *Ctenodactylus gundi*, a North African rodent, from which the protozoan was described) belongs to the "cyst-forming coccidia" of the family Sarcocystidae. *T. gondii* is of importance because toxoplasmosis of humans and domestic animals (especially sheep) is still, contrary to many other parasites, prevalent in industrialized countries. Definitive hosts are felines, for example domestic cat, wild cat or lynx. Almost all mammals can serve as intermediate hosts, even the cat itself, with the house mouse playing an important role. Many birds can be infected, including chickens. The sequenced genome of the strain ME49B7 has a size of approximately 65 Mb, is organized in 14 chromosomes, and encodes for about 9000 genes. In Europe and North America, there are three major and various minor *Toxoplasma* strains, which may only rarely recombine. In South America and elsewhere, the parasite population appears much more diverse. In Europe and North America, the majority of human infections are caused by Type II strains. The disease caused by *T. gondii* in the mouse model is clearly dependent on the strain; evidence strongly supporting a crucial effect of strain on disease outcome also now exists for human toxoplasmosis. As asexual multiplication inside cells of intermediate hosts is easily achieved in cell culture, *T. gondii* has become an important model organism for studies on the cell and molecular biology of Apicomplexa.

Development The development in the definitive (feline) host takes place after ingestion of either sporozoites (hatching from sporulated oocysts) or bradyzoites from tissues of infected intermediate hosts (Figure 2.53). These stages invade intestinal epithelial cells and multiply by schizogony with four generations of schizonts. The merozoites of the last generation develop to macrogametocytes or microgametocytes, from which 24–32 microgametes with two flagella each arise. About 3–9 days after infection, the first unsporulated oocysts are shed with the feces. This excretion lasts 3 weeks at most and is very productive. In infection experiments, cats discharged up to 100 million oocysts per day. Subsequent infections, though, are limited by immune responses and are much less productive. The sporulation process needs free access to oxygen and is completed in 2–4 days. Sporulated oocysts are highly resistant to environmental and other influences, including bleach, acid, and ultraviolet radiation. Under suitable conditions, they can retain infectivity up to 5 years.

Intermediate hosts can be infected by either sporozoites or bradyzoites. The parasites invade subepithelial cells, where an asexual multiplication by endodyogeny follows. This is a special type of schizogony (see Figure 2.41), which proceeds rapidly in diverse types of cells. As the divisions during endodyogeny can occur as rapidly as every 5–9 h, the stages are known as "tachyzoites" (Greek: *tachys* = quick). The number of tachyzoite generations is not genetically fixed. In this phase of rapid multiplication, the parasites are disseminated via the blood and are able to actively penetrate the barriers of tissues and permeate, among others, the placenta, pass into the offspring and multiply in them. This allows vertical

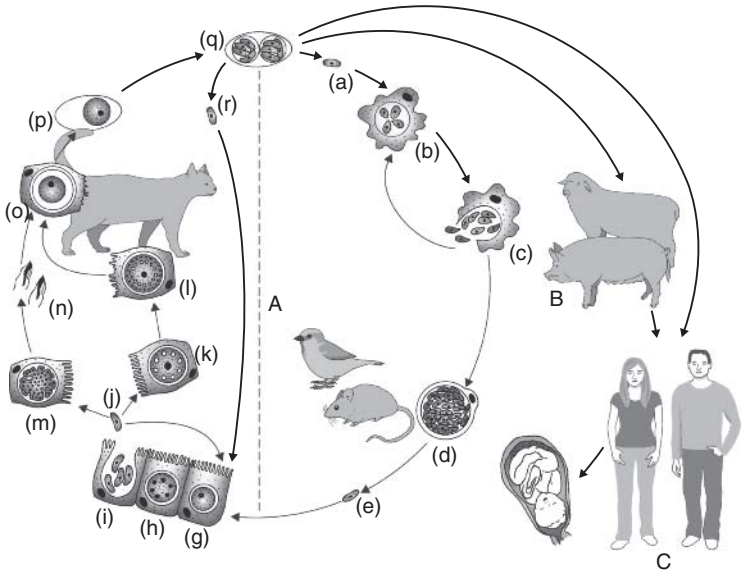


Figure 2.53 Life cycle of *Toxoplasma gondii*. (a) Sporozoite. (b, c) Tachyzoites in macrophage. (d) Bradyzoites in tissue cysts. (e) Bradyzoite. (f) Infection of intermediate hosts with bradyzoites from tissue cysts. (g) Trophozoite in intestinal epithelial cell. (h) Schizont. (i, j) Merozoites. (k) Macrogametocyte. (l) Macrogamete. (m) Microgametocyte. (n) Microgamete. (o) Zygote. (p) Sporont inside oocyst. (q) Sporulated oocyst with two sporocysts, each containing four sporozoites (infectious for cat or intermediate host). (r) Sporozoite, infectious for cat.

transmission, which may be a relevant mode of sustained transmission in rodents and is certainly of great clinical importance in humans.

After some days, the multiplication of tachyzoites in the infected host slows down as a result of a strong host imune response. Now bradyzoites develop (Greek: *bradys* = slow), which divide only slowly within their host cells. At the same time, the wall of the parasitophorous vacuole is remodeled to form a 2-µm thick cyst wall. The new tissue cysts can occur in all organs, but are concentrated in the brain and skeletal or heart muscle. Recently, it has been shown that *T. gondii* tachyzoites can infect endothelial cells, which enables them to cross the blood brain barriere and establish chronic infections. These stages can persist for years, probably in neurons, and are infectious for the cat as definitive host. They are also infective for intermediate hosts, if ingested during carnivorism. Tissue cysts can interfere with neurological functions of the host and can alter the cat-avoidance behavior of rodents: cat urine is atractive for *T. gondii*-infected mice and rats – in contrast to a pronounced aversion shown by infection-free animals for that odor, which is interpreted by some as a parasite-induced mechanism to increase transmission (see also Section 1.7.3). Whether such effects are a direct or indirect action of the parasites is not known.

In the cat, an infection with sporulated oocysts can also lead to the development of tachyzoites and tissue cysts. In this infection mode, the parasites invade the

epithelial cells of the cat's intestine, where they initiate the sexual cycle. This results in schizogony and gametogony and, ultimately, excretion of oocysts. Because of the slower and less efficient sexual cycle when initiated via ingestion of oocysts (relative to tissue cysts), this may not be such an important part of the natural life cycle and spread in the environment.

Morphology The oocysts of *T. gondii* are colorless and measure approximately 12.5 × 11 µm (Figure 2.54). They lack a micropyle and contain two sporocysts with four sporozoites each and a granulated residual body. The tachyzoites are crescent-shaped and measure ~6 × 2 µm. The tissue cysts are round, up to 300 µm in diameter and possess a wall of 2–3 µm thickness consisting of fibril-like material surrounding variable numbers of bradyzoites inside. Bradyzoites are similar in size to tachyzoites.

Toxoplasmosis of Humans In immunocompetent adults, the infection is generally self-limiting, although recent data from South America suggest that ocular disease (retinal lesions) may be a very frequent outcome even in otherwise healthy adults, rarely reaching a clinically significant extent but sometimes producing blindness. Infection may initially cause fever and swelling of lymph nodes, but usually passes unnoticed (with the exception noted above). Throughout the world, 15–85% of adults are infected depending on region. In Germany, the rate

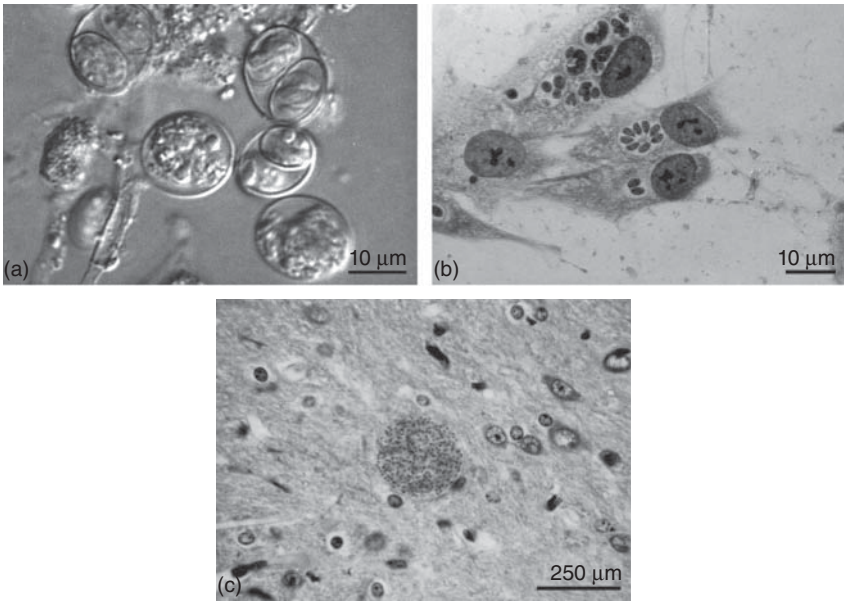
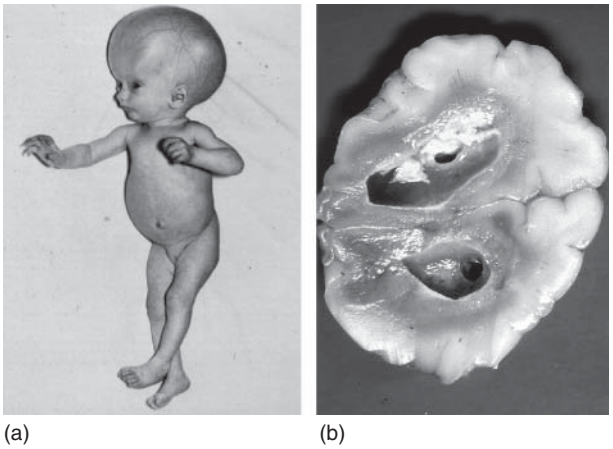


Figure 2.54 Stages of *Toxoplasma gondii*. (a) sporulated oocysts from cat feces. (Image: T. Jäkel.) (b) Tachyzoites in cultured fibroblasts. (Image: R. Lucius.). (c) Tissues cysts in the brain of a laboratory mouse. (Image: Archive of the Department of Parasitology, University of Hohenheim.)

of infection determined by the prevalence of specific antibodies varies between 30% and 60% of the population, depending on the geographic locale. As a rule of thumb, and as expected for an infection acquired from the environment, the rate of infection with *T. gondii* in humans increases proportionally with age. Why some countries have much higher prevalence of infection than others is not known, but is presumed to be related to dietary preferences (e.g., whether meat is eaten and, if so, what type and how well cooked) and/or public health infrastructure (e.g., purity of drinking water and degree to which vegetables are washed before/after they reach the consumer).

Fetuses developing within women who have experienced a primary infection before pregnancy are usually protected against acute toxoplasmosis by the mother's immunity and so the fetus is not endangered. The mother's immune status can be determined by analysis of the antibody levels: an infection acquired in the past is generally indicated by *Toxoplasma*-specific IgG antibodies, while IgM antibodies are present in an acute infection. Sophisticated tests to discriminate acute versus chronic infections have been developed and are in routine use in many countries. In the case of a first infection occurring during pregnancy, the parasites can pass into the fetus and establish mainly in neural tissues. The probability of the parasite crossing the placenta is proportional to the gestational period, perhaps as a result of the larger target represented by the growing placenta; hence, first-trimester infections are much less likely to lead to infection of the fetus than those beginning in the third trimester. However, the opposite is true for the severity of the disease that results: infections during the first and second trimesters often lead to abortions or to birth of children with profound malformations (Figure 2.55). These may include hydrocephalus,



(a) (b)

Figure 2.55 Congenital toxoplasmosis. (a) Child with hydrocephalus. (From Dyke (1960) *Recent Advances in Clinical Pathology*, Vol.3. By kind permission of Elsevier.) (b) Cross section of a hydrocephalus brain with distinctly enlarged ventricles. (From: Schrod *et al.* (1991) Pädiat. Prax 43, 117–125. With kind permission by Hans Marseille Publisher.)

inflammations of retina and choroid (chorioretinitis), and cerebral calcification. Infections of the fetus during the last trimester are usually less extensive and only 5–10% of children who do get infected *in utero* show conspicuous symptoms at birth. However, more than 50% (>90% in some regions) when growing up later, suffer from chorioretinitis, which may lead to blindness, if the parasites are not controlled by chemotherapy. In Germany, about 4500 pregnant women contract a *T. gondii* infection, so that a considerable number of children are affected (Figure 2.56). The relevance of the infection has caused the implementation of compulsory tests during pregnancy in Austria and France. At diagnosis of an acute infection, the mother is usually treated with chemotherapeutics. Given the potentially disastrous sequelae, when infections are diagnosed during early pregnancy, an abortion is often chosen by the mother. Prophylactic treatment of a congenitally infected, asymptomatic newborn is strongly recommended as this can prevent subsequent disease (e.g., blindness) from developing later in life.

Chronic infections with *Toxoplasma* tissue cysts are usually tolerated without symptoms as long as the immune system works efficiently. In persons who develop deficient T cell responses, *Toxoplasma* cysts in the brain can be reactivated, the bradyzoites eventually reverting to tachyzoites. The parasites that are freed from the cysts invade cells of the surrounding tissue, resulting in severe localized inflammation. The dividing parasites can generate extensive damage, partly by invading endothelial cells and by rupturing the vessels. The ensuing hemorrhages and thromboses provoke an oxygen deficiency in distal areas of the brain causing large necroses (Figure 1.47). Such a *Toxoplasma* encephalitis due to reactivation of cysts can be lethal if untreated. In Middle Europe, it was fatal in one out of four AIDS patients in the beginning of the AIDS pandemic. Fortunately, in countries where AIDS chemotherapy is routine, the restoration of the immune system appears sufficient to control the *Toxoplasma* infection and prevent serious disease from this parasite. Such patients are also often treated

662 685 births/year in Germany

28.26% of pregnant women are antibody-positive (putatively immune), that is, 71.74% = 475 410 "risk pregnancies"

0.92% seroconversion during pregnancy
= 4371 maternal infections

29% of children of infected mothers = 1 268 children become infected

22% of infected children with clinical symtoms up to an age of 3 years (according to Wallon et al., 2013)
= 279 sick children/year

Figure 2.56 Estimated incidence of congenital toxoplasmosis in Germany in 2011, according to information of the Robert Koch-Institute Berlin. (Wilking, H., Thamm, M., Stark, K., Aebischer, T., and Seeber, F. (2014) Sci. Rep. 6, 22551. Wallon, M. *et al.* (2013) Clin. Infect. Dis. 56, 1223–1231.)

with anti-*Toxoplasma* prophylaxis when *Toxoplasma* antibodies are detected. Reactivation of dormant cysts can also occur in recipients of transplants who receive immunosuppressive treatment to ensure a better acceptance of the graft. Similarly, infection of a recipient by a donor organ is possible.

Toxoplasmosis in Animals Toxoplasmosis occurs in all domestic and farm animals. This parasite poses a serious problem to sheep husbandry as abortion storms ("infectious abortion") can occur, and it is associated with a risk of infecting 100% of stocks. Sheep and goats can transmit *Toxoplasma* via milk. In pigs, the rate of infection varies depending on the husbandry conditions. In the modern fattening industry, it is <1%, while much higher percentages of sows can be infected under less controlled conditions. Cysts in meat survive for 3 weeks at 4 °C. Toxoplasmosis in dogs can lead to dysfunctions of the central nervous system, which are difficult to distinguish from rabies. In cats, intestinal and eye disease can occur, but is rare. Although cattle develop antibodies against *Toxoplasma*, the parasite appears to be much less efficiently transmitted by beef. Data on infection rates in wild rodents vary from >1% to 35%. The literature reports infection of many dozens of warm-blooded animal species in nature, consistent with the notion that this parasite has an exceptionally broad host range. Which of those species are biologically important versus which are "dead ends" in terms of transmission to other hosts (as humans are today) is not currently known.

Cell Biology *T. gondii* is a well-investigated parasite as the tachyzoites can be cultivated continuously in host cells (for instance, in fibroblasts) and manipulated genetically. The specificity for the host cell is broad and so many host cell types are used experimentally, depending on the question being addressed. Among others, neurons, macrophages, fibroblasts, and cells of the microglia, endothelia, and muscles can be infected. This suggests that receptors of the tachyzoites are able to recognize commonly occurring surface components such as sulfated saccharide groups (for instance, heparin sulfate and chondroitine sulfate). Cell invasion is effected by mechanisms that may be related to gliding motility (Figure 2.44). The surface of *T. gondii* is covered with a variety of GPI-anchored proteins, which are involved in adhesion and virulence. They belong to various protein families, the most studied of which are SAG (surface antigens), SRS (SAG1-related sequences), and SUSA (SAG-unrelated surface antigens). The expression of many of these proteins is stage- and strain-specific. It is thought that this multitude of different surface antigens may allow *T. gondii* to invade different types of cells and host species, although this has not been experimentally demonstrated. It could alternatively be related to avoiding a more potent immune response through, for example, confusing the host's immune response with exposure to such a large number of closely related antigens (a process akin to "original antigenic sin" in HIV infections).

Depending on the strain of parasite, the endoplasmic reticulum and mitochondria of the host cell can be actively recruited to the membrane of the parasitophorous vacuole (Figure 2.57). This process is mediated by a polymorphic

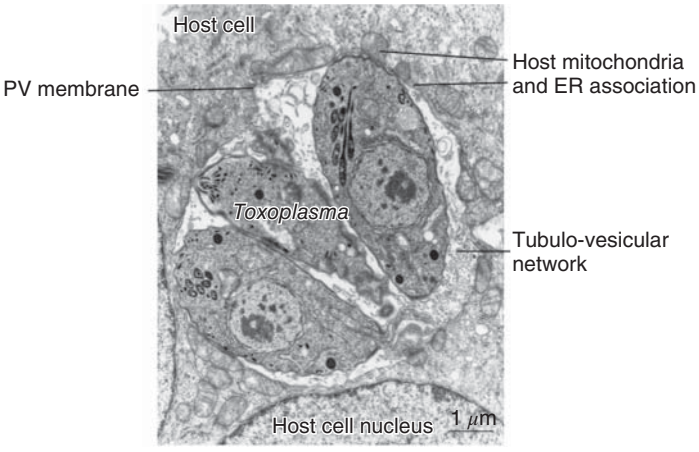


Figure 2.57 Tachyzoites of *Toxoplasma gondii* in a parasitophorous vacuole. The vacuole is surrounded by host-cell mitochondria and endoplasmic reticulum, which the parasite recruits. Image: Coppens and Joiner (2001) Exp. Review in Mol. Med. 2001, 1–20.

dense granule protein known as mitochondrial association factor 1 (MAF1). Initially, this recruitment was assumed to facilitate the effective transfer of host metabolites, but the finding that only some strains (and, notably, not the ubiquitous Type II strain of Europe) engage in such recruitment suggested that the association may be related to other pressures since metabolic needs would be assumed to be largely common to all strains. It is known that many strain-specific differences relate to their engagement with the immune responses of their hosts; indeed, the major pressures driving the emergence of new strains is presumed to be related to differences in the immune responses of the different intermediate hosts, in which they most often find themselves. This suggested that the mitochondrial association was in part related to the recently discovered role of mitochondria in innate immune responses and, as predicted, the response to strains that do or do not recruit host mitochondria are very different.

The parasitophorous vacuole is filled with a tubulo-vesicular network derived from parasite material, but the function of this network is unknown.

The conversion of tachyzoites to bradyzoites and the development of tissue cysts are caused by various stresses, including cytokines (e.g., IFN-γ) that are produced by activated T cells. The conversion can also be triggered by nitric oxide (NO), which is produced as an effector molecule by host cells when these are activated by IFN-γ. Moreover, depending on the host species, the tryptophan and iron metabolism of infected cells can be altered after the IFN-γ activation, such that the production of these components, which are essential for the parasites, is reduced. Therefore, the conversion of the stages and the extremely slow multiplication of the bradyzoites seems to be a reaction to the altered status of the host cell. If the production of IFN-γ is diminished as in AIDS patients, because of a reduced number of T helper cells, the development of tachyzoites can occur unchecked and a devastating reactivation of the infection can result.

Immunobiology The process of *Toxoplasma* infection in the intermediate host is well studied in the mouse model. Here, the first contact between parasite and host takes place in the intestinal epithelium and the lamina propria beneath. The first cells reacting to molecular structures of the invading stages probably are dendritic cells and macrophages with their toll-like receptors (TLRs) and Nod-like receptors (NLRs). They produce IL-12 and TNF-α, which for their part stimulate NK cells and neutrophils to produce IFN-γ. Thus, an environment is created, in which the T helper cells are polarized toward a pro-inflammatory type. After antigen presentation, they produce IFN-γ, which activates the host cells to kill their intracellular parasites.

After activation by cytokines, infected macrophages apparently kill their intracellular parasites by means of NO production and reactive oxygen products. In addition, the depletion of tryptophan and iron induced by IFN-γ, described above in connection with the stage conversion, can contribute to the defence in cells that are not able to efficiently produce NO. Mice with a chronic *T. gondii* infection are protected from subsequent infections due to their T cell response. In immunodeficient mice, however, no tissue cyst is generated and an unchecked multiplication of the tachyzoites occurs, which causes death (Box 2.3).

Box 2.3 How *Toxoplasma* Effectors Manipulate the Host Cell?
by John Boothroyd, Stanford University, Stanford

As mentioned earlier, the rhoptries inject their contents into the host cell during invasion by a yet unknown mechanism. Recent work has revealed that these specialized organelles contain a number of important, polymorphic "effectors" that modulate the immune response of the host. Among these, the best studied are ROP16, ROP5, ROP17, and ROP18. ROP16 is a tyrosine kinase that can directly phosphorylate certain STATs ("signal transducers and activators of transcription"). They therefore mimic the host enzymes known as JAK kinases that normally target the key tyrosine in the STATs to activate them after binding of interleukin receptors. For example, IL-4 binding to its receptor activates JAK2, which phosphorylates a key tyrosine on STAT3 and STAT6. The result of such activation is to depress the generation of IL-12 and drive the host cell toward a Th2-type response (anti-inflammatory). Surprisingly and very interestingly, the ability of ROP16 to intercept this pathway differs dramatically between strains: Types I and III encode a ROP16 version that is very effective at mimicking JAK2, whereas Type II ROP16 is not. As a result, the Th1/2 balance of an infected animal will vary greatly depending on the strain that initiates the infection. The pressures responsible for these differences can never be known for certain but seem likely to be adaptations to a particular host. It may be, for example, that the Type II strain of *Toxoplasma* has evolved in association with an intermediate host that is naturally inclined to a Th2 type of response and that by having a ROP16 that does not push further in this direction, an optimal response, in terms of achieving a high parasite load, and a persistent infection

is achieved. Type I/III strains may be more used to infecting hosts where Th1 is the dominant response and this needs tempering by some Th2 cytokines to prevent a cytokine storm that might overwhelm the host prior to transmission of the parasite to the next host (note, e.g., cats are typically hunters of live prey, not scavengers of dead animals). Whether this inclination toward Th1 versus Th2 is an inherent property of different host species or a reflection of other infections harbored by hosts of the same species is not known: a high prevalence of worm infections, for example, might result in hosts that are already Th2-inclined, making them better suited to the counterbalancing of Th1-inclined Type II strains of *Toxoplasma* (Figure B2.3).

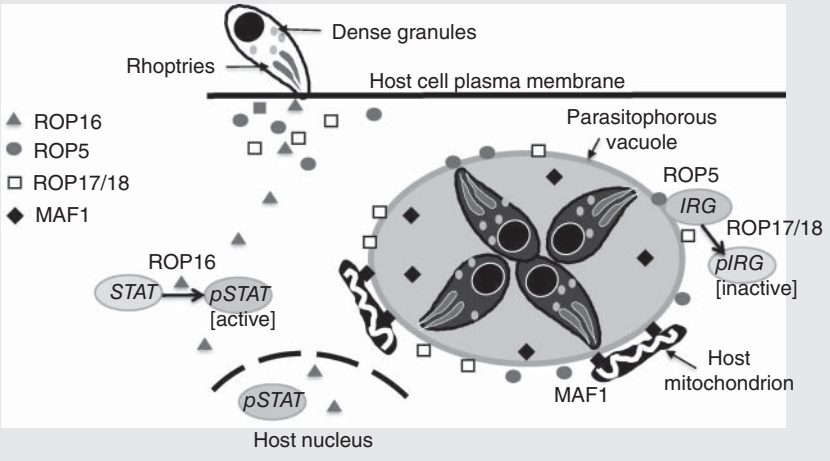


Figure B2.3 Effectors of *Toxoplasma gondii*. (Image: J. Boothroyd.)

ROP5 and ROP17/18 are three additional rhoptry proteins that are homologous to ROP16 and also polymorphic. In this case, however, ROP17/18 is a pair of serine/threonine kinases and ROP5 is a catalytically inactive pseudokinase. These ROPs cooperate in an unusual way: ROP5 binds important host proteins known as immunity-related GTPases (IRGs), thereby preventing them, at least transiently, from oligomerizing. Such oligomerization is key to IRG function in allowing them to disrupt the vacuolar membrane, in which the tachyzoites would otherwise grow. ROP5-binding also holds the IRG in a conformation that allows ROP17/18 to phosphorylate the IRGs, resulting in a permanent block in oligomerization. As for ROP16, ROP5, and ROP17/18 differ greatly between strains and the presumption is that this again reflects a difference between the respective hosts with which each strain coevolved. An obvious difference exists between, for example, rodents and birds as rodents have a highly developed system of IRGs induced by IFN-γ, whereas birds do not; having highly active ROP17/18, therefore, may be a liability to a strain that circulates predominantly in birds but necessary for those that circulate in mice. A further

nuance of the ROP5 family is that it is a series of approximately 5–10 tandemly repeated genes that are closely related but not identical; the exact number and sequence differs between Toxoplasma strains. This is matched by the upwards of 20 polymorphic IRG genes found in a given mouse strain. Each ROP5 appears to have a distinct set of IRGs that it can bind and inactivate. This suggests that there is an ongoing "arms race," in which the parasite and host genomes are trying to stay one step ahead of each other, with the host constantly evolving new IRGs that cannot be bound by an existing ROP5, which the parasite has to counter by evolving a new ROP5 that can neutralize this new IRG threat.

Dense granules also introduce effectors into the host cell. One, GRA15, has a dramatic effect on the key host transcription factor NfKB. Another, the MAF1 mentioned earlier, mediates mitochondrial association, which affects the host cell's innate immune responses. Furthermore, as for the ROPs mentioned earlier, these dense granule effectors differ greatly between strains. It is clear, therefore, that the total armamentarium possessed by any given strain of *Toxoplasma* is optimally tuned to a particular ecological niche (e.g., species of intermediate host). Occasional sex between strains (in a cat) can result in new combinations of these effectors such that when a new niche arises, there is likely to be an F1 progeny that is optimally suited to that niche. As humans have disrupted the environment over the last millennium, there have likely been many times when such adaptability has been a great advantage to *Toxoplasma*, allowing them to continue to be the ubiquitous parasites they are.

As might be expected, not all effectors have been found to vary between the three canonical strains of Europe; some appear to be common to them all, presumably because the challenges represented by their respective host niches are also similar. There are many GRAs and ROPs whose functions have yet to be identified, but they likely represent a remarkably rich collection of novel effectors that, collectively, manipulate the infected host to just the right degree.

In the mouse model, a partial defence against a challenge infection with *T. gondii* can be achieved by immunization with several single recombinant proteins. For that purpose, proteins of micronemes, rhoptries, dense granules, and parasite surface have been used among others. However, the highest rate of protection has been achieved by vaccination with genetically attenuated parasites, in which one or more key proteins have been deleted. Deleting the aforementioned ROP5, for example, attenuates a normally mouse-virulent Type I strain by several orders of magnitude. Even more effective is removing a biosynthetic gene such as that encoding carbamoyl phosphate synthase; the resulting strain is auxotrophic for pyrimidines and cannot grow without excess pyrimidines being provided in vitro. Such strains are likely to form the basis for future vaccines. At present, however, the only commercially available vaccine is based on a strain (S48) that was attenuated by extensive passaging in mice and that now has lost its ability to develop

tissue cysts. As a result, it multiplies only for a short time and induces a protective immune response. This vaccination provides some protection against abortion in sheep. This is of value to farmers in preventing losses from toxoplasmosis; it may have the added benefit of reducing transmission to humans who might eat undercooked lamb.

Toxoplasma infections in the cat also induce a robust protection, which inhibits the development of the parasite and the shedding of oocysts in the case of subsequent infections. For this reason, vaccination of cats is often discussed as a means to efficiently interrupt transmission of *Toxoplasma.* No such vaccine has yet been produced commercially, which requires detailed studies on immunity to *Toxoplasma* in cats.

2.6.2.7 *Neospora caninum*

Neospora caninum is found worldwide and is an important pathogen of cattle, horses, and dogs. It was first described in 1984 as a cause of disease and its biology is similar to that of *T. gondii*, but canines act as the definitive host, allowing for the development of a sexual intestinal cycle. Infected dogs excrete unsporulated oval oocysts measuring 11 × 10 µm that contain two sporocysts (Figure 2.58). Each sporocyst sporulates to give four sporozoites in a process that takes 3 days approximately. The parasite exploits a very wide range of intermediate hosts, and ungulates (hooved animals) play a particularly important role. Intermediate hosts such as cattle can be infected horizontally through ingestion of oocysts or vertically through the placenta. Infection of cattle can result in abortion, reduction of milk production, and reduced post weaning weight of calves. It is therefore of particular economic importance and is the most common cause of bovine abortion in Europe. *N. caninum* infection can be efficiently transmitted congenitally through successive generations of cattle for many years. Consequently, vertical transmission seems to be epidemiologically more important for *N. caninum* than horizontal transmission.

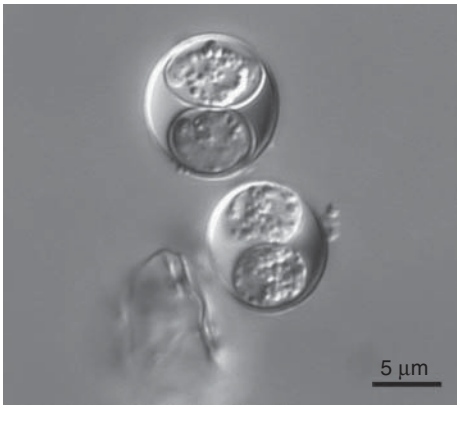


Figure 2.58 Oocysts of *Neospora caninum*. (Image: Institute of Parasitology, University of Bern.)

Details of the intestinal reproduction in the final host very likely resemble the process in *Toxoplasma gondii*. In the intermediate host, asexual division of the fast-dividing tachyzoites present throughout the acute phase is by endodyogeny. This is superseded by a chronic phase of the infection, in which tissue cysts containing bradyzoite stages are formed. These cysts possess relatively thick walls of up to 4 µm, and are found mainly in neuronal tissue. These cysts are assumed to persist as resting stages in the host for the duration of their lives. Reactivation of these cysts during pregnancy is the likely source of congenital infection. The parasites can be transferred to mice and other experimental animals by intraperitoneal injection of infected material containing cysts. Infected mice develop cysts in their brains. These *Neospora* tissue cysts have also been detected in aborted and newborn calves. Infection of adult intermediate hosts, including cattle and dogs, causes relatively few symptoms. However, infection during pregnancy, other than causing abortion, can result in severe congenital disease characterized by central nervous system disorders ultimately resulting in paralysis of the offspring.

2.6.2.8 ***Sarcocystis***

Parasites of the genus *Sarcocystis* (Greek: *sarx* = meat, *kýstis* = bubble) have a mandatory alternation of hosts between carnivorous vertebrate animals (= final hosts) and their vertebrate animal prey (= intermediate). More than 140 *Sarcocystis* species have been described since the initial description of wild and domestic animals in 1975. All these species are specifically adapted to their final and intermediate hosts (see also Table 2.7). Some species of *Sarcocystis* can cause serious disease as they undergo schizogony and result in significant losses in livestock. Several *Sarcocystis* species infect humans as final hosts and exploit pets, including dogs and cats, as intermediate hosts. Species are often named after the combination of intermediate and definitive hosts.

Table 2.7 Frequently occurring *Sarcocystis* species.

Type	Final host	Intermediate host	Muscle cyst (length)
Sarcocystis cruzi	Dog	Cattle	0.5 mm
Sarcocystis tenella	Dog	Sheep	0.7 mm
Sarcocystis arieticanis	Dog	Sheep	1 mm
Sarcocystis capracanis	Dog	Goat	2.5 mm
Sarcocystis bertrami	Dog	Horse	9 mm
Sarcocystis miescheriana	Dog	Pig	1.1 mm
Sarcocystis hirsuta	Cat	Cattle	8 mm
Sarcocystis gigantica	Cat	Sheep	1.5 cm
Sarcocystis muris	Cat	House mouse	Not specified
Sarcocystis hominis	Human	Cattle	Not specified
Sarcocystis suihominis	Human	Pig	1.5 mm
Sarcocystis dispersa	Owls	House mouse	Not specified
Sarcocystis singaporensis	Python	Different rat species	About 1 mm

Sarcocystis suihominis Exploits humans as the final host and pigs as an intermediate host. Humans can become infected with *S. suihominis* by eating undercooked pork that contains the cyst stages. The zoites ("cystozoites") released from the tissue cysts in the intestine invade the intestinal epithelial cells, where they do not undergo schizogony, but rather differentiate directly into macrogametocytes and microgametocytes, which then become gametes (Figure 2.59). After fertilization of the macrogametes by the flagellated microgametes, oocysts are formed. These very thin walled oocysts contain two sporocysts, each of which contains four sporozoites. This development process occurs rapidly (prepatency period of 9–10 days). Sporulated oocysts are excreted via feces of infected hosts over a period of several months.

In the pig, the sporozoites penetrate the intestinal wall and then invade the vascular endothelial cells of the liver and other organs, where they form two generations of merozoites by means of endopolygeny. The last-generation of merozoites

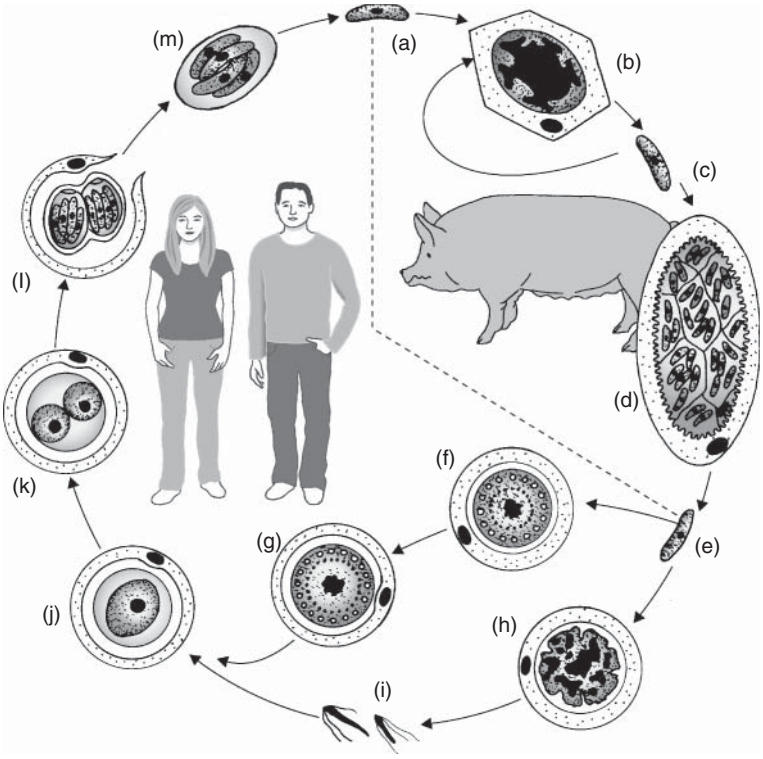


Figure 2.59 Life cycle *of Sarcocystis suihominis*. (a) Sporozoite. (b) Endopolygeny in endothelial cell. (c) Merozoite. (d) Tissue cysts in muscle cell. (e) Cystozoite. (f) Macrogametocyte. (g) Macrogamete. (h) Microgametocyte. (i) Microgametes. (j) Zygote. (k) Sporoblasts. (l) Sporocysts with sporozoites within the oocyst wall. (m) Excreted sporocyst. (Adapted from Mehlhorn, H. (ed.) (1988) *Parasitology in Focus*, Springer-Verlag, Heidelberg.)

predominantly infects muscle cells of the tongue, diaphragm, and masseter muscles, where they grow and divide by endodyogeny. The wall of the parasitophorous vacuole is transformed into a cyst wall to create a tissue cyst. "Metrocyte stages" that are located in the periphery of the cyst divide through endodyogeny, producing thousands of cystozoites, which are incapable of dividing but infectious for humans.

The oocyst wall is very thin and shapes itself to fit the sporocysts. As a result, it often tears in the gut and consequently mostly free sporocysts are found in feces samples (size: 10×13 µm) (Figure 2.60). The tissue cysts are spindle-shaped, measure approximately 1100×100 µm, and have hair-like protrusions on the surface, which are 13 µm in length. The interior of the cysts, which can contain thousands of cystozoites, is divided into chambers by septa. The banana-shaped cystozoites within are 12–15 µm in length.

S. suihominis is potentially pathogenic for humans, although clinical disease rarely occurs in well-nourished individuals. However, in experiments, volunteers who ate raw pork containing cysts developed severe diarrhea and required hospitalization. The pathogenicity of *S. suihominis* can be very high for pigs. Infection can result in fever, massive intravascular coagulation, and capillary damage. This is observed especially during the synchronous development of the schizonts within endothelial cells. Pigs become seriously ill and can even die after a massive *Sarcocystis* infection. The consumption of 1 million or more sporocysts regularly results in the death of the animal. Acute infections in pregnant animals can lead to abortion. Mild infections of animals are of economic importance as they result in reduced weight gain. The muscle cysts that develop following infection are minimally pathogenic.

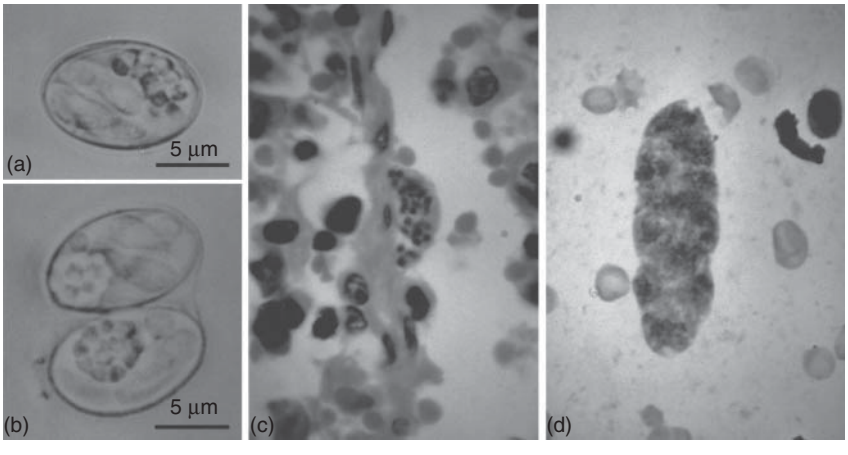


Figure 2.60 Stages of *Sarcocystis suihominis*. (a) Free, sporulated oocysts. (b) Sporocysts within the thin oocyst sheath. (Images (a) and (b): Courtesy of H. Rommel.) (c) Schizont in the endothelial cell of a pig (d) Tissue cyst, Giemsa stain. (Images (c) and (d): Courtesy of A. O. Heydorn.)

Sarcocystis infections are widespread in domestic and wild animals. The muscle cysts, also known as "Miescher's tubes," can be very conspicuous. The cysts of *Sarcocystis gigantea,* for example, typically located in the throats of sheep, reaches a size of $>1 \times 0.5$ cm. Due to their high host specificity and their pathogenicity for the intermediate host, oocysts of ***Sarcocystis singaporensis*** are used for the biological control of rats. This Southeast Asian type uses pythons as the final host, and this enables the production of large quantities of oocysts. When rats eat bait containing oocysts, they later die of internal bleeding, which is associated with the schizogonies.

2.6.3 Haematozoea

Parasites of the Haematozoea class have an obligate host switch between blood-sucking arthropods and vertebrates. Fertilization occurs in the intestinal lumen of arthropods and sporogony leads to the formation of "free sporozoites," which do not lie within a cyst, but are transmitted in the saliva of the arthropods. Vertebrates such as lizards, birds, and mammals serve as intermediate hosts. This pattern suggests that the Haematozoea developed from intestinal parasites of hematophagous arthropods, whose blood hosts were lateron integrated in haematozoan life cycles as intermediate hosts. In vertebrates, cell types such as endothelial cells, macrophages, hepatocytes, leukocytes, and erythrocytes are exploited for schizogony. The apical complex within sporozoites and merozoites is reduced and the conoid, in particular, is only rudimentary. In the Haemosporida order, the final hosts are blood-sucking Diptera with reptiles, birds, and mammals being exploited as intermediate hosts. There is only one family, the Plasmodiidae. The genus *Plasmodium* is of utmost importance because of their medical importance as malaria parasites of humans. In the Piroplasmida order, which includes the Babesiidae and Theileriidae families, most species exploit ticks as final hosts and mammals as intermediate hosts. In ungulates, the piroplasms take the ecological niche of the Haematosporida (containing the genus Plasmodium) and are the cause of significant animal diseases.

2.6.3.1 *Plasmodium*

The genus *Plasmodium* (Greek: *plasmatos*, small entity) is divided into four subgenera with more than 170 species. There are five human pathogenic species: *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium knowlesi*, and *Plasmodium falciparum* (see also Tables 2.8 and 2.9: http://www.who.int/tdr/diseases/malaria/default.htm). They each share a number of common characteristics, but also have distinctive features. *P. knowlesi* differs from the other species as it is a zoonotic parasite, occurring in monkeys in Southeast Asia. It will not be treated in detail. Plasmodium parasites of monkeys, rodents, and birds are covered at the end of the chapter.

Development The four human pathogenic *Plasmodium* species have a very similar biology (Figure 2.61), but they cause different types of malaria. Infection

Table 2.8 Human pathogenic *Plasmodium* species and the forms of malaria they cause.

Species	Disease	Fever attacks – interval
Plasmodium vivax	Malaria tertiana	48 h, Synchronous
Plasmodium ovale	Malaria tertiana	48 h, Synchronous
Plasmodium malariae	Malaria quartana	72 h, Synchronous
Plasmodium falciparum	Malaria tropica	48 h, Non-synchronous
Plasmodium knowlesi	Zoonotic Malaria	fast replicating

is transmitted by female *Anopheles* mosquitoes, which unlike their male counterparts require blood meals for reproduction. Approximately 10–100 sporozoites are injected into the host with saliva of the insect as it feeds. They reach the liver after a short time, migrate through macrophages (Kupffer stellate cells), and invade liver parenchymal cells. In 6–16 days, they mature in a parasitophorous vacuole within hepatocytes and undergo schizogony ("**exoerythrocytic schizogony**"), which leads to the formation of 10 000–30 000 liver merozoites, depending on the species involved. In the case of *P. vivax* and *P. ovale,* dormant stages ("**hypnozoites**") develop parallel to schizogony. Further development of these stages only occurs after some months, facilitating the staggered and prolonged production of liver merozoites.

In a complex budding process, membrane-bound packages of liver merozoites ("merosomes") are released into the blood circulation from the infected hepatocytes. During the lung passage, merosomes rupture and first generation merozoites invade erythrocytes, where they initiate repeated rounds of schizogony ("**erythrocytic schizogony**"). In addition to a residual body, 8–16 merozoites emerge from each schizogony and these infect further erythrocytes. Depending on the species of *Plasmodium*, an erythrocytic schizogony lasts for 48 or 72 h. In *P. vivax*, *P. ovale*, and *P. malariae*, schizogony is strictly synchronized and consequently the entire population of parasites can be found in the erythrocytes at the same development stage and the same time. Several days or weeks after the onset of erythrocytic schizogony, some of the merozoites differentiate into gametocytes and consequently both schizogony stages and gametocytes can then be found in the blood simultaneously.

Following ingestion by the mosquito and stimulated by the physiological conditions in the mosquito gut, the gametocytes develop into gametes within approximately 10 min. In microgametocytes, this differentiation is known as **exflagellation** and is associated with a dramatic change of form. During this process, each microgametocyte quickly forms eight plasma protrusions, into each of which a nucleus migrates. Microgametes separate themselves and fertilize the macrogametes. The fully formed mobile zygote ("**ookinete**") migrates through the epithelial cells of the mosquito stomach and establishes itself between epithelial cells and basement membrane. The ookinete develops into an **oocyst,** in which sporogony occurs. Several thousand sporozoites are then formed after

Table 2.9 Overview of human infections with *Plasmodium* (excluding Plasmodium knowlesi).

Type	Liver forms	Blood forms	Fever attacks	Prepatency/incubation period	Gametocytes	Development in the mosquito
Plasmodium vivax	8000–20 000 merozoites, hypnozoites	Usually 12–16 merozoites Schüffner's dots, Erythrocyte increases in size	Interval: 48 h Relapses after months through hypnozoites	8 days/12–18 days	Round, within 3 days after the onset of blood schizogony	20 °C: 16 days 28 °C: 8–10 days
Plasmodium ovale	Around 15 000 merozoites, hypnozoites	Usually 8 merozoites Schüffner's dots, Erythrocyte increases in size, sometimes oval	Interval: 48 h Relapses through hypnozoites	9 days/12–15 days	Round	Not known
Plasmodium malariae	Number not known No hypnozoites	Mostly 8 merozoites, often referred to as "Daisies." Late trophozoites sometimes ribbon-shaped, Ziemann's dots	Interval: 72 h relapse after years through occult blood forms	14 days/18–40 days	Round	20 °C: 30–35 days 28 °C: 14 days and longer
Plasmodium falciparum	Around 30 000 merozoites No hypnozoites	Usually 8–12 merozoites. Ring forms small, often double infection of erythrocytes, older rings and trophozoites not in peripheral blood	Nonsynchronized	5 days/7–15 days	Bow-shaped, usually 10 days or later after the onset of blood schizogony	20 °C: 22–15 days 28 °C: 9–10 days

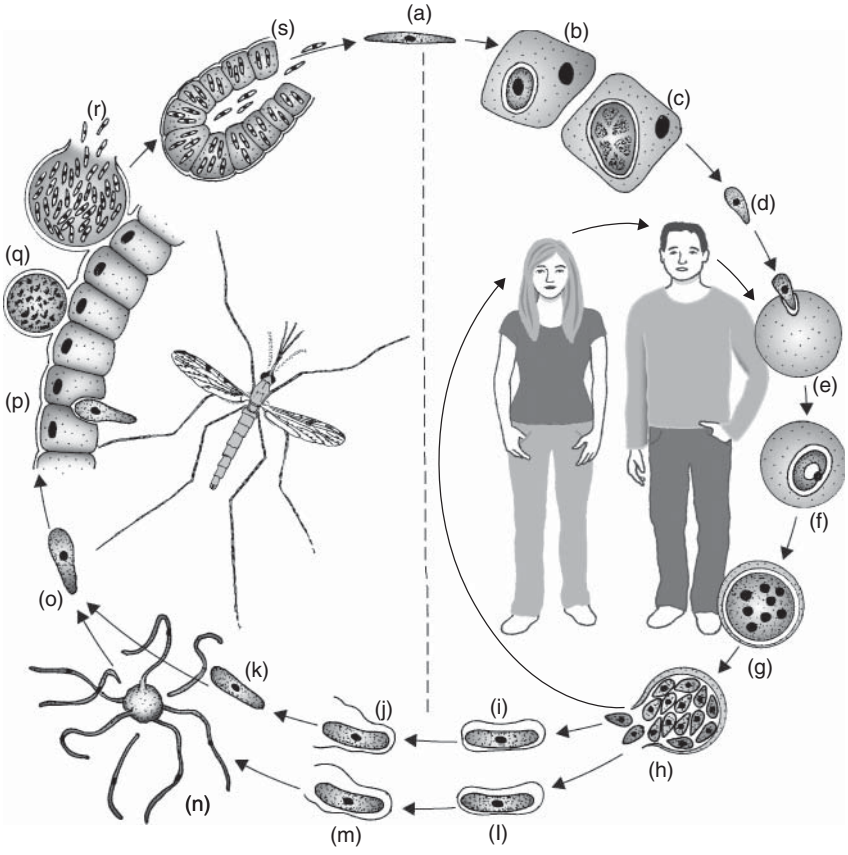


Figure 2.61 Life cycle of *Plasmodium falciparum*. (a) Sporozoite. (b) Trophozoite in the liver cell. (c) Liver schizont. (d) Merozoite from liver cell. (e) Invasion of an erythrocyte. (f) Ring stage. (g) Schizont. (h) Merozoites. (i, j) Macrogametocyte. (k) Macrogamete. (l, m) Microgametocyte. (n) Exflagellation, resulting in the formation of microgametes. (o) Zygote. (p) Ookinete. (q) Oocyst. (r) Sporozoites are released from the oocyst and invade the salivary gland. (s) Transmission of sporozoites with the saliva. (Adapted from Mehlhorn, H. (ed.) (1988) Parasitology in Focus, Springer-Verlag, Heidelberg.)

meiosis has taken place. In a laboratory infection, more than 100 oocysts can develop from an infected *Anopheles*, and hence a large number of sporozoites can be obtained. High environmental temperatures are favorable for development of sporozoites, which can take one to several weeks. The sporozoites migrate through the haemocoel to the salivary glands of the mosquito, where they are infectious for humans.

Morphology The slender, elongated **sporozoites** of Plasmodium parasites are 10–15µm in length. After invasion of the liver cell, they develop into organisms of about 3 µm in size, subsequently growing into liver schizonts measuring

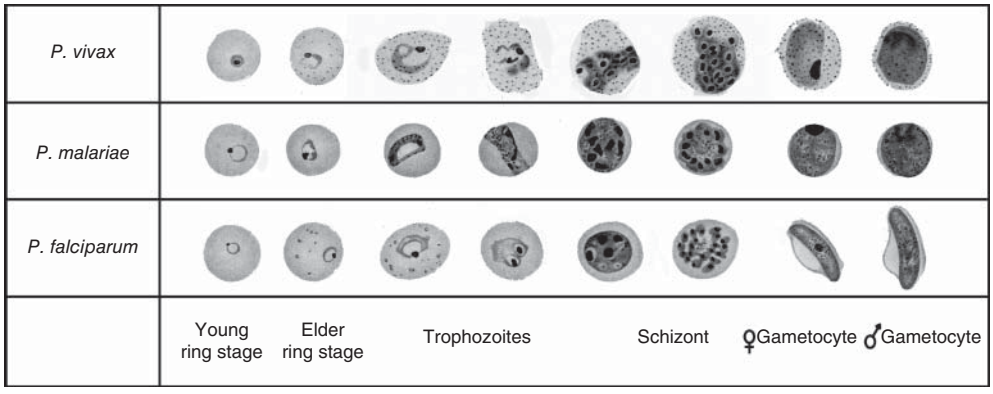


Figure 2.62 Blood stages of human pathogenic Plasmodium species. Top row: *Plasmodium vivax*. Note the relatively large deformation of the erythrocyte and the formation of Schüffner's dots. Middle row: *Plasmodium malariae*. The ribbon shape of the trophozoite (does not always occur) and the shape of the schizont ("daisy") is typical. Bottom row: *Plasmodium falciparum*. The delicate, relatively small ring shapes and the banana-shaped gametocytes are characteristic here. Maurer's clefts are shown in the older signet ring stages and in the young trophozoites. Note that only rings and gametocytes are usually found in the blood smear. *P. ovale* is not shown because the stages have the same appearance as those of *P. vivax*. (Courtesy of Bayer AG Leverkusen, from *The Microscopic Diagnosis of Tropical Diseases* (1955).)

30–70 µm. The **merozoites** resulting from the liver and blood schizogonies are about 1 µm in length and oval in shape. After the invasion of erythrocytes, the parasite initially consists of a plasma bubble with a central food vacuole and a marginal, easily dyeable nucleus. The developing blood stages have species-specific characteristics, based on which they can be differentiated by experienced diagnosticians (Figure 2.62). The microscopic image of the young erythrocyte stage has a shape like a signet ring in Giemsa-stained preparations and is therefore called **ring stage**. As the amount of plasma increases, the ring stages become round or ribbon-shaped, and are refered to as **trophozoites**, from which the **schizont** and subsequently the merozoites develop. The malaria pigment hemozoin, an insoluble degradation product of hemoglobin (Figure 2.62), lies in the food vacuole of the parasite. Small dots of pigment are also scattered through the cytoplasm of infected erythrocytes. Most species of **gametocytes** have a round shape, but in the case of *P. falciparum*, they are crescent-shaped. The ookinetes reach a length of between 18 and 24 µm. The oocysts grow to a size of 80 µm, and are surrounded by a thin layer of fibrillar material.

Genome The sequenced strain 3D7 of *P. falciparum* strain has a haploid, extremely AT-rich genome of 23 Mb, organized into 14 chromosomes and the extrachromosomal DNA of the mitochondrion (about 6 Kb) and the apicoplast (35 Kb). The apicoplast genome encodes 30 proteins, as well as tRNAs and some RNAs, but 551 protein-coding genes found in the nucleus are of apicoplast origin.

Overall, 5268 protein-coding genes are present, 54% of which have introns. The chromosomes of *P. falciparum* seem to be relatively unstable, since length polymorphisms of chromosomes and rearrangements and multiplications of genes are often observed, for example, in different strains or after storage in culture. It has been calculated that about 2% of all parasites change their genome in one generation. This high genetic plasticity also favors antigenic variation and the development of drug resistance. The localization of genes showed that certain classes of PfEMP1 genes and genes that encode for other variable antigen genes of *P. falciparum* lie mainly near the telomere. These regions at the chromosome ends exhibit unusual repetitive sequences that may facilitate recombination and thus lead to increased genetic flexibility and facilitate the creation of antigenic variants and immune evasion.

Cell Biology The invasion of liver cells by *Plasmodium* sporozoites is far more complex than previously assumed. It has been shown that the sporozoites exit blood capillaries by actively invading liver macrophages interspersed throughout the endothelium. They migrate through these "Kupffer stellate cells," to reach the liver parenchyma cells. However, before commencing schizogony in a hepatocyte, they first transmigrate through, several parenchyma cells (without a parasitophorous vacuole being formed) until they finally settle in a cell by forming a vacuole, and schizogony occurs.

The recognition and invasion mechanisms of erythrocytes by merozoites (Figure 2.63) are comparable to those of *T. gondii* tachyzoites, although the erythrocyte as a host cell without a nucleus is in many respects unique. The merozoites presumably bind to glycophorin on the erythrocyte surface. They then induce the formation of a parasitophorous vacuole, which is increased and modified by the incorporation of parasite-derived proteins and lipids. *P. falciparum* forms a series of clefts and tubular structures known as Maurer's clefts within infected erythrocytes, which are used for export of proteins by the parasite to the erythrocyte surface. These transported proteins contain a particular sorting motif, PEXEL, near their N-terminus. At the surface, such proteins of *P. falciparum* interact with the erythrocyte cytoskeleton and form knobs, which are a scaffold for adhesion proteins mediating cytoadherence (see Box 2.4).

The parasite ingests host cell hemoglobin through the micropore into its food vacuole and obtains amino acids through degradation of the protein by specific proteases. The toxic heme group remains as nonrecyclable residual matter and is converted, together with proteins and lipids, into hemozoin, an insoluble and therefore nontoxic product. This is deposited in the food vacuole in the form of brown crystalline material ("malaria pigment"). After the breakup of the infected erythrocytes, the malaria pigment is ingested by macrophages, which then take on a dark appearance. Inhibition of the heme polymerization by the drug chloroquine and other quinolines kills the parasites. Hemozoin has been implicated in both immune activation through TLR9 and activation of Nalp2 inflammasome, and immune suppression through alteration of dendritic cell and macrophage functions.

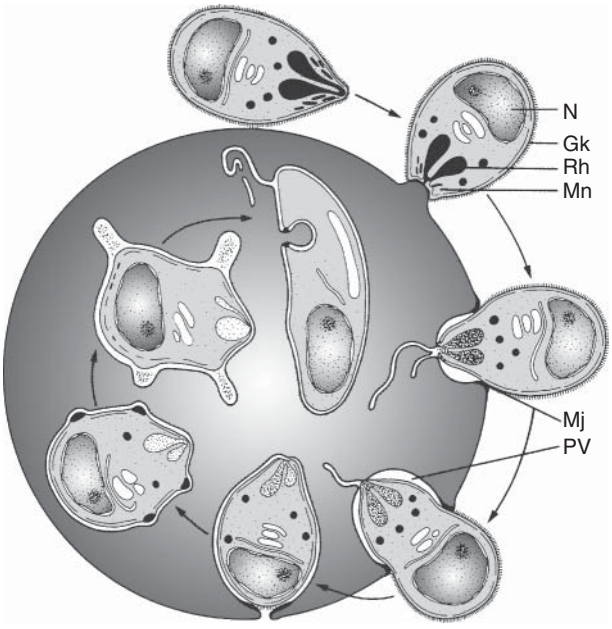


Figure 2.63 Steps in the invasion of an erythrocyte by a *Plasmodium knowlesi* merozoite. Gk, Glycocalyx; mj, *moving junction*; Mn, micronemes; N, nucleus; PV, parasitophorous vacuole; Rh, rhoptries. From Hausmann, Hülsmann, Radeck (2003) Protistology, with permission from Scheizerbart'sche Verlagshandlung, Stuttgart, www.scheizerbart.de.

History and Significance Malaria is one of the most important infectious diseases, endemic in over 100 countries (Figure 2.65). It is estimated in 2015 that 3.2 billion people were exposed to the risk of malaria infection and that 214 million humans suffered malaria attacks every year. About 438 000 persons, mostly children in sub-Saharan Africa, were estimated to annually die from the infection (http://www.who.int/mediacentre/factsheets/fs094/en/). People who acquire infection in an endemic area will become ill after returning to their home country (imported infection). For example, the number of malaria-infected people entering Germany is approximately 1000 per year, of which approximately 20 die. Human pathogenic Plasmodium species are transmitted by female mosquitoes of the genus *Anopheles*, which comprises 450 species. About 70 species of these are important hosts (see also Arthropods, family Culicidae). These species require different types of breeding waters, so Anopheles occurs in almost all habitats. This indicates that malaria can be transmitted in all areas, in which the minimum temperature necessary for the development of Plasmodium in the mosquito is reached. Many parts of the world have such conditions. Altitudes above 1500 m in temperate latitudes and 2500 m in tropical latitudes are usually malaria-free because the temperatures fall below the minimum. The actual distribution of *P. falciparum* and *P. vivax* is shown in Figure 2.65.

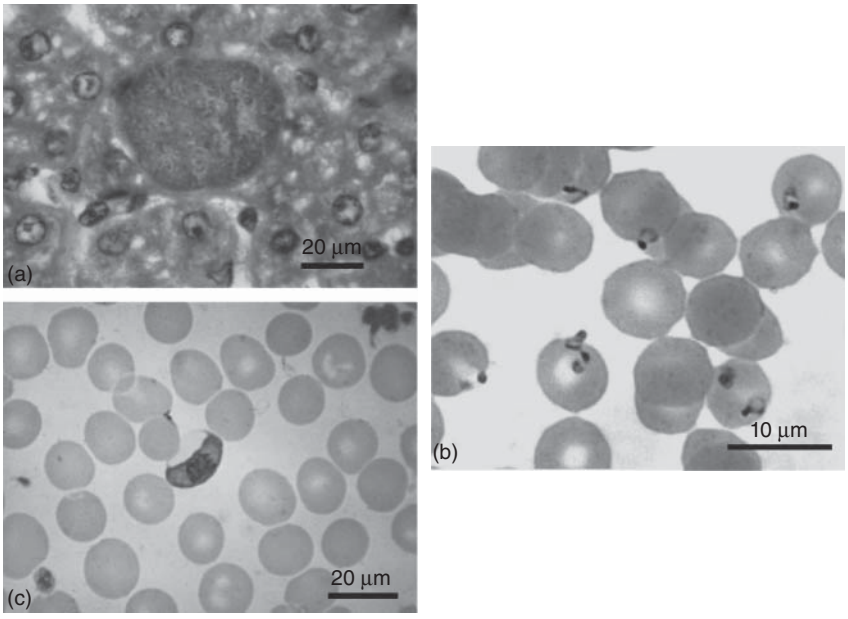


Figure 2.64 Stages of *Plasmodium falciparum*. (a) Liver schizont in a histological section. (b) Ring stages in the blood. (c) Gametocyte in a blood smear. (Images: Archive of the Department of Parasitology, University of Hohenheim.)

Since malaria has been eradicated in wide parts of the world, it is not always appreciated what an extraordinary influence the infection had had on the course of global history. It is claimed that Rome's swampy environment protected the city from attackers much better than the weapons of its defenders and that the fighting forces of numerous campaigns of Germanic tribes and medieval emperors were substantially reduced by malaria before they reached Rome. During World War II in Southeast Asia, the United States allegedly lost more soldiers to malaria infections than through enemy action. Malaria was a major problem for the United States in the Vietnam War. Malaria has also had a lasting impact on the human genome as the disease exerts strong evolutionary pressure. Consequently, certain genetic diseases and polymorphisms that provide protection against malaria were able to spread in endemic areas, and can still be frequently observed there (see Section 1.4.4).

Even in ancient times, the link between malaria and stagnant waters was known – but no one guessed the role mosquitoes played as vectors. The infection was attributed to the bad air that hung over the marshes, hence the name "swamp fever" (Italian: *mal'aria* = bad air). A long time passed before effective drugs against the infection were discovered. The introduction of Cinchona bark from Peru in the early seventeenth century and the extraction of quinine from the bark of the *Cinchona officinalis* tree (fever bark) were important milestones in the treatment of malaria. Chloroquine, the first widely applied synthetic antimalarial drug was synthesized in 1934 by Andersag, a German chemist. Unlike almost

Figure 2.65 Distribution of *Plasmodium vivax* and *Plasmodium falciparum*. (From Global Malaria Mapper 2016, with kind permission.)

any other infection or topic, malaria has preoccupied generations of scientists, ever since the French colonial doctor Laveran first described *P. falciparum* stages in the blood of an Algerian soldier in 1878. In 1897, Ronald Ross, a Scottish doctor seconded to India discovered that oocysts actually formed in *Anopheles stephensi* after it had fed on human blood. One year later, he discovered that the transmission of sporozoites of *P. relictum* triggered infections in sparrows. Similar observations were made almost simultaneously by Italian scientists. It was only in 1948 that Short and Garnham described the exoerythrocytic phase of Plasmodium. Only in 1951 the USA were declared to be free of malaria, and it look until 1974 to eradicate the parasite in Greece.

2.6.3.2 *Plasmodium vivax*, a Causative Agent of Tertian Malaria

P. vivax (Latin: *vivax* = long-lived) causes a form of malaria characterized by recurrent fever, which attacks every 48 h and is sometimes referred to as tertian malaria (Figure 2.66). One feature of the life cycle of *P. vivax* is the occurrence of hypnozoites, which can remain dormant in the liver for several months or even years after the initial infection before becoming reactivated to cause malaria attacks. The merozoites preferentially infect immature erythrocytes (reticulocytes), therefore a maximum of about 2% of the cells are infected. Infected erythrocytes are slightly larger and possess pigment deposits ("Schüffner's dots"). Schizogony usually results in 12–16 merozoites. The gametocytes are round and appear as early as 3–5 days after the first attacks of fever.

P. vivax is the most widely distributed human pathogenic *Plasmodium* and causes about 43% of all cases of malaria. Since the sporogony of *P. vivax* takes place in *Anopheles* mosquitoes at a relatively low temperature, the 16 °C summer isotherm (which, e.g., includes parts of Siberia) is the outermost limit of distribution. The main distribution areas lie within the 25 °C summer isotherm, which runs, for example, through Central Europe. Tertian malaria was once widespread

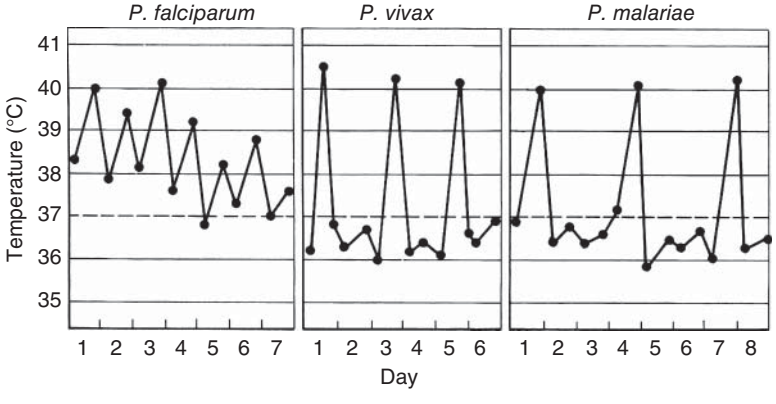


Figure 2.66 Types of fever in Malaria tropica, tertiana und malariae. (From W. Lang (1993), *Tropenmedizin in Klinik und Praxis*; by kind permission of Georg Thieme, Publishers, Stuttgart.)

in Europe. For example, in Strasbourg France, in the eighteenth century, 20% of all patients in the city hospital were infected with malaria, and in warm summers this figure rose rapidly to 70% with the onset of the Rhine valley floods. There is also evidence of former indigenous incidence of malaria in the North of Germany, in areas around Berlin, for instance. On the Upper Rhine, malaria only subsided after the straightening of the Rhine in the first half of the nineteenth century, an act, which led to a drastic reduction of the groundwater level. The elimination of *Anopheles* breeding grounds together with improved hygiene led to the disappearance of malaria. Although, *P. vivax* is generally not considered fatal, in the United Kingdom, 8209 malaria-related death were reported between 1840 and 1910, suggesting that certain cofactors present in the population increase susceptibility. Again, changes to the environment contributed to elimination of the mosquito and thus malaria.

The tertian malaria caused by *P. vivax* is not life-threatening, but is a serious illness. The erythrocytic forms have a synchronized growth with a development time of 48 h, so the fever attacks occur every other day. According to the ("inclusive") Roman way of counting, the day of the first attack of fever was the first day, the subsequent afebrile was the second day and the day of the next attack counted as the third day, and this explains the origin of the term "tertian" malaria. The fever attacks often begin unnoticed, but lead to extremely high temperatures (40–41 °C) and severe chills and lasts for 6–12 h. Left untreated, the disease is healed after 12–15 such attacks. However, the hypnozoites become reactivated to cause more fever attacks after several months or years causing repeated bouts malaria.

2.6.3.3 *Plasmodium ovale*, a Causative Agent of Tertian Malaria

P. ovale causes a relatively rare infection in tropical Africa and has many characteristics in common with *P. vivax*, including the occurrence of hypnozoites. Erythrocytes are enlarged by the infection and become unstable; they are seen to be drawn out and lengthened in blood smear preparations, which gives them their eponymous oval shape. The gametocytes are round-shaped. The duration of schizogony is 48 h in common with *P. vivax* infections, but disease tends to be more benign.

2.6.3.4 *Plasmodium malariae*, the Causative Agent of Quartan Malaria

Merozoites of *P. malariae* only infect mature erythrocytes and consequently less than 1% of the blood cells are infected. The trophozoites can assume a typical ribbon-like shape. The malaria pigment consists of finely distributed granules ("Ziemann's dots"). The immature merozoites (usually eight) are uniformly arranged at the periphery within the schizonts, resulting in a regular structure also known as a "daisy." Neither the schizonts nor the round gametocytes (which are usually formed only several weeks after the first fever attacks) significantly deform the erythrocytes.

The duration of the schizogony is 72 h, resulting in a quartan malaria infection. The fever and the entire course of malaria are somewhat milder than in the case of

a *P. vivax* infection, but the disease lasts longer. One frequently described complication of Quartan malaria is kidney complications caused by immune reactions. Although hypnozoites do not occur with *P. malariae*, a relapse may occur up to 50 years after the initial infection. These relapses are attributed to occult blood forms. *P. malariae* causes 3% of all cases of malaria and is mainly prevalent in West and East Africa and parts of India.

2.6.3.5 *Plasmodium falciparum*, the Causative Agent of Malignant Tertian Malaria or Malaria tropica

Merozoites of *P. falciparum* (Latin *falx*, falcis = the sickle, *parere* = give birth) infect erythrocytes of all ages, and this is why nearly 50% of the blood cells can be parasitized. It has been calculated that 10×12 parasites can occur in a patient, coresponding to 500 g of biomass. The signet ring stages of *P. falciparum* are relatively small (Figures 2.62 and 2.64). The schizonts usually split into 8–12 merozoites. The gametocytes of *P. falciparum* are diagnostically crescent-shaped. The duration of the schizogony in *P. falciparum* is 48 h; however, growth is not synchronized as it is in the case of the other malarial pathogens. *P. falciparum* infection consequently causes a usually high fever, but without the regular bouts of fever. Afebrile infections ("algide malaria") also occur. One indication of a chronic malaria infection is a severe swelling of the spleen up to 20 times its normal size (Figure 2.67). This condition, known as splenomegaly, can be detected by manually feeling the spleen and it can be used in epidemiological studies as a simple diagnostic feature.

P. falciparum is mainly distributed in the tropics, as its development in the mosquito requires a relatively high temperature, but it was previously present in some regions of Europe (e.g., in Hungary). Tropical malaria is the most common

Figure 2.67 Children with splenomegaly caused by Malaria tropica – the photo was taken in a West African village. The second child from the left has an umbilical hernia, which was not caused by malaria. (Image: R. Lucius.)

Plasmodium infection of humans and represents 50% of all malaria cases. It is one of the most dreaded tropical diseases due to its often fatal outcome. Increasing resistance to current drugs has made the ongoing development of new drugs for prophylaxis and new therapeutics necessary. Travellers intending to go to the tropics must therefore find out about the spread of malaria in the target area and take specific prophylactic drugs as directed by a doctor qualified in the treatment of tropical diseases. In the 1950s and 1960s, large-scale control of *Anopheles* by insecticide spraying almost succeeded in eradicating malaria in many areas. However, when insecticide resistance developed in the mosquito populations, malaria spread once again, for example, in India.

Blood stages of *P. falciparum* can be cultivated *in vitro* at low oxygen levels. The discoverers of this method (*Candle Jar* technology) kept their cultures in airtight glass containers, in which a candle was lit. When the candle was extinguished, the oxygen content was optimal for the parasites. The development of this *in vitro* culture method greatly facilitated the study of this pathogen.

Pathology *P. falciparum* is the only species of human pathogenic Plasmodium species that leads to "severe malaria," a syndrome among others triggered by blocking of capillaries by infected erythrocytes. Blocking occurs through adherence of infected red blood cells to endothelial cells ("cytoadherence") and clumping of infected and noninfected erythrocytes (Figure 2.68). Adherence and

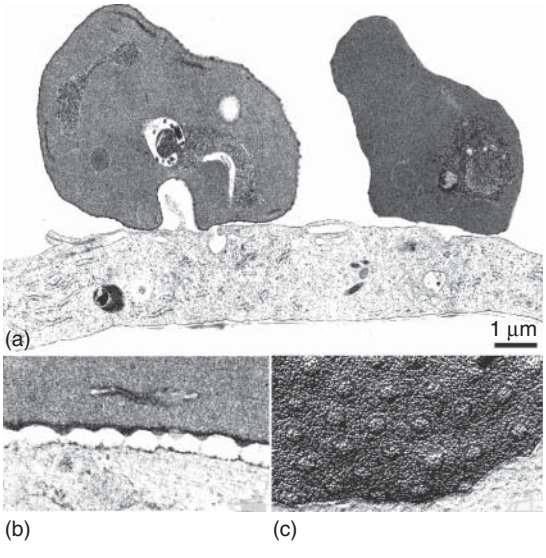


Figure 2.68 Cytoadherence of *Plasmodium falciparum*-infected erythrocytes. (a) Two erythrocytes adhere to an endothelial cell (EM image). (b) Higher resolution of (a). Note the strands of PfEMP1 between the knobs of the erythrocyte and the membrane of the endothelial cell. (c) SEM image of knobs on the surface of an infected erythrocyte. (Images: Courtesy of D. Ferguson, contributed by P. Horrocks *et al.* (2005) J. Cell Sci. 118: 2507–2518.)

clumping is mediated by the parasite protein PfEMP1, which is located in knobs at the erythrocyte surface (see Box 2.4). Binding to the endothelium of capillaries prevents older trophozoites and schizonts from being filtered out and destroyed by the spleen.

Adherence of infected erythrocytes to endothelial cells comprising the capillaries of the brain, heart, lungs, kidneys, and visceral organs leads to reduction of blood flow or interruption of the microcirculation, entailing hypoxia, edemas, and hemorrhages in affected tissues of various organs (Figure 2.69). The placenta is particularly affected in pregnant women. A very severe form of tropical malaria is cerebral malaria, where cytoadherence occurs in capillaries of the brain and results in coordination disorders, confusion, paralysis, coma, and death (see Box 2.4). Damage to other organs can lead to pulmonary edemas, renal dysfunction, diarrhea, and pain. A mass infection of erythrocytes causes hemolysis and consequently anemia, reducing the transport capacity for O_2. Pathogenicity is further strengthened by acidification of the blood due to the high CO_2 content ("acidosis") and by metabolic stress. Together, these factors can result in the overall clinical picture of "severe malaria," which is very often fatal. Tropical malaria in pregnant women often leads to miscarriages and death.

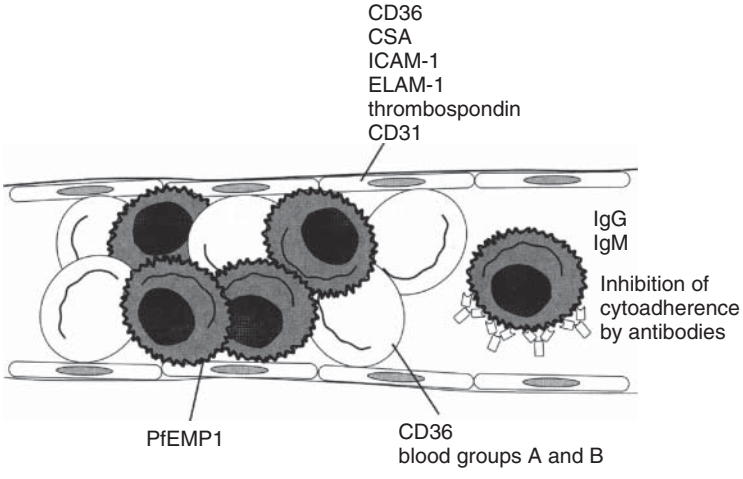


Figure 2.69 Schematic representation of cytoadherence of *Plasmodium falciparum*-infected erythrocytes. *Gray*-highlighted erythrocytes are infected. They express PfEMP1 proteins, which bind to different ligands on endothelial cells, erythrocytes, and/or IgM and IgG in the plasma. Specific antibodies against PfEMP1 can block this binding process, resulting in clinical immunity. PfEMP1, Plasmodium falciparum erythrocyte membrane protein 1; CD36, CSA, ICAM-1, ELAM-1, thrombospondin and CD31 are cell surface components. (Adapted from Schlichtherle *et al.* (1996) Parasitology Today 12, 329–332.)

Box 2.4 Cytoadherence and Antigen Variation in *P. falciparum*

The peripheral blood of patients infected with *P. falciparum* contains ring stages, young schizonts and gametocytes, while erythrocytes with mature trophozoites bind the endothelial cells of capillary walls, thus escaping filtration through the spleen. Binding is mediated by PfEMP1 (*P. falciparum erythrocyte membrane protein 1*), a member of the *var* gene family of adhesion proteins. This parasite protein is expressed on the surface of infected cells and interacts with host adhesion molecules, including CD36 and ICAM1, on endothelial cells, macrophages, erythrocytes, and other cells. PfEMP1 is embedded in protrusions, termed *knobs*, that are formed by parasite-encoded scaffold proteins on the surface of infected cells, from where its N-terminus sticks out. The genome of the sequenced *P. falciparum* 3D7 strain bears 59 PfEMP1 variants, with a molecular weight ranging between 200 and 300 kDa.

Each *P. falciparum* clone expresses only one particular PfEMP1 gene variant at a given time point. Each PfEMP1 variant has a conserved transmembrane domain and a highly variable region composed of several modules that enables it to bind to different ligands on the surface of host's endothelial cells. Erythrocytes infected with PfEMP1 types that primarily bind to ICAM1 preferentially locate to capillaries of the brain, while other variants preferentially bind chondroitin sulfate on endothelial cells of the placenta (Figure 1.44). Many clones have the capacity to bind to CD36, a receptor on endothelial cells and macrophages. When triggered by the engagement of CD36, macrophages release IL-10, resulting in suppression of inflammatory responses. Antibodies produced by the host can block PfEMP1 binding to host ligands. Consequently, the infected erythrocytes are no longer adherent and are eliminated in the spleen. The parasite can evade the antibody response through expressing a different PfEMP1 protein in a process termed antigen variation. During the course of an infection, the PfEMP1 proteins of a clone therefore switch sequentially within a period of weeks. A patient can simultaneously harbor a number of different clones and consequently has to contend with a complex and dynamic complement of PfEMP1 variants. Once a patient has acquired antibody responses to the prevalent local PfEMP1 variants, the person has a labile immunity, which prevents most forms of severe malaria, without necessarily stopping parasitaemia.

Evolution Several studies have shown that *P. falciparum* started to spread relatively recently. A study of 35 isolates from a wide range of regions found no silent mutation in 10 genes examined (i.e., base substitutions in the third base pair of a codon). This type of mutation is not under selection pressure and therefore takes place very easily. Their absence has been used to argue that all the tested isolates originated from a single progenitor strain about 10 000 years ago. A more recent study that analyzed full-length mitochondrial sequences from Chimpanzee and

Gorilla *P. falciparum* found that the human parasite is a monophyletic line derived from Gorillas. Detailed studies have shown that *P. falciparum* have evolved from a single strain spread from a Gorilla to infect humans. The timing of this event is unclear, but it has been postulated that a rapid spread occurred about 10 000 years ago, aided by higher temperatures after the last ice age, the increasing population density with the introduction of agriculture and the increasing anthropophily of *Anopheles* mosquitoes. Irrespective of the exact timing, the hypothesis that *P. falciparum* is a relatively new parasite of humans is consistent with its high pathogenicity compared with other human well-adapted Plasmodium species that have affected humans for longer.

Immunobiology Malaria is estimated to have caused the deaths of 627 000 persons, mainly children in Africa in 2012. In those that survive, repeated infections with *Plasmodium*, creates an unstable immunity that is maintained by constant exposure to infections. Most adults living in malaria-endemic areas have a degree of immunity that protects them from the clinical manifestations of disease (Figure 2.70). The symptoms of a malaria infection are usually similar to those of flu for these immune individuals. This level of immunity is reduced, however, if stress levels are high (caused by, for example, other infections or pregnancy). Infection during pregnancy can cause maternal death, low birth weight, miscarriage, or fetal death. The level of immunity usually fluctuates where seasonal transmission is the norm. Immunity can be parasite strain-specific, so an infection with "foreign" strains can result in infection.

Protective immunity to *Plasmodium* is stage-specific and dependent on many aspects of the immune response. *Plasmodium* induces a strong innate immune

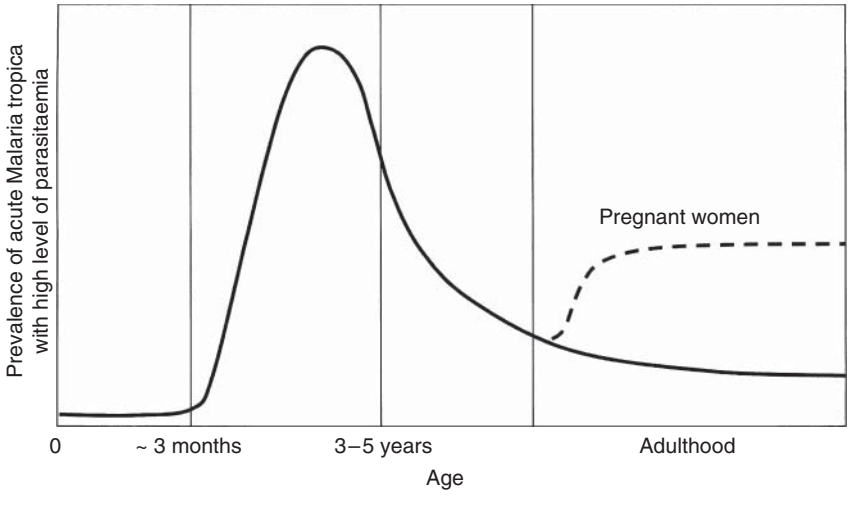


Figure 2.70 Prevalence of malaria in different age-groups changed. (Adapted from Wyler, D.J. (1990) *Modern Parasite Biology*, W. H. Freeman & Company, New York.)

response due to the presence of TLR agonists such as GPI-anchors that bind TLR2 and TLR4. This induces inflammatory mediators, including TNF-α, IL-6, and nitric oxide, that contribute to both protection and pathology, which is an active area of research. $CD4^+$ T helper cells contribute to protection by production of IFN-γ that augments nitric oxide production. $CD4^+$ T cells also provide support to antibody production. These antibodies are stage-specific and act primarily by binding sporozoites and merozoites and preventing them from entering their target cells. Studies have demonstrated that passive transfer of antibodies from the serum of immune individuals protects children from severe malaria. Antibodies can also bind gametocytes, which does not directly help the host, but might disrupt the life cycle of the parasite as it is entering the mosquito. $CD4^+$ T cells are also required for providing IL-2 for the successful development of cytotoxic T cells that are effective against the infected hepatocytes. The existence of such protective immune responses and the fact that vaccination of volunteers with attenuated sporozoites induces a long-lasting immunity would suggest that the development of malaria vaccines is feasible. In spite of a wide range of different approaches, however, there are currently only a few experimental vaccines in clinical trials (see Box 2.6).

Plasmodium parasites have developed efficient defense strategies against such immune responses. The intracellular localization in erythrocytes is seen as an important part of immune evasion. The parasites are inaccessible to antibodies when they are inside the erythrocytes. Erythrocytes do not express MHC I proteins and are consequently not targeted by $CD8^+$ cytotoxic T cells. One very important principle of immune evasion used by *P. falciparum* is to avoid the spleen passage by means of cytoadherence (see above). The variable surface antigen PfEMP1, which is expressed on the surface of infected erythrocytes, plays a central role in this process by binding a number of different ligands, for example CD36 and ICAM-1 CD36 and ICAM-1 on vascular endothelial cells (see Box 2.4 Cytoadherence and Antigen Variation in *P. falciparum*). *P. falciparum* causes polyclonal B lymphocyte activation and this might function as another form of immune evasion by disorienting the immune system. Plasmodium parasites also create large amounts of immunodominant antigens that induce irrelevant antibodies, while relevant epitopes are only partially and minimally immunogenic (see Box 2.5, "Plasmodium antigens"). The infection also induces a marked immunosuppression that could cause malaria patients to respond more poorly to vaccination after acute attacks.

Box 2.5 Plasmodium Antigens

Many proteins of *P. falciparum* have been cloned and molecularly characterized as part of vaccine research programs. One of the most intensively studied is the circumsporozoite protein (CSP), which forms a homogeneous layer over the surface of the sporozoite. In common with many other proteins of *P. falciparum*, the 35-kDa protein has repetitive regions. The C-terminus of CSP bears a

hydrophobic anchor sequence with a GPI-anchor addition signal that mediates insertion into the surface of the sporozoite. This anchor sequence is followed by a highly conserved domain, which functions as a receptor of the parasite for ligands of the liver cell surface. Following is a region in the center of the protein consisting of 41 repeats of the tetrapeptide NANP, which induce strong antibody responses (four of these repeats vary slightly). The N-terminus bears a variable region that is thought to mediate the initial contact of sporozoites with the cell and the signal peptide (Figure B2.4). CSPs of other *Plasmodium* species also have a similar structure. Other proteins of *P. falciparum* can have up to several hundred repeats. For example, the S-antigen produced by schizonts and released into the parasitophorous vacuole has 100 repeats of an 11 amino acid sequence. Although the DNA sequences of these proteins are not homologous, the encoded proteins have structural similarities and induce strong, cross-reactive antibody responses, which, however, do not protect the host. It has been postulated that the formation of irrelevant antibodies distracts the immune system from the production of protective antibody responses.

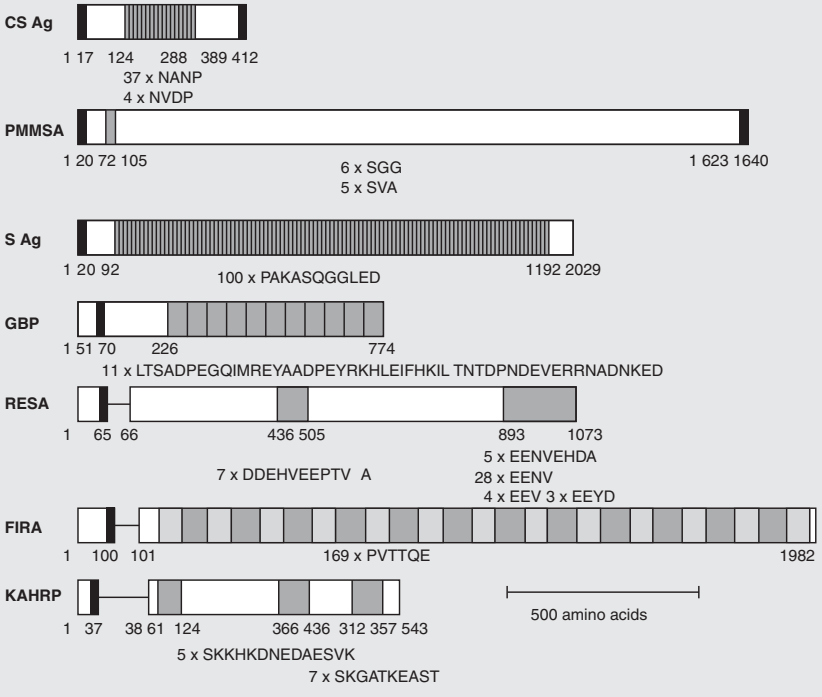


Figure B2.4 Gene structure of various *Plasmodium falciparum* proteins. Dark areas: repetitive sequences. Black boxes: Signal sequences or hydrophobic anchor sequences. (Adapted from Kemp, D.J., Coppel, R.L., and Anders, R.F. (1987). *Annu. Rev. Microbiol.*, **41**, 181–208.)

Box 2.6 The Long Road Toward a Malaria Vaccine

by Kai Matuschewski, Humboldt Universität zu Berlin.

Despite decades of efforts to accelerate the development of promising malaria vaccine candidates, a safe, affordable, accessible, and lasting vaccine with a protective efficacy of more than 80% against clinical disease remains elusive. Malaria vaccine research largely benefits from the possibility to infect immunized volunteers, an approach termed controlled human malaria infection (CHMI), because individuals can be closely monitored and treated with a licensed antimalarial drug upon microscopic detection of parasites in the peripheral blood. This approach, which cannot be applied to the two other global public health threats, HIV/AIDS and tuberculosis, allows early selection of vaccine candidates for larger clinical trials. Thus far, only two malaria subunit vaccine formulations, termed SPf66 and RTS,S/AS01, have entered advanced (phase III) clinical testing. Both formulations were safe and immunogenic and reduced the risk of clinical malaria, albeit only partially and lasting only for a few months.

In order to advance malaria vaccine discovery and meet the goal of licensing an efficacious prophylactic vaccine in the next decade, two promising complementary approaches are being pursued. A multigene, multistage combinatorial subunit vaccine is expected to build on the partial success of RTS,S/AS01. The latter formulation has been iteratively optimized over a course of 25 years and is able to elicit the highest antibody responses in vaccine history, largely because of the potent proprietory adjuvant (AS01). Vaccine-induced antibodies target the major surface protein of *P. falciparum* sporozoites, termed CSP. However, antibody titres do not correlate with partial efficacy, underlining the need for systematic immunological profiling toward the identification of correlates of protection. A next-generation subunit vaccine will likely include additional target antigens of the asexual parasite replication cycle in host erythrocytes and ookinetes that colonize the *Anopheles* vector. Major roadblocks include the identification of invariant antigens on the surface of the parasite or the infected erythrocytes, evidence for robust inhibition of parasite-stage conversion by standardized assays, and compatibility with the present formulation.

In perfect agreement with the complex *Plasmodium* biology and the striking slow natural acquisition of partial antidisease immunity in malaria-endemic countries, the first and most effective experimental malaria vaccines were live-attenuated sporozoites. Impressive results in human volunteer studies have been obtained with radiation-attenuated *P. falciparum* sporozoites and unaltered sporozoites under simultaneous chemoprophylaxis, such as chloroquine suppressive treatment. The original discoveries were made in murine malaria models and reproduced exceptionally well in human volunteer studies. Vaccine efficacy often reaches complete sterile protection

and correlates with effector memory T cells, indicating that the parasite population expansion in the liver is the major target of protective immunity. Accordingly, vaccination with live attenuated, metabolically active *P. falciparum* sporozoites remains the benchmark for experimental vaccine research. This approach requires hand dissection and purification of sporozoites from infected *Anopheles* mosquitoes, subsequent storage in liquid nitrogen, and intravenous injection, thus representing major, if not insurmountable, disadvantages for deployment in malaria-endemic areas. Substantial research and bioengineering investments, such as axenic sporozoite culture systems and proof of efficacy by the intramuscular route, are necessary before whole sporozoites can be translated into a licensed product for infants and children in sub-Saharan Africa. A plausible way forward is a global effort to gain a better molecular and cellular understanding of how attenuated sporozoites achieve superior protection not seen in nature, and to design a first evidence-based multi-epitope formulation that closely mimics a complex parasitic infection.

A vaccine against *P. vivax* is considered by many to be more feasible because of the temporary nature blood infection by this parasite. However, periodical reactivation of hypnozoites in the liver adds another level of complexity, suggesting that a *vivax* malaria vaccine needs to be 100% efficient during the pre-erythrocytic phase of the infection. Since inclusion of a malaria vaccine into the Expanded Programme on Immunization (EPI) of the WHO in Africa remains a distant vision, global access to the existing malaria control tools, that is, vector-control programs, exposure prophylaxis (for example through insecticide treated bednets), and rapid malaria diagnosis and treatment, remains a public health priority.

The relatively slow buildup of protective immune responses results in children in endemic areas being highly vulnerable to malaria. In some regions, up to 50% of infant mortality is due to Malaria tropica. Partial protection against infection usually exists in the first year of an infant's life, which is due to several factors. In the first months, children still exhibit a proportion of fetal hemoglobin, which *Plasmodium* cannot exploit efficiently; and protective IgG antibodies transferred transplacentally from the mother may also have a beneficial effect. These protective mechanisms are no longer effective after small children have been weaned, so the children are at great risk until they have built up their own immune responses to the parasites. They must now develop efficient antibody responses to the local *P. falciparum* strain's repertoire of PfEMP1 proteins and other parasite antigens of sporozoites, liver and blood stages, a process that can take approximately 5 years in children (Figure 2.70).

High mortality rates for infected people indicate that *P. falciparum* is an important selection factor. Individuals who develop effective immune responses against certain Plasmodium antigens therefore have a better chance of survival and reproduction. The potential to respond to certain antigens is significantly influenced by genes of the MHC system and certain MHC alleles are associated

with resistance to cerebral malaria and other susceptibility to disease. Other normally disadvantageous genetic factors that reduce the severity of malaria can be a selective advantage in geographical areas, where malaria is prevalent. Examples include genetically acquired hemoglobin abnormalities, including sickle cell anemia and hereditary deficiency of glucose-6-phosphate dehydrogenase. Several polymorphisms in cytokine genes have also been demonstrated to have a protective effect against malaria (see Section 1.4.4).

2.6.3.6 *Plasmodium* species of Monkeys, Rodents, and Birds

Numerous species of Old and New World monkeys are naturally infected with various species of *Plasmodium* and the host parasite relationship can have certain similarities with that of humans. Consequently, these host and parasites have gained importance as animal models in research. *Plasmodium cynomolgi* was first discovered in Macaques monkeys (*Macaca irus*) in Java and its biology has parallels with that of *P. vivax*, including the ability to form hypnozoites. *P. cynomolgi* was exploited in the development of Primaquine, a drug that is effective against hypnozoites. *Plasmodium knowlesi* was isolated from macaques in India, and causes a disease similar to Malaria tropica. The parasite can be transmitted to rhesus monkeys, for example, in which it can cause deadly infections.

Closely related to the human pathogenic Plasmodium parasites ***Plasmodium berghei*** is often used as an animal model for biomedical studies. *P. berghei* is a natural parasite of the gallery forest rat *Grammomys surdaster*, which occurs in the higher regions of Central Africa, but can be used experimentally in a variety of rodents. The susceptibility of different rodent species varies considerably and is somewhat age-dependent. While old rats are resistant to infection, it is fatal in mice and young rats, which develop cerebral malaria. *P. berghei* has therefore been used in many experimental studies and as a teaching tool. The deposition of hemozoin in macrophages of the liver and spleen as well the presence of blood schizonts can be easily demonstrated. ***Plasmodium yoelii***, ***Plasmodium chabaudi,*** and ***Plasmodium vinckei*** are three other species that infect African gallery forest rats and are used as model organisms. Due to its importance, the genome of *P. yoelii* was sequenced at the same time as that of *P. falciparum.* Further genome analyses have uncovered further variable antigen gene families implicated in adhesion in a number of *Plasmodium* species. These include *rif/stevor*, *vir*, *kir*, *cir*, *bir*, and *yir* found in *P. falciparum*, *P. vivax*, *P. knowlesi*, *P.chabaudi*, *P.berghei*, and *P. yoelei*, respectively. These discoveries should facilitate comparative genetics and immunological studies between important human pathogens and the animal models used to understand them.

Plasmodium gallinaceum is the best known species of *Plasmodium* to cause avian malaria. The natural host is jungle fowl in South-East Asia, but the species can be easily transmitted to the domestic and other fowl. It develops in more than 30 species of different mosquito genera (e.g., *Culex*, *Aedes*, and *Anopheles*). *Aedes aegypti*, which is relatively easy to maintain, is the best experimental intermediate host. Deviating from the biology of human-pathogenic species *P. gallinaceum* sporozoites invade macrophages in the skin (instead of liver cells), where about 200 merozoites form from one sporozoite within 48 h. These

infect more macrophages to produce second-generation merozoites that infect erythrocytes to initiate blood schizogony. In parallel with blood schizogony, exoerythrocytic schizogony occurs in capillary endothelial cells of various organs, leading to the formation of gametocytes. In their natural hosts, the disease is mild, but chickens can die if infected. ***Plasmodium relictum*** is another avian parasite that affects 10–20% of sparrows in Central Europe. *Culex* mosquitoes are responsible for transmission. Avian Plasmodium species have been used as models of mammalian infection and have played an important role in increasing our understanding of the cell biology of this group of parasites.

2.6.4 Piroplasms

The Piroplasmida order includes the *Babesiidae* and *Theileriidae* families, which infect mammals and exploit hard ticks (see Chapter 4.1.1.2) as final hosts and vectors. Erythrocytes or lymphocytes are exploited for schizogony. They derive the name from the erythrocytic, **pear-shaped mitotic forms** (Latin: *pirum* = pear, *plásma* = entity, body). Unlike many other members of the Apicomplexa, they do not form a parasitophorous vacuole, but rather lie directly in the plasma of erythrocytes. Unlike Plasmodium, they do not form any type of pigment. Schizogony is atypical as only two or four merozoites develop from infected erythrocytes. The gametocytes were first discovered in gut preparations from ticks fed on animals with *Theileria* by Robert Koch. They are referred to as "ray bodies" and possess two protruding structures that contain microtubules, but lack the structure of flagella. Two ray bodies fuse to form a **kinete,** which is a mobile worm-like stage (this contrasts with oocysts that are formed in other Apicomplexa). These kinetes escape the gut to the hemolymph, where in some species they can invade other organs and replicate. Sporogony occurs in the salvary glands, resulting in infective sporozoites. The kinetes of some species (including *Babesia divergens*) are able to infect the ovaries and eggs of ticks, resulting in vertical transmission of the parasite through generations of ticks. Piroplasms are pathogens of major animal diseases, especially in the tropics, subtropics, and southern Europe.

2.6.4.1 *Babesia*

Babesia parasites are a heterogeneous group of piroplasms with a life cycle alternating between ixodid ticks and vertebrates (Figure 2.71). The process of schizogony and gamogony occurs exclusively in erythrocytes of the vertebrate host. In the tick, some species can be transmitted transovarially to subsequent generations. Babesia infections are of great economical importance for animal husbandry in tropical and subtropical regions, but also in some areas with temperate climate. We introduce two species of significant importance as representatives of the babesias.

Babesia divergens is a common infection in European cattle, where it causes hemoglobinuria also known as "red water fever." The pathogen is transmitted by the castor bean tick *Ixodes ricinus* and piroplasmosis occurs mainly in spring and early autumn when the tick is active.

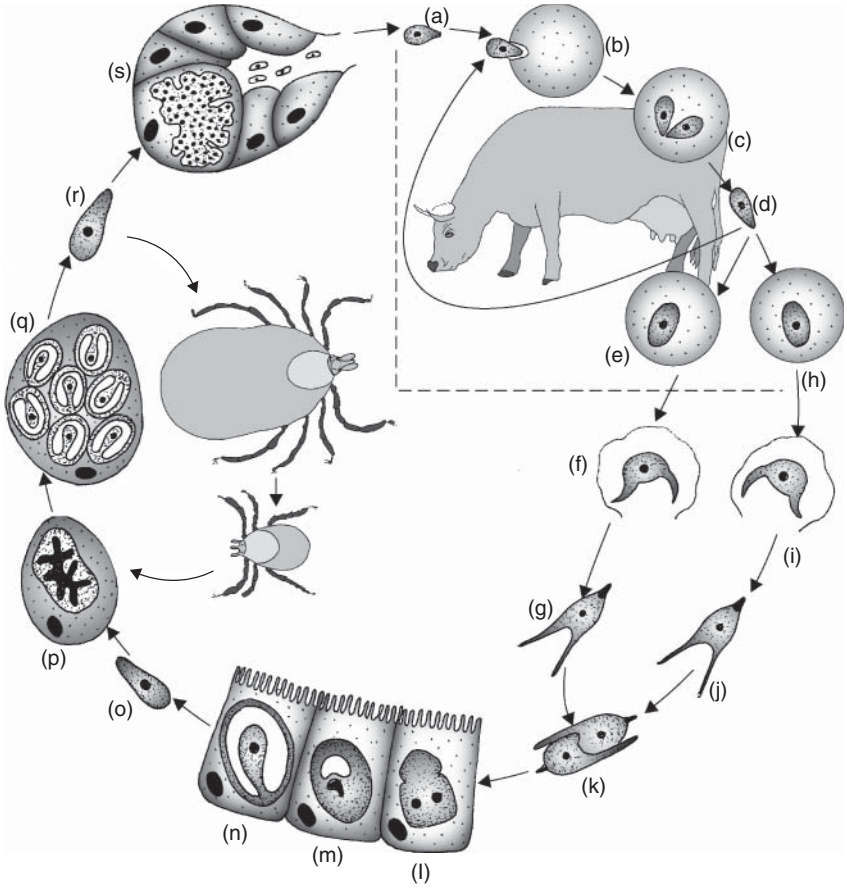


Figure 2.71 Life cycle of *Babesia divergens*. (a) Sporozoite. (b) Invasion of an erythrocyte. (c) Schizogony. (d) Merozoite. (e) Macrogametocyte. (f, g) Ray bodies. (h) Microgametocyte. (i, j) Ray bodies. (k) Fusion of the ray bodies. (l) Zygote. (m, n) Formation of kinete in intestinal epithelial cell of the tick. (o) Kinete. (p, q) Multiple divisions in somatic cells with formation of other kinetes. (r) Kinete. (s) Sporozoite formation in the salivary gland. (Adapted from Mehlhorn, H. (ed.) (1988) Parasitology in Focus, Springer-Verlag, Heidelberg.)

Babesia bovis is an important global pathogen of hemolytic anemia of cattle. It occurs in Southern Europe, Asia, Africa, Australia, and Central and South America. The main vector ticks are species of the genus Boophilus. Transovarial transmission is of particular importance for these one-host ticks, the larvae, nymphs, and adult stages of which live on one individual host, as otherwise the offspring of the parasite would not reach another host.

Development and Morphology The sporozoites of *Babesia* only infect erythrocytes (Figures 2.71 and 2.72). Pear-shaped merozoites of about $1 \times 2\,\mu m$ in size

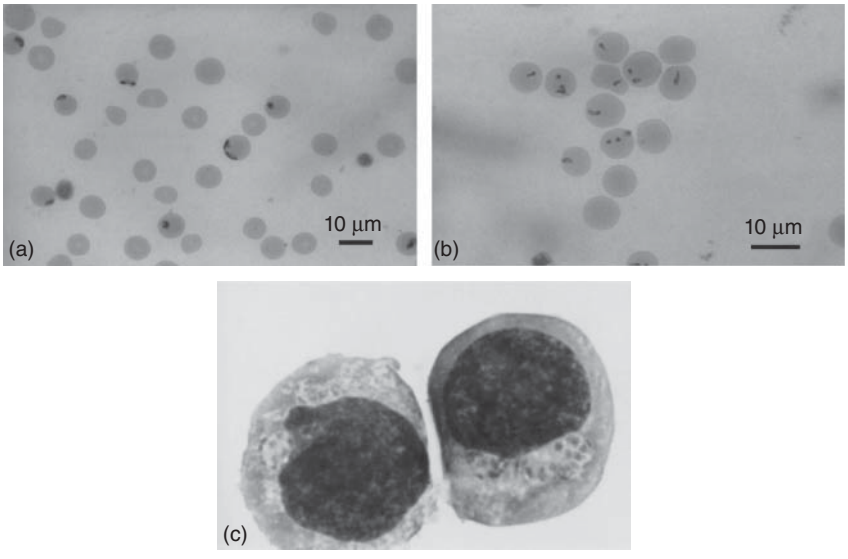


Figure 2.72 Infection of cells with *Babesia* and *Theileria*. (a) Blood smear of a cow with *Babesia divergens* in erythrocytes (by kind permission of H. Mehlhorn). (b) Blood smear with *Theileria parva* in erythrocytes. (Image by Archive of the Department of Parasitology, University of Hohenheim). (c) T lymphocytes infected with schizonts of *Theileria parva*. (Image: Courtesy of D. Dobbelaere.)

are created by schizogony, and they invade more red blood cells. Rounded gametocytes also develop, but their morphology is difficult to distinguish from merozoites. If infected blood is ingested by a tick, the gametocytes differentiate into gametes termed "ray bodies," while they are still within the erythrocytes. The ray bodies have several short, thorn-like appendages. They become free and two ray bodies merge to form the zygote that invades epithelial cells of the tick gut. One single worm-shaped kinete develops within the zygote. This kinete gains access to the hemocoel, where it can invade cells of various organs, including the ovaries. Here multiple divisions occur, creating many kinetes and these in turn undergo more cycles of propagation in different host cells. When a tick starts its blood meal, the kinetes invade the salivary gland cells and several thousand sporozoites are formed. Just one day after attachment, the infectious sporozoites are transmitted to the mammalian host in the tick saliva.

Kinetes that have invaded an egg cell do not disrupt the development of the tick. However, they do eventually form division stages equivalent to kinetes in the intestinal epithelial cells of the resulting tick larva. They can undergo more cycles of division in somatic cells, or alternatively invade cells of the salivary glands to produce sporozoites. The kinetes can also infect oocytes and persist in the developing egg, and finally infect intestinal epithelial cells of the tick larva. This transovarial transmission ensures the transition of *Babesia* from one tick generation to

the next. In this way, *Babesia* can persist in a region, even if there is a temporary shortage of suitable vertebrate hosts.

More *Babesia* species of ruminants, carnivores, and rodents are listed in Table 2.10. One special feature that is worth mentioning is that ***B. divergens***, ***B. bovis***, and ***Babesia microti*** can cause infections in humans in rare cases. There is a risk of this infection being misdiagnosed as malaria. Antimalarial drugs, however, are ineffective against *Babesia*.

Babesiosis Animals infected with *Babesia* usually develop severe anemia, high fever, diarrhea, and hemoglobinuria. The metabolic changes induced by the parasites lead to a hemolysis (even of uninfected erythrocytes), and this results in a drastic reduction in the oxygen-carrying capacity of the blood. The respiratory rate is increased to compensate for this. Clumping and adherence of infected erythrocytes on the endothelial cells of capillaries also lead to interruptions of the microcirculation and to severe shock, both of which can often directly cause death. Antigenic variation has also been documented for *Babesia*, a process that is reliant on variable cytoadherence proteins (VESA1 proteins), which are located in knobs of the erythrocyte membrane. Babesiosis in calves is usually mild, whereas in adult cattle, severe cases associated with a rapid decrease in milk production often occur and can lead to death. This is why some countries have live vaccines available that allow young animals to be immunized prophylactically. Prevention of Babesiosis can be achieved by systematically eradicating the ticks by treating cattle with acaricides.

Immunobiology of *Babesia* Antibody-mediated immune responses seem to play an important role in Babesia-infections, as, since *B. bovis*-infected cattle can be protected by immune sera. Just how this protection works is unclear. The cloning of *Babesia* antigens (identified by their antibody reactivity) revealed highly variable proteins with repetitive sequences, which were able to induce antibody responses but no protection. More recently, proteins from the surface of merozoites and rhoptries were genetically engineered, and these provided protection against challenge infections with *B. bovis*. Therefore, the development of recombinant vaccines against *Babesia* is not without hope of success.

Babesia microti is similar to *Theileria* in some aspects of its biology and is an emerging human pathogen. It has the smallest nuclear genome of all apicomplexans studied to date. It is considerably divergent from other *Babesia* and *Theileria* and represents a distinct clade within the group.

2.6.4.2 *Theileria*

The *Theileria* species cause diseases with occasional but very large losses in domestic ruminants in Africa, Europe, and Southeast Asia (Figure 2.73). Transmission is by two-host or three-host hard ticks (see Table 2.10), but each *Theileria* species has adapted to a specific tick species. Ticks become infected when feeding on infected mammals and following molting transfer sporozoites

Table 2.10 Overview of important Piroplasms.

Species	Intermediate host tick	Disease	Pathogenicity	distribution
Babesia/final host				
Cattle				
Babesia divergens	*Ixodes ricinus*	Red water, Babesiosis in humans	+	Europe
Babesia bovis	*Boophilus* sp., *Ixodes ricinus*	Plague-like hemoglobinuria	+	Worldwide in warm climates
Babesia bigemina	*Boophilus* sp.	Texas fever		Worldwide in tropics and subtropics
Babesia major	*Haemaphysalis* sp.	Babesiosis	−/+	Europe, Africa, South America
Sheep/goat				
Babesia motasi	*Rhipicephalus* sp., *Haemaphysalis* sp.	Babesiosis	+/−	South Europe, Near East, Vietnam
Babesia ovis	*Rhipicephalus* sp.	Babesiosis	−/+	South Europe, Near and Middle East, Africa, South America
Dog				
Babesia canis	*Rhipicephalus* sp., *Haemaphysalis* sp. *Dermacentor* sp.	Babesiosis	+	Worldwide
Rodents				
Babesia microti	*Ixodes* sp.	Babesiosis in humans	+	Worldwide
Theileria/final host				
Cattle				
Theileria parva	*Rhipicephalus* sp.	East Coast, Corridor fever	+	Africa south of Sahara
Theileria annulata	*Hyalomma* sp.	Tropical Theileriosis	+	Mediterranean, North Africa, Near East, and Central Asia
Theileria mutans	*Amblyomma* sp.	−	−	Africa south of Sahara
Sheep, goat				
Theileria ovis	*Rhipicephalus* sp., *Dermacentor* sp. *Ixodes ricinus*	−	−	Europe, Middle East, Asia, Africa
Theileria hirci	*Hyalomma* sp.	malignant Theileriosis	+	South Eastern Europe, Africa, Caucasus, India
Horse				
Theileria equi (formerly *Babesia*)	*Hyalomma* sp., *Rhipicephalus* sp. *Dermacentor* sp.	Theileriosis	+	Worldwide

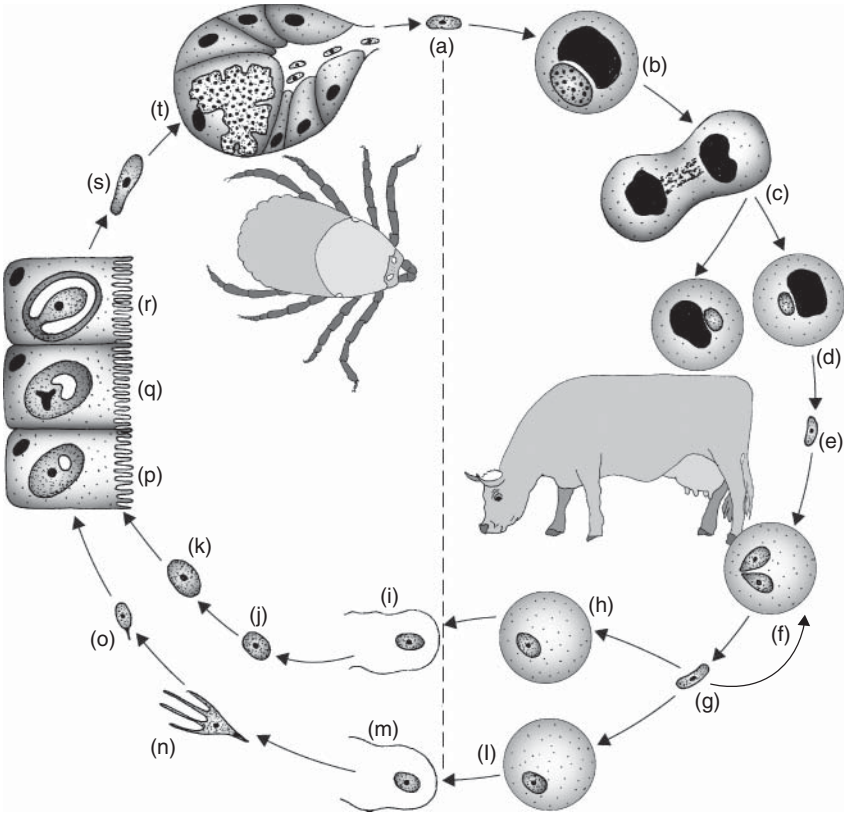


Figure 2.73 Life cycle of *Theileria parva*. (a) Sporozoite. (b) Schizont in a lymphocyte. (c, d) Division of the lymphocyte with simultaneous division of the schizont. (e) Merozoite. (f) Schizogony in the erythrocyte. (g) Merozoite. (h–j) Macrogametocyte. (k) Macrogamete. (l–n) Microgametocyte. (o) Microgamete. (p) Zygote in intestinal epithelial cells of the tick. (q, r) Formation of kinetes. (s) Kinete. (t) Formation of sporozoites in the salivary gland. (Adapted from Mehlhorn, H. (ed.) (1988) Parasitology in Focus, Springer-Verlag, Heidelberg.)

to a new mammalian host during the blood meal. The tick totally rids itself of sporozoites, so no infection takes place the next time blood is taken. In contrast to the *Babesia*, no transovarial transmission to the offspring of the tick is possible.

Theileria parva is the pathogen of East Coast or Corridor fever in cattle in sub-Saharan Africa. Buffalo act as reservoir hosts. The three-host ticks *Rhipicephalus appendiculatus* and *Rhipicephalus zambeziensis* act as vectors. In some areas, East Coast fever is thought to be the costliest disease of cattle in terms of animal loss. Consequently, vast areas where the disease thrives are unsuitable for intensive cattle rearing. Robert Koch, aware of the importance of this, performed studies on the transmission of theileriosis and focussed on the life cycle of the parasite. *T. annulata*, the causative agent of Tropical Theileriosis, was formerly considered

relatively benign, but it turned out that newly introduced European breeds are severely affected by this parasite.

Development and Morphology Within 10 min of being transferred by the tick saliva, the sporozoites invade the lymphocytes, where they free themselves from the parasitophorous vacuole and lie within the cytoplasm, where they develop into schizonts of 10–15 µm in size. The sporozoites preferentially invade T lymphocytes. The schizonts transform the lymphocytes, that is, they stimulate their host cell to divide, while themselves dividing in synchrony (Figure 2.73b). They are associated with the mitotic spindle of the host cell, so that when the host chromosomes are separated during mitosis, the schizont is also distributed to the daughter cells. This mechanism is so efficient that when cultured *in vitro*, more than 95% of cells in cultures can be infected with *T. parva*. Infected lymphocytes, which are capable of actively dividing, are often present en masse in lymph nodes. The parasitized cells are easy to stain and the schizonts that can be seen lying in these cells are known as Koch's bodies. Large macroschizonts with 5–20 large nuclei are observed first and microschizonts with up to 100 comma-shaped merozoites are formed later. At 8–12 days after infection, numerous merozoites measuring 1–2 µm in length develop from the schizonts and infect the erythrocytes (Figure 2.73c.). Up to 40% of the erythrocytes of a host animal may be infected. Round gametocytes also develop in the blood cells.

Ray bodies of 8–12 µm in length first develop from the microgametocytes in the tick gut. The macrogametocytes are round and also give rise to ray bodies. The fusion of the gametes leads to the formation of a mobile zygote that invades an epithelial cell of the tick gut. One single kinete (14–22 µm in length) develops from the zygote. The kinete migrates to the salivary gland and invades follicle cells within. When the tick begins its blood meal, multinucleated cytomeres develop, followed by up to 50 000 small sporozoites that are released with the tick saliva around 3 days after the onset of bloodfeeding.

Genome and Cell Biology Genome sequencing of *Theileria annulata* and *Theileria parva* reveals that they have relatively small genomes of 8 and 8.3 MB with 3.792 and 4.035 protein–encoding genes, respectively, organized in four chromosomes. As in the case of Plasmodia, many genes, which encode for variable proteins, lie in chromosome regions near the telomeres. In the case of *Theileria*, some of these proteins are probably secreted into the host cell cytoplasmand possibly perform a function in the transformation of the host cell.

As primary host cells, sporozoites of *T. parva* infect T lymphocytes and transform them so that they continually divide. The transformation is reversible and is caused by the presence of the parasites. Division of T cells is normally only in response to an antigenic stimulus in the form of peptide presented on the MHC of an antigen-presenting cell with appropriate co-stimulation signals and cytokines. However, *T. parva* reprogram their host cell to divide by interference of multiple signaling pathways.

One major mechanism of T cell activation by *T. parva* is the constitutive activation of the transcription factor nuclear factor kappa B (NF-kB). This protein is a key switch in cell activation. It causes the expression of genes of the cell cycle, the inflammatory immune response, and the inhibition of apoptosis. NF-kB is usually activated after receptor stimulation of the cell by cytokines or other ligands. The constitutive activation of NF-kB is achieved by recruiting I kappa B kinase (IKK) protein complex to the surface of the *T. parva* schizonts, where it is permanently activated. IKK then degrades two inhibitors of the transcription factor, after which NF-kB can migrate to the nucleus and cause the simultaneous transcription of several genes. Another activation is apparently achieved by a parasite enzyme, called peptidyl-prolyl-isomerase (PPIase), which binds to an ubiquitin ligase of the host cell. This ligase controls the levels of cJun, an important enzyme in a major pathway leading to cell proliferation. Binding of PPIase to the ubiquitin ligase leads to its degradation with the consequence of higher cJun levels. These eventually lead to increase cell proliferation. A recent study demonstrated that the micro RNA oncomir *miR-155* is upregulated in infected cells similar to observations in a number of tumors, including Hodgkin's lymphoma, where it is important in maintaining the transformed phonotype.

2.6.5 Ciliophora

- Free-living or parasitic
- Surface has many cilia
- Micronucleus and macronucleus
- Conjugation as a mechanism of genetic exchange
- Ingestion of nutrients by cytostome (cell mouth)

The Ciliophora have a characteristic **pellicle**, which typically has many **cilia**. The structure of these cilia corresponds to that of flagella. They are usually arranged in rows or wreaths. A pinocytotically active organelle, termed the **parasomal sac,** forms an indentation near the root of each cilium. Flat **Alveoli** underlie the plasmalemma. Under these, a protein layer reinforced by microtubules contributes to a relatively rigid surface. The cilia swirl food particles to a **cytostome,** where they are phagocytosed and enclosed in vacuoles. These vacuoles circulate through the cell and are excreted at the **cytopyge** (cell anus). Osmoregulation occurs via **contractile vacuoles**. Ciliophora have a **macronucleus**, which regulates cell activity, and **micronucleus,** the genetic material of which is exchanged during conjugation. Reproduction takes place asexually by transverse division and distribution takes place through cysts. Parasitic *Ciliophora* populate the surface (e.g., of fish) or the digestive tract of their hosts.

2.6.5.1 *Balantidium coli*

Balantidium coli (Greek: *balantídion* = small purse, due to its shape) usually lives as a commensal in the cecum and colon of pigs and other animals (including humans), but it can become pathogenic if the degree of resistance is reduced.

Development and Morphology Transmission occurs via round cysts, which have a diameter of 40–60 µm. The cysts are excreted in the feces and their strong wall helps them to survive for several weeks under favorable conditions in the external environment (Figure 2.74b). The trophozoites that emerge are 50–200 µm in length and irregularly oval. The surface is covered with rows of cilia that beat synchronously, allowing targeted movements. The cilia are thicker near the cell mouth, which lies at the bottom of a slit-shaped peristome, where they play a role in food ingestion. The macronucleus is elongated, but the micronucleus is unremarkable. The trophozoites feed on bacteria and detritus.

Humans in frequent contact with pigs, such as butchers and farmers, are at an increased risk of infection.

The disease causes a diarrheal disease known as Balantidiosis. In severe infections, masses of Balantidia can develop and cause inflammation of the colon mucosa and ulcers. The pathogen can also cause severe disease in monkeys.

2.6.5.2 *Ichthyophthirius multifiliis*

Ichthyophthirius multifiliis (Greek: *ichthys* = fish, *phteiros* = louse) is a globally distributed parasite of farmed and ornamental fish. It settles in the skin and gills, causing "white spot disease." The infection is mainly found in larvae and young fry. Free-swimming, elongated stages of about 40 µm in length, termed "theronts" penetrate the surface of the host (Figure 2.75). The parasites then

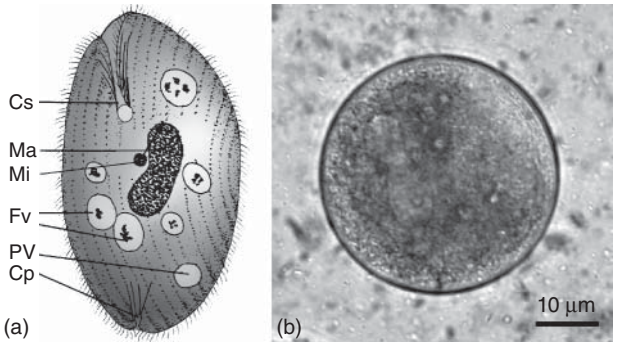


Figure 2.74 Stages of *Balantidium coli*. (a) Schematic diagram of a trophozoite. Zs, cytostome; Ma, macronucleus; Mi, micronucleus; Nv, food vacuoles; PV, pulsating vacuole; Zp: =Cytopyge = cell anus. (b) Cyst from the feces of a pig. (Image: Courtesy of B. Bannert.)

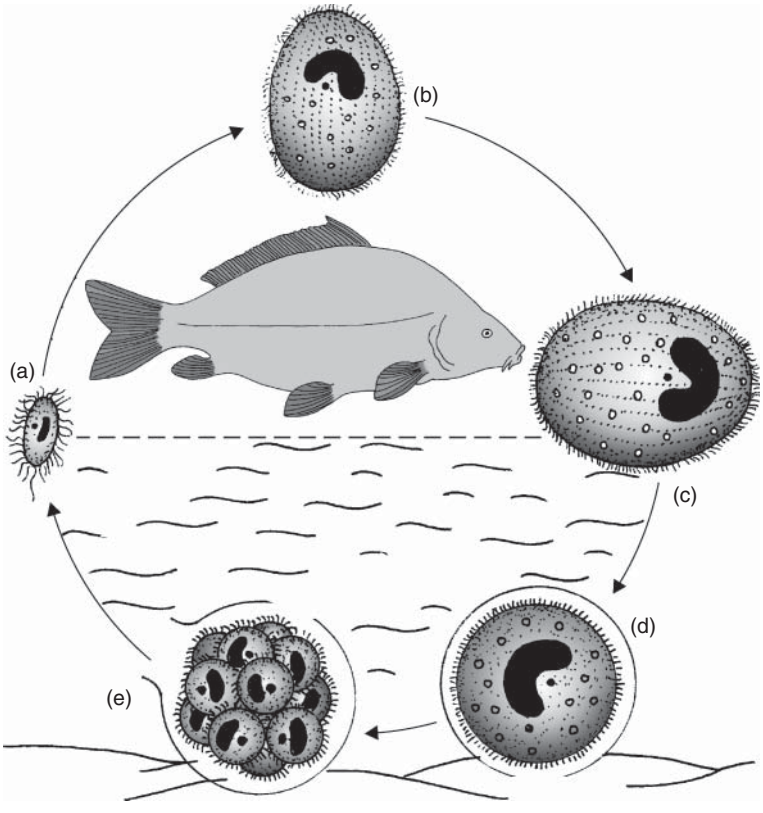
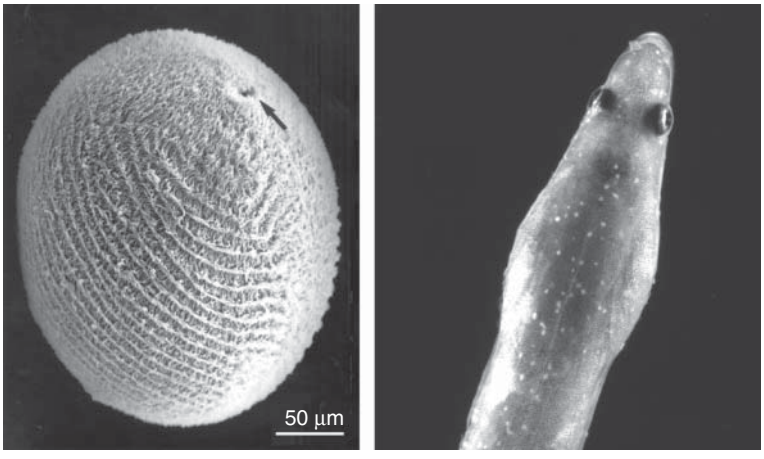


Figure 2.75 Life cycle of *Ichthyophthirius multifiliis*. (a) Theront. (b, c) Trophont. (d) Cyst. (e) Tomites, which will break free of the cyst shell.

settle beneath the epidermis and transform into a growth stage, termed the "trophont." This constantly rotating parasite lies in a pustule filled with necrotic host material and mucus. The parasites develop into oval trophonts with a horseshoe-shaped macronucleus and a round micronucleus within 2–20 days, depending on the ambient temperature. Trophonts are between 0.3 and 1 mm in size (Figure 2.76) and their surface is covered with rows of cilia. A short cytopharynx is attached to the cytostome, which the trophonts use to ingest leukocytes and epithelial cells of the host. Many contractile vacuoles are found within the cell. The nucleus of the mature trophont is highly polyploid. During this stage, the parasite breaks out of the skin and encysts at the bottom of the water or on a hard surface. Up to 2000 daughter organisms ("tomites") are now created by division. They free themselves from the cyst shell, exchange genetic material via conjugation, and differentiate into theronts. They must find and infect a fish within 24 h.



(a) (b)

Figure 2.76 *Ichthyophthirius multifiliis*. (a) Trophont, SEM image: Courtesy of W. Foissner. (b) Young eel with white spot disease. (Image: H. Taraschewski.)

The parasites in the gills usually sit directly below the epithelium near blood vessels. The resulting inflammation and proliferation of connective tissue interfere with osmoregulation and the exchange of substances, and might even result in necrosis. The skin can be densely covered with white dots ("white spot disease") and lose its color (see Section 1.5). Severe infection can cause the death of fish through the failure of osmoregulation and secondary infections, a situation exacerbated with high density of fish and high temperatures. Fish that have already survived an infection demonstrate a temporary, but broadly effective immunity to new infestations. It is interesting to note here that this protection comes about by antibodies of the fish that bind to a surface antigen of the parasite. The antibody binding causes the parasites to leave the host within minutes.

2.6.5.3 *Trichodina*

A few Ciliophora of the genus *Trichodina* (Greek: *trichos* = hair, *dine* = vortex) are found on the gills and body surface of almost all freshwater and saltwater fish. This genus encompasses many species. The parasites are hat-shaped (Figure 2.77). The cilia move in constant, swirling motions and several cilial wreaths beat in phased or out-of-phase movements, providing a highly aesthetic appearance under the microscope. The *Trichodina* use a hook ring (which also serves as an identifying feature) to anchor themselves in the epithelium and mucous layer. They reach a diameter of about 60 µm. High levels of parasite reproduction can lead to the death of fish that have already been weakened by other infections.

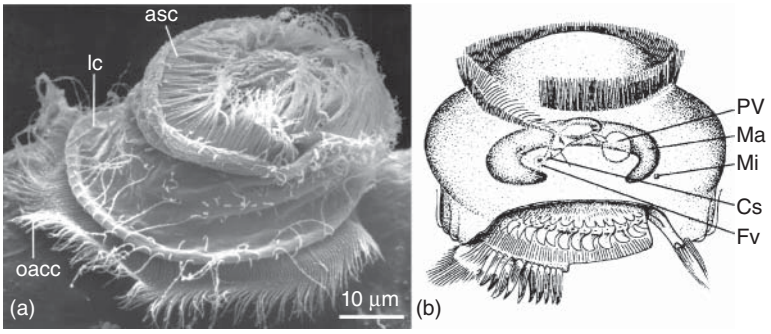


Figure 2.77 Trophozoite of *Trichodina* sp. (a) SEM image: W. Foissner. acs, adoral cilial spiral; oacc, outer adoral cilial crown; lc, lateral cilia. (b) Drawing of *T. myicola*, semi-schematic. Part of the foreground has been omitted to show the structure of the ventral cilial crowns. PV, pulsating vacuole; Ma, Macronucleus, Mi, Micronucleus, Cs, Cytostome, Fv, Food vacuole. (From Hausmann, Hülsmann, Radeck (2003) Protistology, with permission from Scheizerbart'sche Verlagshandlung, Stuttgart, www.schweizerbart.de.)

Further Reading

Allred, D.R. and Al-Khedery, B. (2004) Antigenic variation and cytoadhesion in *Babesia bovis* and *Plasmodium falciparum*: different logics achieve the same goal. *Mol. Biochem. Parasitol.*, **134**, 27–34.

Beeson, J.G. and Brown, G.V. (2002) Pathogenesis of *Plasmodium falciparum* malaria: the roles of parasite adhesion and antigenic variation. *Cell. Mol. Life Sci.*, **59**, 258–271.

Chapman, H.D., Barta, J.R., Blake, D., *et al.* (2013) A selective review of advances in coccidiosis research. *Adv. Parasitol.*, **83**, 93–171.

Checkley, W., White, A.C., Jaganath, D., *et al.* (2015) A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for Cryptosoridium. *Lancet Infect. Dis.*, **15**, 85–94.

Dubey, J.P., Schares, G. (2011) Neosporosis in animals – the last five years. *Vet. Parasitol.*, **180**, 90–108.

Feachem, R.G.A., Phillips, A.A., Hwang, J., Cotter, C. *et al.* (2010) Shrinking the malaria map: progress and prospects. *Lancet*, **376**, 1566–1578.

Francia, M.E., Striepen, B. (2014) Cell division in apicomplexan parasites. *Nat. Rev. Microbiol.*, **12**, 125–136.

Gilbert, R.E., Freeman, K., Lago, E.G., *et al.* (2008) Ocular sequelae of congenital toxoplasmosis in Brazil compared to Europe. *PLoS Negl. Trop. Dis.*, **2** (8), e277.

Grigg, M.E., Bonnefy, S., Hejl, A.B., *et al.* (2001) Success and virulence in *Toxoplasma* as the result of sexual recombination between two distinct ancestries. *Science*, **294**, 161–165.

Ingmundson, A., Alano, P., Matuschewski, K., Silvestrini, F. (2014) Feeling at home from arrival to departure: protein export and host cell remodelling during Plasmodium liver stage and gametocyte maturation. *Cell Microbiol.*, **16**, 324–33.

Konradt, C., Ueno, N., Christian, D.A., *et al.* (2016) Endothelial cells are a replicative niche for entry of Toxoplasma gondii to the central nervous system. *Nat. Microbiol.*, **1**, pii:16001.

Krause, P.J., Daily, J., Telford, S.R., *et al.* (2007) Shared features in the pathobiology of babesiosis and malaria. *Trends Parasitol.*, **23**, 605–610.

Huang, D.B. and White, A.C. (2006) An updated review on *Cryptosporidium* and *Giardia*. *Gastroenterol. Clin. North Am.*, **35**, 291–314.

Lang, C., Groß, U., and Lüder, C.G.K. (2007) Subversion of innate and adaptive immune responses by *Toxoplasma gondii*. *Parasitol. Res.*, **100**, 191–203.
Liu, W., *et al.* (2010) Origin of the human malaria parasite Plasmodium falciparum in gorillas. *Nature*, **467**, 420–425.
Liu, W., *et al.* (2014) African origin of the malaria parasite Plasmodium vivax. *Nat Commun,* **5**, 3346.
Maier, A.G., *et al.* (2009) Malaria parasite proteins that remodel the host erythrocyte. *Nat. Rev. Microbiol.*, **7**, 341–54.
Marsolier, J., Perichon, M., DeBarry, J.D., *et al.* (2015) Theileria parasites secrete a prolyl isomerase to maintain host leukocyte transformation. *Nature*, **520**, 378–382.
Nacer, A., Movila, A., Sohet, F., *et al.* (2014) Experimental cerebral malaria pathogenesis – hemodynamics at the blood brain barrier. *PLoS Pathog*. 10: e1004528
Perkins, D.J., Were, T., Davenport, G.C. (2011) Severe malarial anemia: innate immunity and pathogenesis. *Int. J. Biol. Sci.* **7**, 1427–1442.
Pernas, L., Adomako-Ankomah, Y., Shastri, A.J., *et al.* (2014) Toxoplasma effector MAF1 mediates recruitment of host mitochondria and impacts the host response. *PLoS Biol.*, **12** (4), e1001845.
Schnittger, L., Rodriguez, A.E., Florin-Christensen, M., and Morrison, D.A. (2012) *Babesia*: a world emerging. *Infect. Genet. Evol.*, **12**, 1788–1809.
Schuster, F.L. and Ramirez-Avila, L. (2008) Current world status of *Balantidium coli*. *Clin. Microbiol. Rev.*, **21**, 626–638.
Seeber, F., Steinfelder, S. (2016) Recent advances in understanding apicomplexan parasites. *F1000 Faculty Rev.*, 1396.
Thera, M.A., Plowe, C.V. (2012) Vaccines for malaria: How close are we? *Ann. Rev. Med.*, **63**, 345–357.
Vinayak, S., Pawlowic, M.C., Sateriale, A., *et al.* (2015) Genetic modification of the diarrheal pathogen Cryptosporidium parvum. *Nature*, **523**, 477–480.
Weiss, L.M. and Kim, K. (2013) *Toxoplasma gondii. The Model Apicomplexan – Perspectives and Methods*, 2nd edn, Elsevier, 1160 pp.
White, N.J., Pukrittayakamee, S., Hien, T.T., *et al.* (2014) Malaria. *Lancet*, **383**, 723–735.
WHO http://www.who.int/mediacentre/factsheets/fs094/en/ (accessed 28 April 2016).

Test Questions

1. The apical complex consists of which organelles?
2. How is the pellicle of Alveolata structured?
3. What is the origin of the apicoplast?
4. Which three stages in their life cycle do most Apicomplexa have?
5. Name the infectious stage of the Apicomplexa.
6. On which motion mechanism is cell invasion of Apicomplexa based?
7. In which compartment do Apicomplexa lie after invasion has taken place?
8. Describe the structure of a typical coccidial oocyst.
9. Name the *Eimeria*-like pathogen in humans.
10. Which final host has *Toxoplasma gondii*?
11. Name the early and late stages of development in the intermediate host of *Toxoplasma gondii.*
12. Why is fresh cat feces not infectious as regards *Toxoplasma gondii*?
13. When is an unborn child not at risk from a *Toxoplasma* infection?
14. Why is *Toxoplasma gondii* dangerous for immunocompromised persons?
15. What is the main host cell for *Toxoplasma gondii* resting stages?

16. What kind of damage is caused by *Neospora caninum*?
17. What are the stages formed by *Sarcocystis suihominis* in pigs?
18. What forms of malaria are caused by the four human pathogenic Plasmodium species?
19. Which two types of host cells are colonized by human pathogenic *Plasmodium* species?
20. What is the "exoerythrocytic schizogony" in *Plasmodium*?
21. Which different stages of the schizogony phase of *Plasmodium* are found in erythrocytes?
22. Why does malaria no longer exist in Central Europe?
23. On what types of erythrocytes is *Plasmodium vivax* specialized?
24. How does *Plasmodium falciparum* evade the spleen passage?
25. Name the major surface antigen of the sporozoite of *Plasmodium.*
26. What pathomechanisms led to death by Malaria tropica?
27. What are the long-lived stages of *Plasmodium vivax* called?
28. Through which arthropod host *is Babesia divergens* transmitted?
29. Why can Babesia remain in tick populations for a long time?
30. Which important diseases are caused by *Theileria*?
31. Which host cells exploits *Theileria*?
32. Which main host is infected by *Balantidium coli*?
33. How is *Ichthyophthirius* transmitted?

3
Parasitic Worms

Brigitte Loos-Frank and Richard K. Grencis

The Biology of Parasites, First Edition. Richard Lucius, Brigitte Loos-Frank, Richard P. Lane, Robert Poulin, Craig W. Roberts, and Richard K. Grencis.

Many multicellular parasites with an elongate form and lacking or reduced appendages are aggregated as parasitic worms, called helminths (Greek: *helmis*, *helminthos* = worm in internal organs). This term is widely used in the medically oriented field of infection biology, where "helminthologists" are scientists focusing on parasitic worms. In general, helminths comprise the Platyhelminths (flatworms), Nematoda (roundworms), Acanthocephala (spine- or thorn-headed worms), and even Pentastomida (tongue worms) and leeches. The concept of treating helminths as a zoological group is not supported by systematics, as they belong to very different taxa such as Lophotrochozoa, nematodes, annelids, or even arthropods (Figure 3.1).

In spite of their heterogeneity regarding the systematic position, the helminths have a number of biological traits in common. Many helminths are large, some reaching a length of several centimeters or even meters. Due to their visibility

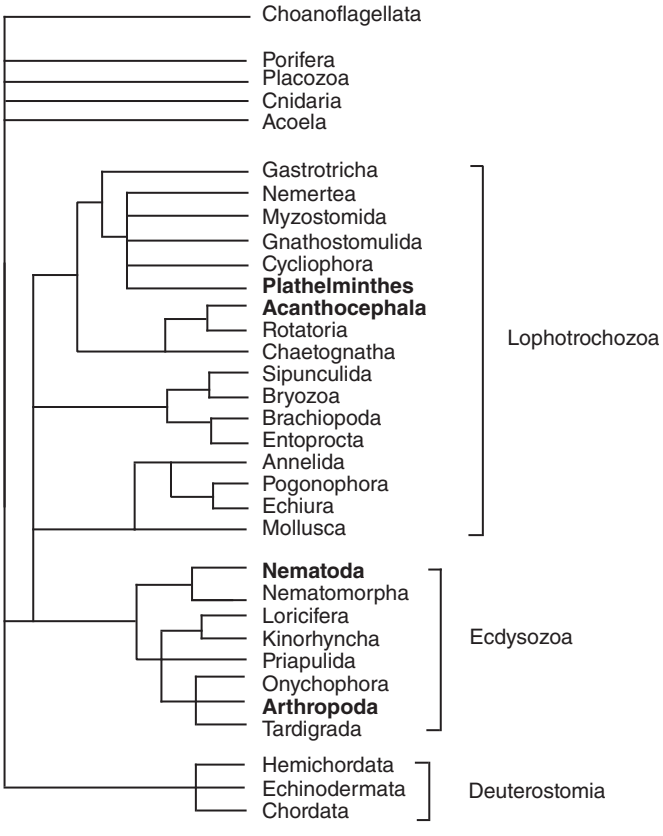


Figure 3.1 Phylogeny of the Metazoa (according to Blaxter, M.L. (2003) *Adv. Parasitol*., **54**, 101–195, some taxa omitted). Taxa containing parasites dealt with in this book are in **bold.**

by the naked eye, they were the first pathogens described by scientists. Another peculiarity is that adult stages of the worms do not multiply in the definitive host. Thus, quite in contrast to infection with viruses, bacteria, or protozoan parasites, infection with a pair of worms typically does not result in a population, but will rather lead to eggs or larval stages that have to leave the host for further development. In exact terms, this fact is described with the word "infestation." The eggs of helminths are of great diagnostic value, as their characteristic size and shape often allow a relatively specific diagnosis (see Figure 3.41). As the pathology of helminth infections is usually correlated with the number of worms, the intensity and duration of exposure play a major role in the clinical picture. Due to these features, the helminths are sometimes termed "macroparasites," in contrast to "microparasites" comprising viruses, bacteria, and protozoan parasites.

The different taxa of the helminths also have a number of commonalities regarding the immune responses induced in their hosts. Infections with parasitic worms typically induce high levels of eosinophil granulocytes and

immunoglobulin E, which are major components of antiworm effector mechanisms. Eosinophil numbers in the peripheral blood are usually very low, but these immune cells strongly increase in number, and serum IgE can reach extreme levels. These traits are ascribed to the fact that parasitic worms typically induce Th2-type immune responses (see Figure 1.37), as they seem to lack certain molecules polarizing immune responses into other directions. The efficiency of these Th2 responses – as well as of other types of immune responses – varies individually. Therefore, worm burdens in a population usually vary widely, most individuals bearing a low number of worms, while a few individuals may harbor very large numbers. These "wormy" – and therefore probably very sick – individuals are very important for the dissemination of infections. The typical uneven distribution of parasites in the population is called "overdispersed" (see Figure 1.24).

A further common trait of helminths is their relative long life span and the fact that most parasitic worms cannot be easily kept in culture and genetically transformed. Therefore, many modern approaches, for example, the production of mutants in the context of functional studies, are not yet applicable to parasitic worms. Due to these difficulties, helminths are largely understudied, in spite of their major medical and socioeconomic importance.

3.1 Platyhelminths

Platyhelminths or flatworms are part of the large group Lophotrochozoa (Figure 3.1) and comprise mainly free-living turbellarians, parasitic flukes, parasitic tapeworms, and some minor groups. Most of them are flattened dorsoventrally and all of them are bilaterally symmetrical and hermaphroditic. They have three germ layers, but no coelomic cavity. This is replaced by a mesenchyme (parenchyma), filling the space between ectoderm and endoderm. A solid outer layer, respiratory organs, and circulating system are not present.

One of the orders of turbellarians, the Rhabditophora, gave rise to the monophyletic taxon of **Neodermata** (Greek: *neos* = new, *dermis* = skin) comprising the Monogenea, Cestoda, and Trematoda. The name is based on the fact that their larva, when emerged from the egg, sheds its ancestral epidermal cells (usually bearing cilia used for locomotion) upon contact with the first intermediate host. A new body surface is developed from cells of mesodermal origin. Their cell bodies with the nucleus are situated beneath the basal matrix and extend processes toward the surface. These fuse to form a nonciliated syncytium above the basal membrane (Figure 3.2). This new coat, the tegument, is metabolically active, can absorb low-molecular-weight nutrients, discharge excretions, and release substances reported to inhibit immune reactions.

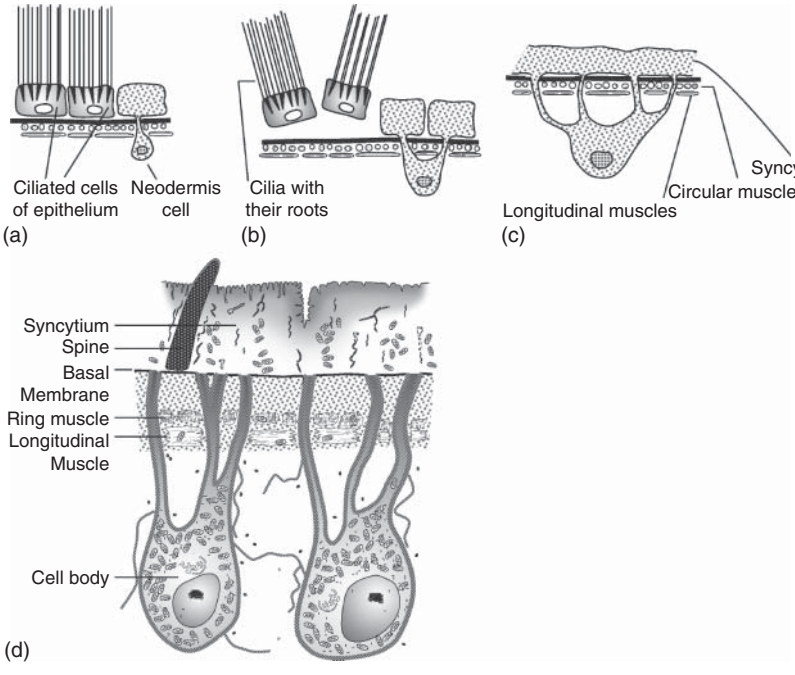


Figure 3.2 Neodermis. (a–c) Schematic presentation of the development. (d) Cross section of the neodermis of a digenean trematode.

Neodermata have compound **reproductive organs**. In the female reproductive system, there is an ovary and paired follicular vitellarium. One oocyte unites with a spermatozoon from a previous fertilization and is packaged together with several yolk cells providing nutrients and eggshell material. This packaging occurs in the ootype and subsequently the egg passes through a long uterus. The male reproductive organs consist of two to multiple testes. From each of them, *Vasa efferentia* lead towards an unpaired *Vas deferens*, which widens just before entering the genital opening to form a seminal vesicle (*Vesicula seminalis*) ending in an evertible copulation organ, the cirrus. This organ is surrounded by a sac-like cirrus pouch, which contains some prostatic glands. Usually, male and female efferent ducts open into a combined (male and female) genital atrium.

The **excretory system** of the Neodermata consists of nucleated terminal cells with a bundle of cilia. Their movement generates low pressure and an influx of fluid from the surrounding parenchyma. This movement can be seen under the microscope, and it gives the impression of a flickering flame, which has led to the term flame cell. The number and arrangement of the flame cells is species-specific, especially for larval form of the flukes, the cercaria. The efferent ducts of the flame cells join to form wider ducts and finally large canals that open into a large excretion vessel.

3.1.1 Digenea

- Adults are obligate parasites of vertebrates
- Conventionally called trematodes
- Usually infect two consecutive intermediate hosts
- Life cycles usually bound to water and involve two swimming larvae (miracidium and cercaria)
- Alternation of sexual and asexual reproduction
- Operculated eggs (except Schistosomatoidea)
- Multiplication predominantly in larval stages
- Adults usually colonize the intestine of the final host.

Digenea occur in all groups of vertebrates. They usually measure less than 10 mm in length. The conspicuousness of their internal organs rendered them favored subjects for observation by natural scientists ever since the invention of the microscope. The name Digenea (Greek: *di* = two, *génos* = ancestry, generation) indicates the characteristic alternation of generations: sexually reproducing adults and asexually multiplying larval stages (metagenesis). With the exception of blood flukes (superfamily Schistosomoidea), most digeneans inhabit the intestine of their final hosts. The term "trematodes" always indicates Digenea.

3.1.1.1 Development

The life cycle of most Digenea (Figure 3.3) includes two intermediate hosts and a definitive host (although again, Schistosomoidea are different). The first intermediate host is nearly always a mollusk, usually a freshwater or marine snail, although in rare cases it can be a bivalve or even a polychaete. There is a strong host specificity regarding the first intermediate host. This is why mollusks were regarded as the first evolutionary constituents of the life cycle, but of late hypotheses and molecular data support the idea that the most recent common ancestor of the Neodermata-adopted vertebrate ectoparasitism as its initial life cycle pattern and that intermediate hosts were only subsequently added. The number of offspring of the adult trematode is often much less than the number of larvae produced asexually in the first intermediate host. Two types of swimming larvae develop: the miracidium and the cercaria. The second intermediate host is typically an animal in the food chain of the final host.

Eggs are discharged with the feces of the definitive host (or with its urine or sputum, according to the locality of the adult worm). If the eggs are unembryonated when shed, the first larva, an actively swimming ciliated **miracidium**, develops in water within the eggshell, hatches and invades the **first intermediate** host. If the egg is shed embryonated, already containing a miracidium, it has to be eaten by the first intermediate host. In both cases, the miracidium sheds its cilia while invading the host, bores through the skin or the gut wall, settles in one of the organs, and becomes a **mother sporocyst**, containing many omnipotent germ balls. These

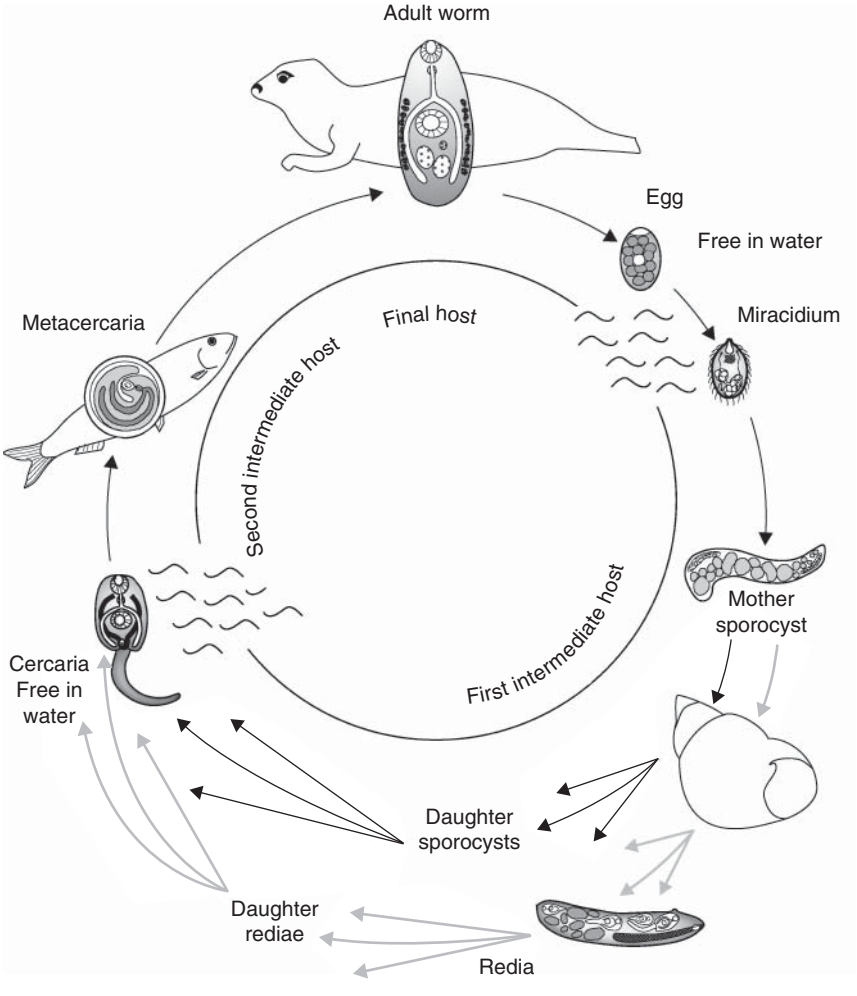


Figure 3.3 Typical life cycle of a digenean trematode (water-bound, marine). First intermediate host: marine snail. Depending on the taxon, development occurs through either sporocysts (black arrows) or rediae (gray arrows). The second intermediate host (a fish in this case) is an animal being part of the food chain of the definitive host (a seal in this case). Multiple arrows: asexual propagation of larval stages. Wave symbols: free swimming larval stages.

give rise to either **daughter sporocysts** or **rediae**, again filled with germ balls, which leave the mother sporocyst and migrate to the hepatopancreas ("liver") of the mollusk. Sometimes further generations are produced, granddaughter sporocysts or daughter rediae. Their germ balls develop to the final larval generation, the **cercariae**. These leave the first intermediate host and swim to find the second intermediate host (or in the case of the Schistosomes, the definitive host). Due to the vegetative reproduction in sporocysts and rediae up to thousands of cercariae

can develop from a single miracidium over a period of several weeks. When the cercaria enters the **second intermediate host** (or, as in schistosomes, the definitive host), it sheds its tail. Also all other larval features are remodeled. The cercaria migrates to the muscle tissue, hemocoele, liver, or other body organs and settles there, but is never found in the intestine. From secretions of its cystogenous gland cells, it produces an elastic hyaline envelope or cyst wall. This stage is the **metacercaria** (Greek: *metá* = after), which again is missing in the schistosomes. It does not develop any further, but has to wait to be ingested by a **definitive host**, a vertebrate. During the passage through the stomach and duodenum of the vertebrate, the cyst wall of the metacercaria is dissolved. The preadult fluke migrates to its final location in the small intestine (but sometimes to other organs such as the bile duct, gallbladder, respiratory system, pancreas, *bursa fabricii* of birds, kidney, eye, or subcutaneous connective tissue) and becomes an adult.

3.1.1.2 Morphology

The **miracidium** has a pear-shaped body (Figure 3.4a–c). The body is covered by four to five rings of several large epithelial cells bearing cilia. The proximal part of the larva contains an apical gland excreting a substance facilitating the invasion of the first intermediate host (usually a snail). It also comprises a cerebral ganglion, often two eyespots (pigment-cup cells) and one or two pairs of protonephridia (for waste excretion). The distal part of the body contains clusters of germ balls that eventually give rise to future sporocysts or redia. A mouth and gut are missing from the miracidium. During invasion through the epidermis of the snail, the ciliated epidermis cells are shed. Subsequently, glands, eyes and ganglion involute and the larva becomes a mother sporocyst.

The mother sporocyst is a sac-like organism with no motility organs, mouth, or gut. Its surface is covered with microvilli interlocked so tightly with the host's tissue that it is often difficult to remove it intact. Mother sporocysts are filled with constantly dividing germ balls, from which either **daughter sporocysts** or **rediae** develop (named after the Italian biologist Redi). Daughter sporocysts may be oval, long, or sac-like, rarely branched larvae without conspicuous organs and contain germ balls (Figure 3.4e) Rediae possess an oral opening, pharynx, and short blind gut (Figure 3.4d). They are able to devour host tissue and even alien trematode larvae. They have a birth pore and, in some families, two short appendages, which serve as locomotion devices within the mollusk. Again, germ balls are present. The germ balls of daughter sporocysts or rediae transform to cercariae (Figure 3.4g–l).

The **cercaria** shows similarities to the adult stage in some respects, namely in shape and number of suckers and often in the digestive system, but it has also larval characteristics enabling it to fulfill the requirements of the short free-living phase that is, the detection and invasion of the host. These larval structures include a tail for rowing movements, sensillae on the surface, and groups of glands producing secretions for the invasion of the host and in many cases for building of the metacercarial cyst. Furthermore, cercariae using an arthropod as second intermediate host possess a stylet for piercing the cuticle. There are no microvilli on the surface, but often spines or scales. Protonephridia (flame cells) can only be seen in the

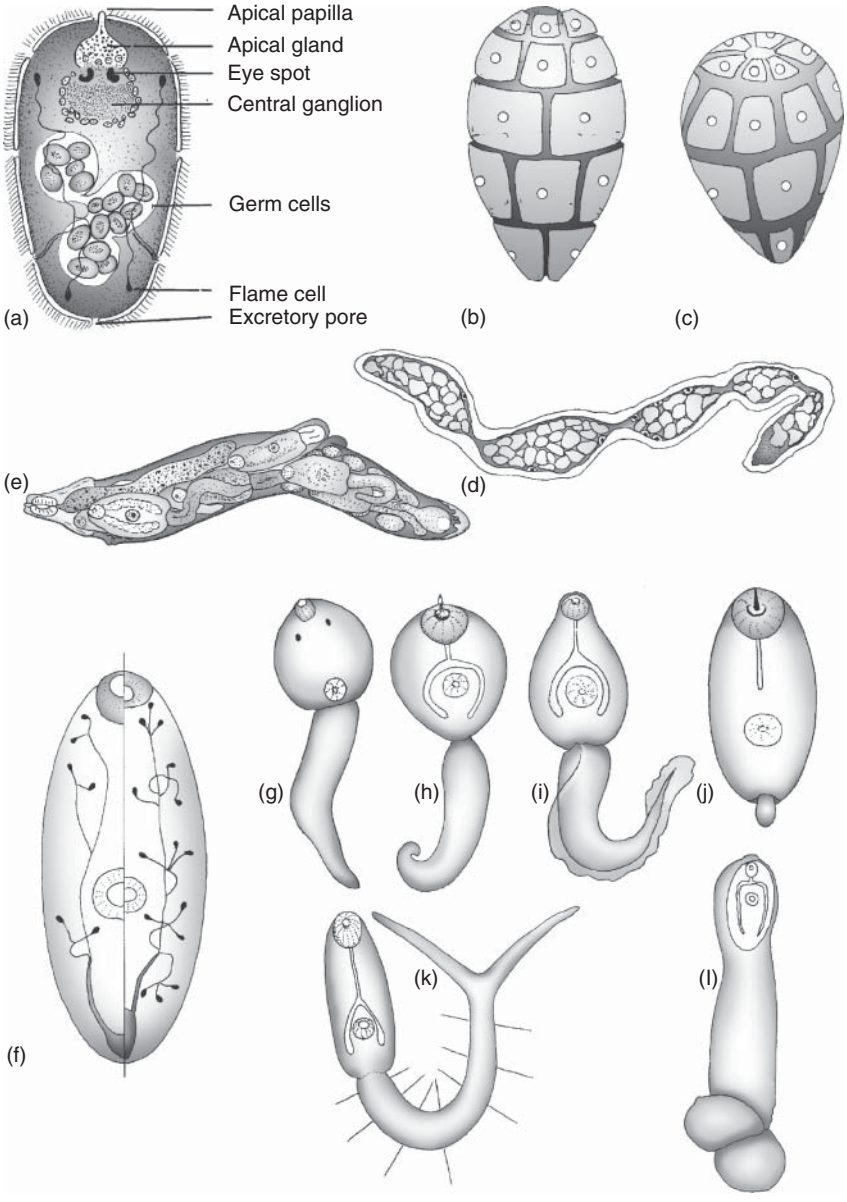


Figure 3.4 Developmental stages of Digenea. (a) Structure of a miracidium. (b) Epithelial cells of a miracidium, family Fasciolidae with five tiers of 6, 6, 3, 4, and 2 cells. (c) Epithelial cells of a miracidium (family Schistosomatidae) with four tiers of 6, 8, 4, and 3 cells. The cilia are omitted for ease of viewing. (d) Sporocyst of a *Schistosoma* species. (e) Redia of *Fasciola hepatica* filled with germ balls. (f) Schematic pattern of the cercarial excretory system, flame cell formula for the left side: (2+2)+2, for the right side: (3+3+3)+3+3). (g–l) Types of cercariae. (g) gymnocephalic amphistomous (*Paramphistomum*), (h) gymnocephalic xiphidiocerc (*Dicrocoelium*), (i) gymnocephalic lophocerc (*Psilochasmus*), (*j*) microcerc xiphidiocerc (*Paragonimus*), (k) furco-trichocerc (*Alaria*), (l) furco-zystocerc (*Azygia*).

Table 3.1 Terms of cercariae according to shape of tail and other characteristics.

Name of cercarial type	Word stem and translation	Shape of tail and other features	Examples
Furcocercous	Latin: *furca* = fork	Tail end bifurcate	*Diplostomum, Schistosoma*
Microcercous	Greek: *mikro* = small	Tail stub-like	*Paragonimus*
Lophocerc	Greek: *lophos* = cockscomb	Tail with lateral fins	*Opisthorchis*
Cystocercous	Greek: *kystis* = bladder	Body enclosed in proximal part of large tail	Families in fish
Gymnocephalic	Greek: *gymnós* = naked, *kephalé* = head	Body not enclosed in tail	*Fasciola*
Xiphidiocerc	Greek: *xiphós* = sword	Stylet in front of or above oral sucker	*Dicrocoelium*
Trichocercous	Greek: *thrix, trichós* = hair	Tail with long sensillae	Strigeidae

living cercaria and only as long as the bundles of cilia are moving, their number and configuration are species-specific, and can be expressed in simple formulas and help in identification. Their collecting ducts empty into a distal Y- or V-shaped excretory bladder. The excretory system may be filled with refractive granules, which probably have an osmoregulatory function. The genital organs of the future adult fluke can sometimes be seen as a group of cells in front of the excretory bladder. The tail is the most conspicuous structure. It contains musculature and glycogen reserves, which serve as energy sources for the swimming phase. The diverse shapes of the tail are reflected in the names of cercarial types (Table 3.1).

3.1.1.3 **Adults**

They have many varieties of size and shape (Figure 3.5a–d). They even differ regarding their form of sexuality, most of them being monoecious or hermaphroditic, but with a few types (e.g., Schistosomatidae) being dioecious, that is, having two sexes.

The length of adult trematodes can vary between 0.5 mm and 8 cm. In general, they are flattened dorsoventrally, but there are also globular or long thread-like species. Normally, they have two suckers, an oral sucker at the proximal end surrounding the mouth, and a ventral sucker (also known as acetabulum) somewhere in the middle of the body. Flukes with two suckers are distome species (Greek: *di* = two, *stóma* = mouth). In monostome flukes, the acetabulum is missing; in amphistome forms, the ventral sucker is situated at the distal end of the body. The tegument is underlaid by longitudinal and ring muscles. The space between muscle layer and the organs is filled with parenchymal cells, collagen-containing fibrils, and dorsoventral muscles. A coelom (body cavity) is not present. The alimentary tract starts with the mouth opening, followed occasionally by a short prepharynx, a muscular pharynx, and a straight esophagus, which leads into two blind-ending ceca. An anus is missing and waste material is regurgitated through

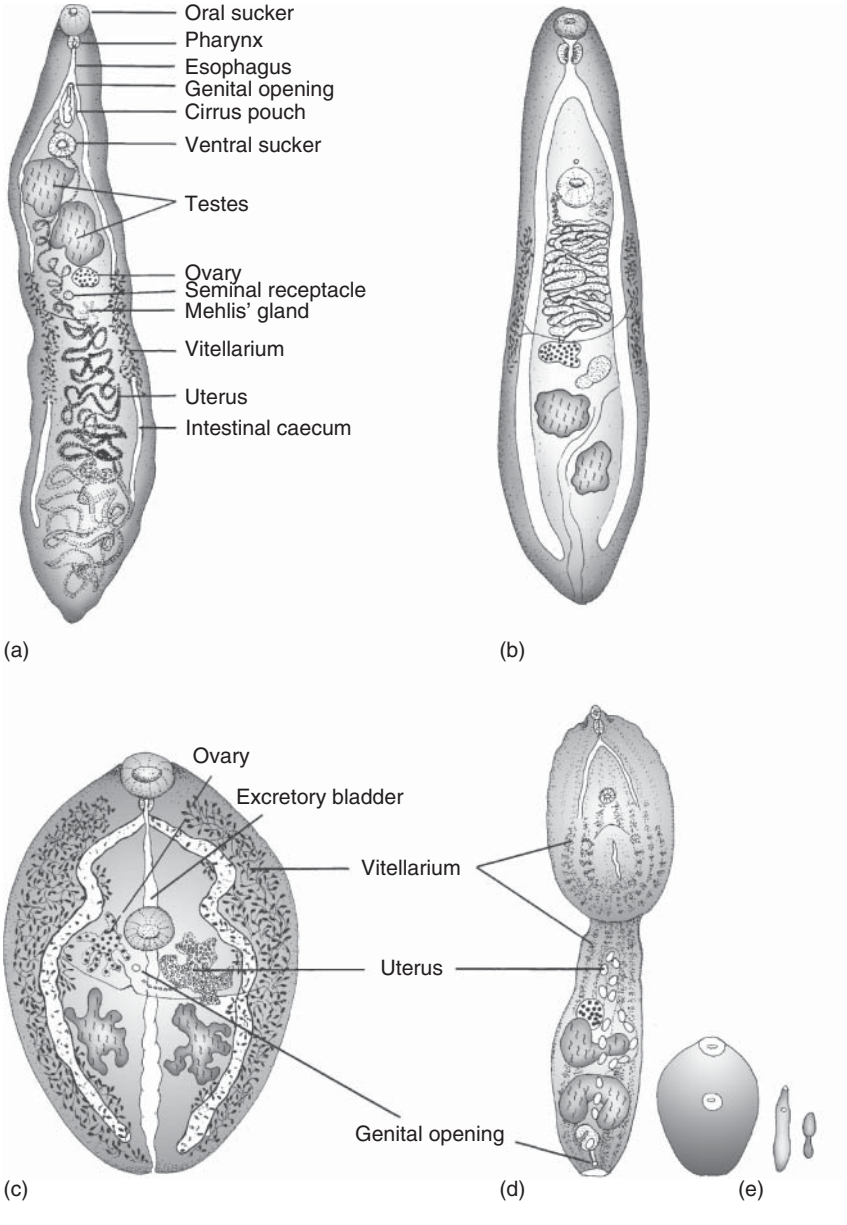


Figure 3.5 Adult Digenea. (a) *Dicrocoelium dendriticum*. (b) *Opisthorchis felineus*. (c) *Paragonimus westermani*. (d) *Diplostomum spathaceum*. (e) Size proportions of the species depicted, from left to right: *P. westermani, D. dendriticum, D. spathaceum.*

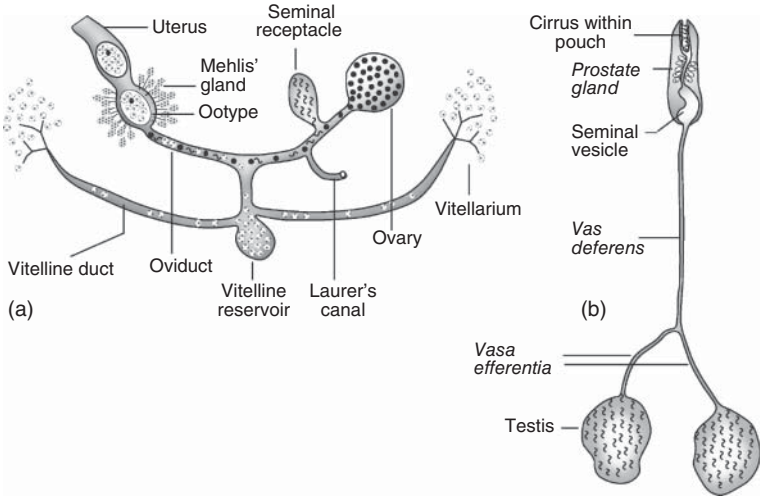


Figure 3.6 Genital organs of Digenea. (a) Female. (b) Male.

the mouth. A nervous system is present, but only visible with certain staining methods. Of the protonephridial system, which is active in osmoregulation, at most two lateral collecting ducts and a distal excretory bladder are visible.

Digenea, as all other platyhelminths, are hermaphrodites except in the case of rare exceptions such as the Schistosomatidae.

The female genital organs (Figure 3.6a) are composed of:

- An ovary producing egg cells,
- paired, lateral, follicular vitellaria, delivering yolk and eggshell material,
- two vitelline ducts, sometimes with a bladder-like vitelline reservoir,
- sometimes, a duct opening at the dorsal surface of the body, called Laurer's canal, possibly for insemination,
- often a large conspicuous seminal receptacle, filled with spermatozoa,
- the ootype, which, surrounded by the Mehlis gland, contributes eggshell material and defines the shape of the future egg,
- the uterus, usually leading as a long, winding tube towards the forebody, where it opens into
- the common genital atrium.

The term "yolk," by the way, is misleading. The vitellaria do not only supply nutrients for the developing embryo, but also precursors of the eggshell substance, present in the vitelline granules. These precursors are soluble proteins rich in tyrosines, which are hydroxylized to 3,4-dihydroxyphenylalanin (DOPA) in the fully developed "yolk" cells. In the ootype, the granules are released from the yolk cells, fuse with each other, and the eggshell precursor proteins are cross-linked in a tanning process, which is probably activated by secretions of the Mehlis' gland. The result is an eggshell-forming sclerotin, which is initially elastic and hardens during the migration through the uterus, sometimes becoming

dark brown. Quinone-tanned materials are extremely resistant, insoluble in water, detergents, and most acids and bases. They are also weakly immunogenic and resistant to proteolytic enzymes. This is why trematode eggs can pass the intestine of the final host undigested. Another type of mature "yolk" granules contains glycogen-like polysaccharides. These granules fuse, grow, and coalesce to form two cushions filling the space between eggshell and embryo. The cleavage of the polysaccharides probably provokes an influx of water into the egg, which causes a preformed lid, the operculum, to burst open, allowing the miracidium to hatch.

The eggs of digeneans are pale yellow or brown colored. They are released from the final host, according to localization, with feces, urine, or sputum.

The male genital organs (Figure 3.6b) consist of (usually) two testes, lying behind the ventral sucker. In two *vasa efferentia,* sperm is carried to a *vas deferens*. Shortly before the common genital opening, the *vas deferens* widens to form a seminal vesicle, which is followed by a prostate gland and protrusible cirrus (a sort of penis). In general, all three are surrounded by a cirrus pouch. Insemination is mutual if more than one fluke is present. Self-insemination apparently happens when a partner is not available.

3.1.1.4 Systematics and Evolutionary History

A recent revision of the classification (Olson *et al*., 2003) based on analyses of ribosomal RNA genes of 170 taxa suggests that the Digenea have only two orders, the Diplostomida and the Plagiorchiida (Table 3.2). As to the evolutionary history of the Digenea, it is obvious that originally their life cycles are bound to water.

Table 3.2 Gross systematics of the Digenea.

Order	Suborder	Superfamily	Family	*Species*
Diplostomida	Diplostomata	Schistosomoidea	Schistosomatidae	*Schistosoma mansoni*
		Diplostomoidea	Diplostomidae	*Diplostomum spathaceum*
		Brachylaimoidea	Leucochloridiidae	*Leucochloridium paradoxum*
Plagiorchiida	Echinostomata	Echinostomatoidea	Fasciolidae	*Fasciola hepatica*
	Pronocephalata	Paramphistomoidea	Paramphistomidae	*Paramphistomum cervi*
	Opisthorchiata	Opisthorchioidea	Opisthorchidae	*Opisthorchis felineus*
	Xiphidiata	Gorgoderoidea	Paragonimidae	*Paragonimus westermani*
			Dicrocoeliidae	*Dicrocoelium dendriticum*

Only taxa mentioned in this book are included.
Source: After Olson et al. (2003) Int. J. Parasitol 33, 733–55.

To date, the majority of the larval stages occur in aquatic environments. The fact that nearly all trematodes use gastropods as first intermediate hosts and that the host specificity for the snails is very high, both indicate gastropods as primary hosts. In addition, the high degree of exploitation of the snail hosts, for example, the nearly complete replacement of its "liver" by parasite tissue, parasitic castration, and giant growth, is considered to indicate a very long-lasting association between these parasites and their gastropod hosts. Taking this long-lasting association into account, it was suggested that ancestors of the contemporary trematodes originally colonized mollusks as adult trematodes during the Paleozoic era 570 million years ago. These forms then altered their life cycles by expanding their larval phase within the gastropods and shifting the adult phase to vertebrates. As a final step in some life cycles, a second intermediate host (very rarely a third) was included, in which the metacercaria encysts to reach the final host as part of the food chain. In most cases, the metacercaria is a stage of little pathogenicity without a great requirement of energy. It may be able to change the behavior of its host to increase the probability of reaching a final host.

3.1.1.5 *Schistosoma*

The genus *Schistosoma* (Greek: *schízo* = to split, *sóma* = body, because the worms live as pairs) is by far the most important trematode genus in the world. In the tropics of Africa, East Asia, and South America, more than 200 million people are infected. The disease is sometimes still called bilharziosis, named after the discoverer of the worms, the German physician and natural scientist Theodor Bilharz (1825–1862). The order Diplostomida, to which the schistosomes belong, is by no means a homogeneous one. There are, for instance, life cycles with two, three, and even four hosts; there are families with monoecious and dioecious adults; there are members inhabiting the intestine and others living in the circulatory system, and some develop via sporocysts, others via rediae.

The Schistosomatidae contain 15 genera. Most of them are parasites of birds, five inhabit mammals, and one occurs in an Australian freshwater crocodile. In the genus *Schistosoma*, there are seven species infecting humans and three infecting domestic animals. It is noteworthy that *Schistosoma* species of humans are not a monophyletic group. The transition to humans has occurred several times (Figure 3.7).

Schistosomatidae differ from other digeneans in the following features:

- Adults are dioecious. Males and females live in permanent copulation.
- They inhabit blood vessels, that is, species-specific locations within veins.
- They do not have a second intermediate host and no metacercaria. The cercaria is the stage infective for the final host
- The eggs do not have an operculum.
- The egg and not the adult worm is the pathogenic stage, with eggs transported into certain tissues, where they cause pathology.
- In contrast to other trematodes, they can live for decades.

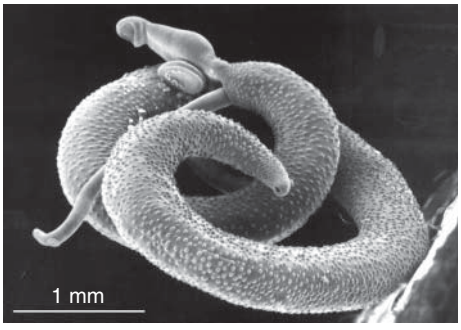


Figure 3.7 *Schistosoma mansoni*, male and female in copula. (Top) Anterior end of male. (Left) Slender anterior part of female. (Image: Archive of the Department of Molecular Parasitology, Humboldt-University, Berlin.)

Development The eggs, produced by the female worm living (together with the male) in the venous system (Figures 3.8, 3.9d, f, h, and 3.10), are shed unembryonated. A certain proportion travels from the veins to the intestine or bladder wall (see Table 3.3 for route of shedding) by releasing proteolytic enzymes and by causing inflammation, which renders the host tissue permeable. They are then shed with the feces or urine, while another proportion of eggs gets stuck in various tissues and causes fibrosis. On the way to the exterior, the miracidium matures. It hatches immediately when reaching water by tearing open the eggshell at any point. It is only viable for a few hours and has to find a suitable snail very quickly. Following invasion of the snail, mother sporocysts produce daughter sporocysts near the penetration site, which migrate to the hepatopancreas ("liver") of the snail, where cercariae are produced after 3–7 weeks.

Intermediate hosts (Table 3.3, Figure 3.9e, g, i) of the *haematobium* and *mansoni* group are freshwater pulmonates (family Planorbidae) of the genera *Bulinus* and *Biomphalaria,* respectively. Both occur in very different types of fresh water with a temperature of at least 20 °C. *Biomphalaria* is easily kept and propagated in laboratories, as long as the water does not contain copper. As a number of strains of laboratory mouse are good definitive hosts for *Schistosoma mansoni*, this is the most frequently and most thoroughly investigated *Schistosoma* species, although *Schistosoma haematobium* is the most clinically important species in humans. The intermediate hosts of the *japonicum* group are prosobranch snails. Those of the genus *Oncomelania* measure only 5–9 mm and have an amphibious life style. They live of diatoms, which they graze off humid soil on the margins of slowly flowing rivers, ditches, and small irrigation channels, for example, in paddy fields.

The free-swimming cercaria needs to invade the skin of a final host within 6 h. It recognizes a suitable host by the body temperature and certain skin fatty acids. Adherence to the skin as well as penetration through the layers of the skin is achieved by a well-orchestrated process, which involves proteolytic enzymes secreted by the large pre- and postacetabular glands. The tail is shed during penetration. The cercaria invades the skin, which is softened by water, at hair

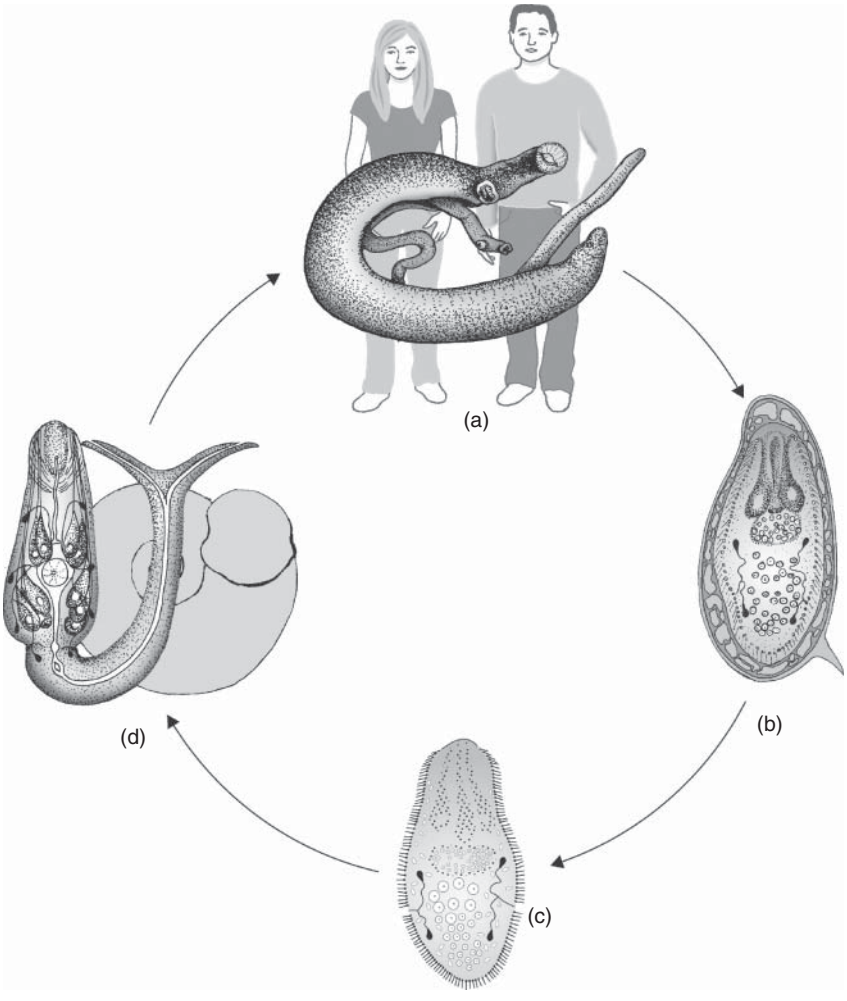


Figure 3.8 Life cycle of *Schistosoma mansoni*. (a) Pair of adults in humans. (b) Embryonated egg. (c) Miracidium. (d) Furcocercous cercaria having developed within sporocysts in a planorbid freshwater snail *Biomphalaria glabrata*.

follicles or skin folds. It reaches the basal membrane of the epidermis and requires a further day or two before arriving at the corium (= dermis). During this process, the glycocalyx is shed and replaced by the tegument of the adult.

The young worm, now termed a skin schistosomulum (Figure 3.11), is a highly sensitive stage and is easily destroyed by the host. If it succeeds in reaching a blood or lymph vessel, it is transported to the lung within 3–4 days, where it takes on a slender elongated shape. It arrives in the arterial vascular system via transit through the left side of the heart and finally resides in the portal vein. The male worms are already sexually mature at this time. Each male takes a

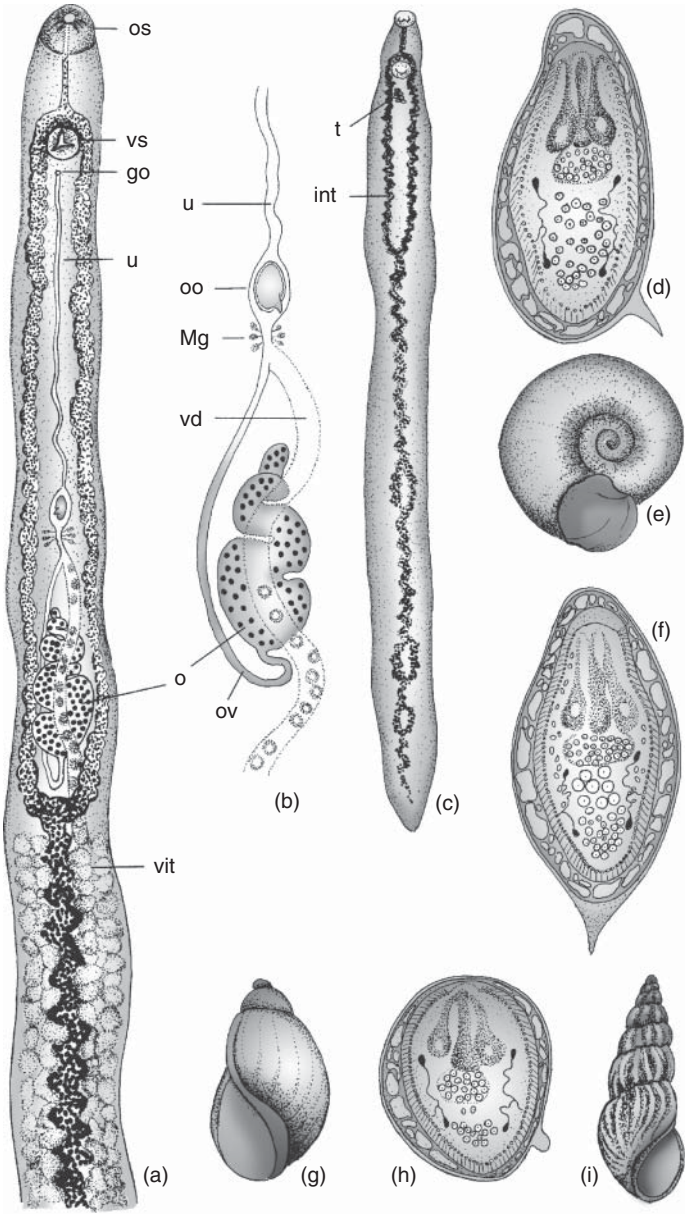


Figure 3.9 Some details of human-pathogenic schistosomes. (a) Anterior end of a female *S. mansoni*. (b) Genital organs of female. (c) A male of *S. mansoni*, body laterally extended (os, oral sucker; vs, ventral sucker; go, genital opening; u, uterus; o, ovary; vit, vitallarium; oo, ootype; Mg, Mehlis' gland; vd, vitelline duct; t, testes; g, gut). (d) Embryonated egg of *S. mansoni*. (e) Intermediate host of *S. mansoni*: *Biomphalaria glabrata*. (f) Embryonated egg of *S. haematobium*. (g) Intermediate host of *S. haematobium*: *Bulinus truncatus*. (h) Embryonated egg of *S. japonicum*. (i) Intermediate host of *S. japonicum*: snail *Oncomelania hupensis*.

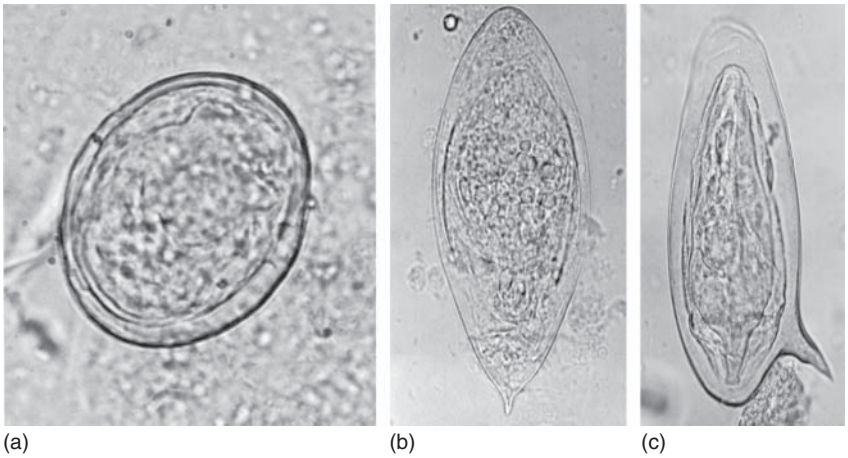


Figure 3.10 Eggs of *Schistosoma japonicum* (a), *Schistosoma haematobium* (b), *Schistosoma mansoni* (c). (Image: Archive of the Department of Parasitology, University of Hohenheim.)

female, arriving somewhat later, into its gynecophoric channel, which stimulates the differentiation of the female reproductive system. After mating, the males carry their females to species-specific localities. These are the mesenteric veins in *S. mansoni* and *Schistosoma japonicum*, and the veins of the urinary bladder in *S. haematobium*. Eggs of *S. mansoni* and *S. japonicum* are shed with the stool after 35 days, and of *S. haematobium* in urine after 70 days.

Morphology The **eggs** are nonoperculate and have an almost distinct lateral or terminal spine (Figure 3.9d, f, h).

The **cercariae** are furcocercous. There is no proper oral sucker, but a so-called apical or oral organ. This consists of an oral gland and a surrounding funnel-shaped muscle, which supports the mouth when it protrudes during penetration. A pharynx is not present. The ventral sucker is small. There are two tiny intestinal ceca at the end of a long esophagus. The larva has two pairs of large preacetabular and three pairs of postacetabular penetration glands, the collecting ducts of which penetrate the apical organ. Eye spots are not present.

The **adults** of both sexes are much longer than broad. The two suckers lie close together. A prepharynx and pharynx are not present. The genital pore is posterior to the ventral sucker. Behind the esophagus, the intestine divides into two long ceca, which unite again at a species-specific position to form a stem reaching the distal part of the body.

The female worm (Figure 3.9a) is longer and thinner than the male. Since it never has to travel in the blood vessel by its own, but is carried by the male, it has a weakly developed musculature and poorly developed suckers. The ovary is of a winding shape, narrowing anteriorly, and lies in front of the intestinal bifurcation (Figure 3.9b). Follicles of the vitellarium fill the lateral spaces next to the unpaired part of the intestine extending from the ovary to the distal body part. The number of eggs produced depends on the species (Table 3.3).

Table 3.3 Important human pathogenic *Schistosoma* species.

Schistosoma species	Distribution	Intermediate host	Mammalian reservoir hosts	Localization of adults	Form of eggs	Eggs shed with
mansoni	Africa, Madagascar, west of South America, the Caribbean	*Biomphalaria* (Planorbidae, lung snails)	Primates, rodents	Mesenterial veins of colon	Oval with lateral spine	Feces
haematobium	Africa, Madagascar, Middle East	*Bulinus* (Planorbidae)	Primates	Veins of lesser pelvis, esp. bladder	Oval with terminal spine	Urine
intercalatum	Central Africa	Bulinus (Planorbidae)	Rodents	Veins of colon and colon sigmoideum	Spindle-shaped with terminal spine	Feces
japonicum	China, Indonesia, Philippines	*Oncomelania* (Pomatiopsidae)	>40 species of mammals	Mesenterial veins of small (and large) intestine	Rotund with rudimentary lateral spine	Feces
mekongi	Laos, Cambodia	*Neotricula* (Pomatiopsidae)	Dog	As japonicum	As japonicum	Feces

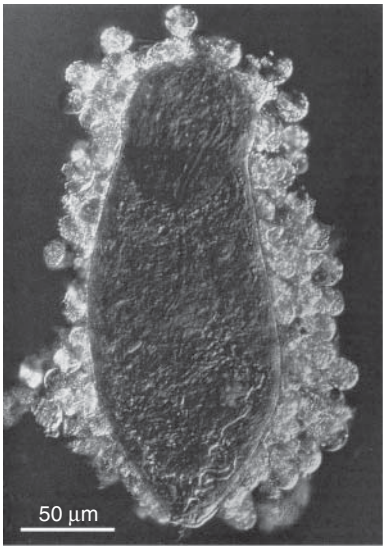


Figure 3.11 *Schistosoma mansoni*, antibody-coated schistosomulum with adhering eosinophils. The antibody-dependent cellular cytotoxicity is fatal for the parasite within 24–48 h. (Image: Courtesy of A.E. Butterworth)

The male worm is shorter and broader than the female (Figure 3.9c). The short proximal part of the body is rotund. The remaining longer part is flat and broad. Its lateral margins are folded ventrally to form a long fold, the gynecophoric channel (= female-bearing channel), in which the longer and thinner female resides, projecting proximally, in the middle and distally. The ventral sucker is large and strong. Multiple testes lie behind the sucker (seven of them in *S. mansoni*,) but are very small and closely packed. The dorsal surface of the male is covered by tubercles.

Genome The genome of *S. mansoni*, *S. haematobium,* and *S. japonicum* is of similar size (363, 385, or 397 Mb, respectively) and is arranged in 16 chromosomes (2n), whereby the synteny between *S. mansoni* and S. *haematobium* is much higher than with *S. japonicum*. Approximately 40% of the genome consists of repeats, and only between 4% and 5% of the genome are protein-encoding sequences. The sequenced schistosomes have between 10 850 and 13 500 putative protein-encoding genes, sharing more orthologs with vertebrates as compared to *Caenorhabditis elegans*. About 11% are organized in a polycistronic manner, indicating that several protein-encoding genes are regulated by a common promoter, and that the resulting precursor transcripts are trans-spliced, a mechanism known from bacteria and Trypanosomes (see Section 2.5). More than 300 genes encode for proteases. This high number is supposedly related to their role in host invasion digestion of blood as a protein source. Genome analysis revealed that schistosomes are not capable of *de novo* synthesis of sterols and free fatty acids, which have to be acquired from the host using diverse transporter proteins. Metabolic pathway analyses revealed a reduced ability to synthesize amino acids, and that carbohydrates can be used as energy source. As expected, male worms display a stronger expression of genes related to muscle functions necessary for

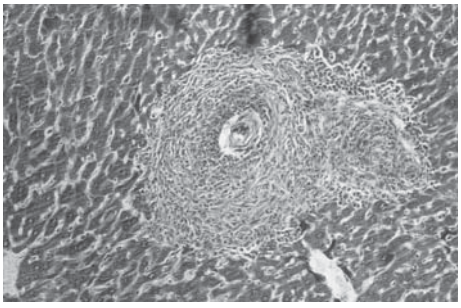


Figure 3.12 Cross-section of an egg of *Schistosoma mansoni* in mouse liver surrounded by a granuloma. (Image: Archive of the Department of Parasitology, University of Hohenheim.)

motility, adhesion, and clasping the female worm, while females strongly express genes related to egg formation.

Pathology Serious pathology is not caused by adult worms, but by eggs delivered from the extremely long-lived worms. A range of 100–300 eggs are shed per day by a female of *S. mansoni*, 20–200 by *S. haematobium,* and 500–3000 by *S. japonicum*. Only about half of them successfully penetrate the intestinal or bladder wall into the lumen to be excreted with the stool or urine. The others become lodged within the tissues, or especially in case of *S. mansoni* and *S. japonicum*, are swept, via the blood of the portal vein into the smallest veins of the liver. The miracidium growing inside the eggshell, over a 3- to 4-week period in the host, produces immunogenic glycoproteins, which are secreted through the eggshell and induce the development of the typical schistosome granuloma (Figure 3.12). These little white foci, visible to the naked eye, consist of a distinctly organized aggregation of different host cell types, which finally give way to fibrous tissue. The granulomas cause blockage of the blood vessels, and impairment of the blood circulation in the area of the portal vein. This may result in portal hypertension, enlargement of liver and spleen (hepatosplenomegaly), ascites, esophageal varices, and finally – if rarely – death. In the infection with *S. japonicum,* carcinoma of colon or rectum can develop. Also, as a result of the high egg production in this species, eggs may be swept into the brain and – as the result of repeated infections from childhood on – can lead to delay of physical, sexual, and mental development. Urogenital schistosomosis by *S. haematobium* is characterized by hematuria as well as pain and difficulty in urinating. Moreover, bladder cancer may develop. Typically, the severity of disease symptoms is correlated with the numbers of eggs, which depends on the worm burden, and serious pathology may start after years of repeated exposure.

Immunobiology Schistosomes provide excellent examples of how parasitic worms very efficiently evade, manipulate, and exploit host immune responses. The long life span of the adult schistosomes is thought to result from, at least in part,

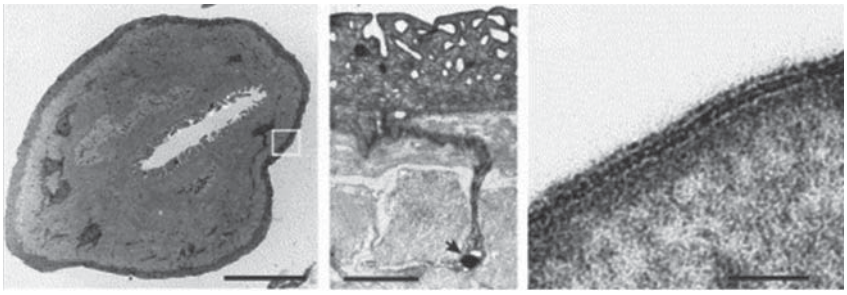


Figure 3.13 Tegumental outer surface of adult *Schistosoma mansoni*. In the lowest magnification (a), the tegument is visible as a dark band surrounding an adult female *S. mansoni* worm (Bar = 20 μm). Panel (b) shows an enlargement of the area indicated by the white rectangle in panel (a). The folds in the outer tegumental membranes (surface pits) increase the area of the epithelium. The arrow indicates a multilamellar body moving from the cell body toward the tegument in a cytoplasmic connection spanning the superficial muscle layers (bar = 1 μm). Panel (c) shows a higher magnification image of the double phospholipid bilayer of the tegumental membranes (Bar = 75 nm). (From Van Hellemont *et al.* (2006) *Int. J. Parasitol.*, **36**, 691–699, with kind permission of Elsevier.)

the unusual structure of their surface, which is limited by a double-elementary membrane (Figure 3.13). This structure is probably generated through the overlay of the actual elementary membrane of the schistosomulum, by membrane material exported from the inner tegumental cells, which then coalesces at the surface to form a "membranocalyx." The outer surface of these two membranes is extremely fluidic, which allows easy repair to damage and incorporation of host proteins and glycolipids. Such a disguise with host components is believed to hamper detection of the worms by the immune system. Elegant studies by Smithers and Terry, who in the 1960s transplanted worms between different hosts, clearly showed that this disguise has a survival value to the parasite. Another immune evasion mechanism of schistosomes consists in cleaving off the Fc ends of antibodies bound to the surface of the parasite by a secreted protease. In addition, potentially harmful complement components are inactivated by parasite proteins. It has been shown that schistosome eggs release potent hepatotoxins that need to be contained. Furthermore, glycoproteins are released, which polarize T helper cell responses toward the Th2 type that are involved in tissue repair, but can also lead to fibrosis (see Figure 1.37). Over time, the size of the egg granulomas has been observed to decrease. This response is also thought to be immune-mediated and regulatory T cells have been suggested to play an important role.

Interestingly, schistosomes at the same time exploit the host immune system, to facilitate the transportation of eggs through capillary walls and tissues of the gut and bladder on their journey to the external environment. This requires inflammatory responses, and the cytokine TNF-α has been shown to be of prime importance. In addition, established schistosome infections induce protective immune responses ("concomitant immunity") that kill newly intruding

schistosomula, without harming established worms. This mechanism regulates the worm population density and prevents premature death of the host. A major mechanism of protective immunity against schistosomula involves the production of IgE antibodies against tegumental antigens, which act in concert with eosinophils to destroy the invading young worms (Figure 3.11). In experimental animals, strong immunity can readily be induced by vaccination with sublethally irradiated cercariae, although the underlying mechanism of immunity is different to that seen in naturally infected humans. Vaccination trials in humans against infection with *S. haematobium* using recombinant antigens are currently underway.

Epidemiology Schistosomosis is prevalent in 76 countries in the world. More than people are infected, 85% of them in Africa. A total of 20 000 deaths occur worldwide annually. Despite considerable efforts at controlling this disease, schistosomosis is increasing. This is especially seen in areas of rice cultivation and alongside new irrigation systems that increase habitats for snails. In such tropical areas, contact with water is an important and unavoidable part of daily life. Eggs are discharged into water by defecating and urinating. Cercariae can infect people during their daily activities such as washing, bathing, fishing, or planting. Children of age 5–19 years are most vulnerable to this infection.

For *S. mansoni,* the maintenance of the life cycle is secured by reservoir hosts (Table 3.3). In Africa and the Middle East, baboons are increasingly living in the proximity of humans. Rodents, although susceptible to infection, do not play an important role as they are small and the number of eggs produced by them is less. Reservoir hosts of *S. japonicum* are numerous, but of greatest significance are pig and water buffalo that produce an enormous amount of schistosome eggs. It is hoped that in the future, transmission of infection may be reduced through relatively simple means, for example, by replacing infected working animals by tractors, or perhaps, as carried out in China, by the low-tech precautionary measure of fixing plastic bags under the tails of buffaloes to prevent the feces from falling into the water.

Cercarial dermatitis, the so-called swimmer's itch, is caused by schistosomal species of water birds that also occur in temperate regions. These include the genera *Trichobilharzia, Ornithobilharzia, Gigantobilharzia, Bilharziella,* and the Australian marine (!) *Austrobilharzia.* Their cercariae can invade the human skin, but do not develop further. Following sensitization during the first contact, they cause a severe itching lasting several days upon renewed contact. In some parts of the world, for example, South Germany, swimming lakes have sometimes to be closed during summer months due to swimmer's itch.

Control Chemotherapy is possible, for example, by means of praziquantel, a safe and reliable drug. Yet, in endemic areas, continued campaigns are necessary for complete elimination of worm burden in the whole population, as infection does not induce protective immunity. For organizational and financial reasons, this cannot be realized in most endemic countries. Finally, the best levels of control will

be through the supply of safe, that is, cercaria-free water sources, and construction of sufficient sanitary facilities in conjunction with broad education programs.

Schistosomes in Domestic Animals *Schistosoma matthei* in cattle (mesenteric and portal veins) induces a strong host-protective response, which eliminates a large part of the worm burden, thus preventing major pathological damage although heavy infections may lead to death. Sheep are not able to regulate their worm burden and suffer substantially or die. *Schistosoma bovis* (which have a similar localization to *S. matthei*) produces a chronic infection with relatively mild clinical symptoms, but is associated with a distinct delay in host growth. *Schistosoma nasale* inhabits the veins of the nasal mucosa of cattle. With this species, egg granulomas are generated within the first 10 cm behind the nostrils and granulomas cause cauliflower-like growths. Heavy nasal discharge is the consequence and infected animals exhibit a typical snoring, which explains why this type of schistosomosis is known as snoring disease.

3.1.1.6 *Leucochloridium paradoxum*

This trematode has a remarkable biology and belongs to the superfamily Brachylaimoidea (Table 3.2). This family has only one intermediate host, in which the cercariae develop into metacercariae. These then have to be eaten by the definitive host to continue the life cycle.

Leucochloridium paradoxum inhabits the cloaca of various songbirds. The embryonated eggs are ingested by *Succinea putris*, a terrestrial pulmonate snail that lives in damp vegetation. Within the snail tailless cercariae develop in long, branched sporocysts. Hundreds of such cercariae move into the distal part of the sporocyst, which forms a swollen "broodsac," which extends into one of the snail's tentacles. The larvae meanwhile transform into metacercariae with thick gelatine-like cyst walls. The broodsac has a bright greenish-brown transverse ring pattern (Figure 3.14). During daylight, the colored sporocyst inside the tentacle exhibits pulsating movements, thus mimicking a caterpillar or butterfly grub, which attracts birds. When the broodsac is picked off the tentacle and eaten, adults develop in the bird.

3.1.1.7 *Diplostomum spathaceum*

Trematodes of the superfamily Diplostomoidea, to which *Diplostomum spathaceum belongs*, are intestinal parasites of birds (Figure 3.15). Their body consists of two different sections, the proximal part, which is flat or spoon-shaped, and the distal part, which is elongated and rotund. *D. spathaceum* is a parasite of gulls feeding on freshwater fish. It has a certain economic significance, since its metacercariae cause damage in the eyes of fish.

Development Eggs are shed unembryonated. The miracidia, developing in freshwater, invade the pulmonate snail *Radix peregra* (= *R. ovata* = *R. balthica*). Cercariae developing in daughter sporocysts are furco-trichocercous (Table 3.1

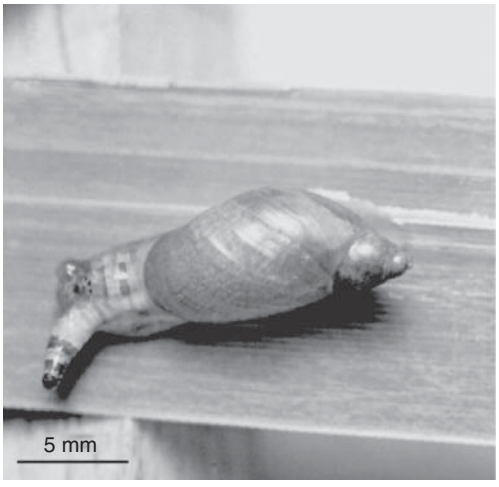


Figure 3.14 Infection of the terrestrial pulmonate snail *Succinea putris* with *Leucochloridium paradoxum*. The left tentacle is filled with a pulsating sporocyst. (Image: Archive of the Department of Parasitology, University of Hohenheim.)

and Figure 3.15i,j). They invade the second intermediate host, various freshwater fish, mainly in the region around the gills. Then, they migrate via subcutaneous connective tissue and musculature into the eye lens, where they become unencysted metacercariae, called diplostomula. After 8 weeks, these are able to infect sea gulls upon ingestion.

Morphology The adult worm measures 4 mm in length, with the flat oval forebody 1.3 × 0.6 mm and the elongated hind body 2.3 × 0.6 mm in size. The anterior end of the body is trilobate, with small lateral protuberances (lappets) on either side of the oral sucker. The forebody contains a pharynx, a very short esophagus, two long intestinal ceca, and behind the ventral sucker a large sucker-like organ, the tribocytic organ. Vitellarium follicles surround the tribocytic organ and continue throughout the hindbody. The hindbody contains a small ovary, and behind it two large testes. Only up to five light yellow eggs of size 100 × 60 µm are usually present. They are shed via a genital pore at the distal end of the hindbody.

The furcocercous cercaria (220 µm long) does not possess an oral sucker. Rather there is a long "anterior organ" of unknown function. It surrounds a long esophagus. The pharynx lies behind the anterior organ and the ceca surround the large ventral sucker. Behind the latter, four penetration glands are found, their ducts passing forward, opening next to the oral opening. Both, the stem of the tail and the two furcae are slightly longer than the body.

The metacercaria (unencysted!) measures about 400 × 233 µm. Its body consists mainly of the future forebody of the adult and contains the oral and ventral sucker and the comparatively large tribocytic organ surrounded by the ceca. The future hindbody is only seen as a short appendage.

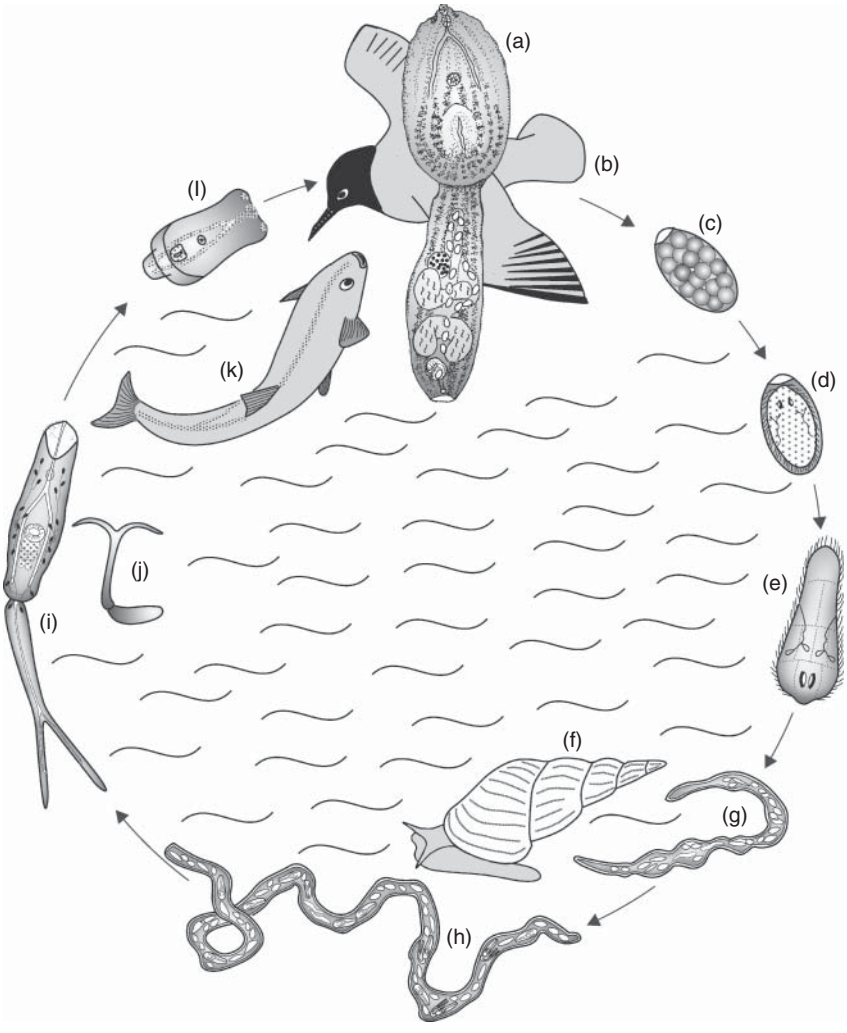


Figure 3.15 Life cycle of *Diplostomum spathaceum*. (a) Adult fluke. (b) Definitive host, the black-headed gull. (c) Unembryonated egg. (d) Embryonated egg. (e) Miracidium. (f) First intermediate host, a freshwater pulmonate *Radix peregra*. (g) Mother sporocyst. (h) Daughter sporocyst. (i) Furcocercous cercaria. (j) Cercaria in swimming position. (k) Freshwater fish. (l) Metacercaria (diplostomulum) in the eye of a fish.

Pathology The cercariae migrate to the eye in the second intermediate host, the fish, destroying tissues of several body parts during this process. This can be fatal for young fish such as fry, with death occurring within 3 days of infection. Moreover, the metacercariae, having invaded the vitreous body and the eye lens, cause an opacity (cataract), leading to blindness, so that the fish cannot find food efficiently. Furthermore, the escape behavior of the fish is altered and it

has a reduced capacity to escape predators such as gulls. This modification of the behavior increases the parasite's chances of survival at the expense of the second intermediate host. In large ponds, where control of snails is not possible, epidemics of *Diplostomum* can occur during summer months.

3.1.1.8 *Fasciola hepatica*

The common liver fluke, a member of the superfamily Echinostomatoidea, is one of the most important parasites of ruminants in temperate climates and the most important representative of the family Fasciolidae. Unfortunately, the common liver fluke is often taken as a prime example for the digenean trematodes. However, this is misleading as *Fasciola* is atypical of the flukes. First, ruminants are not typical hosts for Digenea (as they rarely ingest animals – normally the second intermediate hosts); second, bile ducts are rarely inhabited by trematodes; third, a metacercaria encysting on plants is a rarity for digeneans, and finally, the morphology of adult *Fasciola hepatica* is markedly modified compared with "typical" Digenea and does not provide a good model for comparison of structures common in other trematodes.

Development The eggs are unembryonated when shed with the feces of the final host (Figures 3.16 and 3.57). In water, the eggs mature within 2–3 weeks and release miracidia that invade the first intermediate host. This is an aquatic pulmonate gastropod of the family Lymnaeidae, the pond snails, *Galba truncatula* in Europe. (Intermediate hosts are *Galba humilis* in the New World, *Fossaria bulimoides* in the United States, and *Pseudosuccinella columella* in South America.) *Galba truncatula* is only 6–8 mm in size and lives at the margins of water bodies, where it feeds on layers of algae. Because of this, the snail is difficult to cultivate in the laboratory. After 7 weeks, cercariae – produced via mother sporocysts and several generations of rediae – leave the snail. The cercariae encyst on green submersed leaves of water plants preferentially on the underside. There they form a hemispheric metacercaria with a multilayered cyst wall. Under humid conditions, the metacercariae stay infective for up to several months and can survive temperatures below freezing. However, they are quite susceptible to desiccation.

Following ingestion (together with the vegetation) by the final host, excystation occurs in its stomach and duodenum. The young worms then bore through the intestinal wall and move from the abdominal cavity through the liver capsule. During the following 6–8 weeks, they migrate in the liver parenchyma and then invade the bile ducts, where they become sexually mature. Eggs shed by female worms are transported with bile via the common bile duct into the intestine. The prepatent period (time until eggs are shed) is between 55 and 105 days. Approximately 5000–20 000 eggs are laid by an adult female worm per day.

Morphology The leaf-shaped worm (up to 300 × 139 mm in length) has a proximal cone-shaped projection containing the small oral sucker and the first part of the intestinal ceca with diverticula, although the main parts of the highly branched

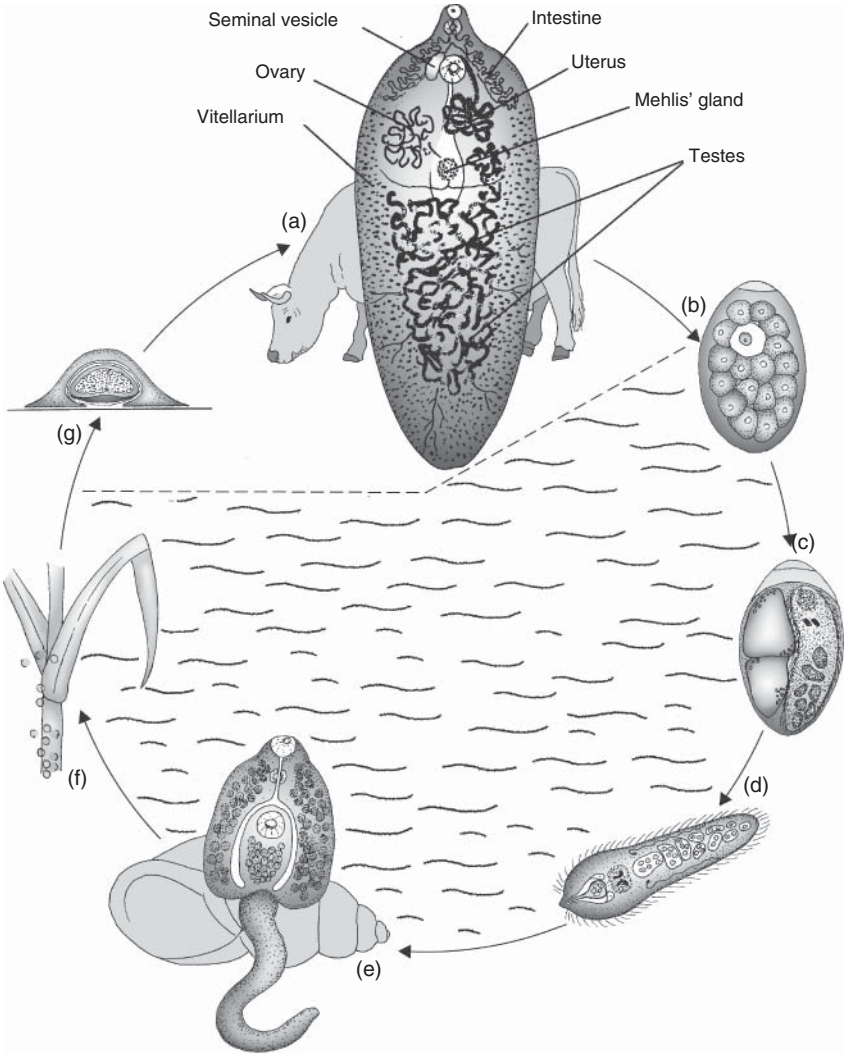


Figure 3.16 Life cycle of *Fasciola hepatica*. (a) adult trematode in the bile ducts of ruminants (only the first part of the intestinal ceca are drawn). (b) Unembryonated egg. (c) Embryonated egg. (d) Miracidium. (e) Gymnocephalic cercaria developing within rediae in the first intermediate host *Galba truncatula* (Pulmonata). (f) Metacercariae on water plant. (g) Cross section of a metacercaria.

intestinal ceca are not visible without the use of special stains (Figure 3.17). In the widening area of the body, the larger ventral sucker, a large cirrus pouch, and the genital opening are visible. Behind the ventral sucker the tightly coiled uterus and ovary are situated, both slightly located laterally. Between and behind them lies the Mehlis' gland. The two highly branched, almost inseparable testes are

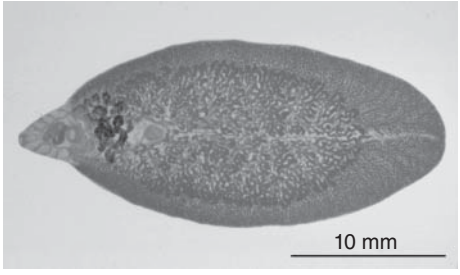


Figure 3.17 *Fasciola hepatica*, stained whole mount (see also Figure 3.16a). (Image: Archive of the Department of Parasitology, University of Hohenheim)

arranged in tandem behind the ovary, where they occupy a large part of the body. The vitellaria surround the genital organs as broad lateral bands and are confluent behind the testes. The eggs appear pale yellow and measure 130–140 × 70 µm. When observed in large numbers in the uterus, they appear as a dark brown cluster.

Epidemiology The common liver fluke is distributed across all the continents, mainly in humid lowland areas, because the snail host, *Galba truncatula* occurs on the margins of flat stagnant water bodies or small, slowly flowing streams. However, the snail also inhabits areas that have become temporarily flooded, or temporary accumulations of water such as those caused by the hoof prints of cattle at drinking troughs. In such wet habitats, the reproductive potential of *G. truncatula* is extraordinarily high. Thus, in areas where cattle graze on good, wet grassland, the infection can cause considerable economic losses. Nevertheless, in places where sheep farming predominates, fasciolosis has great economic significance, with sheep being more susceptible and less able to control infection than cattle.

F. hepatica can infect a wide range of hosts. It can infect nearly all types of herbivorous farm animals as well as wild animals such as hares and rabbits. It is believed to infect more than 2 million people worldwide, especially in areas where people eat water-dwelling plants such as watercress, *Nasturtium officinale*. If this is harvested from *Fasciola* endemic areas, it can readily carry metacercariae.

Pathology The main damage is caused by the young worms migrating through the liver parenchyma for several weeks. They give rise to sinuous blood-filled ducts, which later fibrose. In sheep, this acute phase causes anemia, loss of weight, and lethargy. Furthermore, hemorrhage and death can occur during the prepatent period of 12–20 weeks post infection. In cattle, this phase of infection can be asymptomatic. The chronic phase begins when the worms reach the bile ducts. Changes in the biology of the epithelial cells at this site cause the tissue to become porous and permeable, so that components of blood plasma can pass from the liver into the bile ducts. Albumin deficiency results, which leads to oedema, which is most apparent in the submandibular region ("bottle

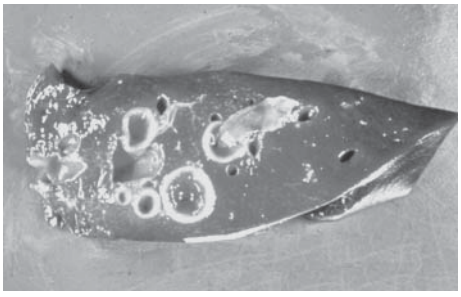


Figure 3.18 Section through a cattle liver with *Fasciola hepatica*. Note the thickened walls of the bile ducts. Right middle: a whole fluke can be seen leaving a bile duct. (Image: Archive of the Department of Parasitology, University of Hohenheim.)

jaw syndrome"). In addition, the muscles can also become fluid-filled. In cattle, hydroxylapatite is often deposited in the submucosa of the bile ducts, which is visible as white, hard, finger-sized tubes along the liver surface (Figure 3.18). In cattle, protective immunity is observed and leads to expulsion of adult worms. Subsequent infections show lower worm burdens and smaller parasites. In sheep, however, there is little evidence of protective immunity. Infections can persist for years and allow re- and superinfections.

Fasciola gigantica of ruminants, occurring in southern Asia, Southeast Asia, and Africa, is very similar to *F. hepatica* and sophisticated molecular techniques are required to correctly identify and diagnose the infection.

3.1.1.9 *Opisthorchis felineus*

Opisthorchis felineus, the cat liver fluke infects piscivorous (fish-eating) mammals such as cats, foxes, otters, and also humans in Eastern Europe and Russia. In some rural areas of Russia, 85% of the population were said to be infected. *O. felineus* belongs to the family Opisthorchiidae, whose members inhabit the gallbladder and bile duct of many species of vertebrates except fish. The name Opisthorchis indicates that the testes (Greek: *orchis*) are situated behind (Greek: *opistho*) in the body. The vernacular name of the adult worm is liver fluke, which is somewhat misleading, as the parasites inhabit the gall ducts, but do not actually live in the liver parenchyma.

Development *O. felineus* produces embryonated eggs containing miracidia, which invade the freshwater snail *Bithynia leachi* (Figure 3.19). The cercariae produced via rediae are released by the snail and exhibit a characteristic floating position in water with the curved body hanging from the perpendicular tail. In contact with the waterbed or hard objects, they immediately gyrate upward again, thus enhancing chances of contact with a second intermediate host, which are cyprinid fish such as minnows and barbels. The cercariae invade the subcutaneous connective tissue or musculature of the fish, where they become very robust, resistant metacercariae withstanding fridge temperatures, drying,

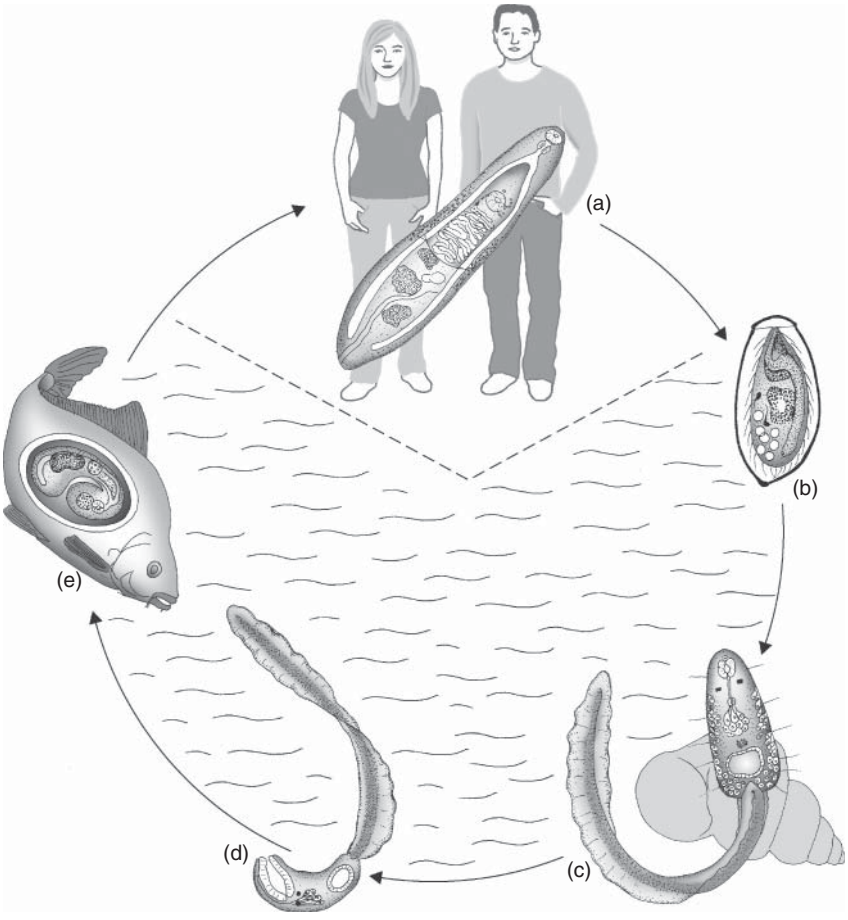


Figure 3.19 Life cycle of *Opisthorchis felineus*. (a) Adult trematode in the bile ducts of humans (see also Figure 3.9d). (b) Embryonated egg. (c) Lophocercous cercaria in the first-intermediate host snail *Bithynia leachi*. (d) Cercaria in characteristic floating position. (e) Enlarged metacercaria in the musculature of second intermediate host, a carp.

salting, marinating, or pickling for a considerable time. Once ingested by a definitive host, the young flukes migrate from the duodenum into the bile duct and in heavy infections also into the pancreatic duct. The prepatent period is 3–4 weeks. There have been reports of infections lasting up to 25 years.

Morphology *O. felineus* is an elongated and slender fluke (Table 3.4, Figure 3.5a). The long and heavily coiled seminal vesicle lies next to the ventral sucker. The cirrus is not surrounded by a cirrus pouch. The strongly coiled uterus occupies a considerable portion of the body. It is followed by the ovary and then the two testes. The lateral vitellaria have approximately the same length as the uterus. The

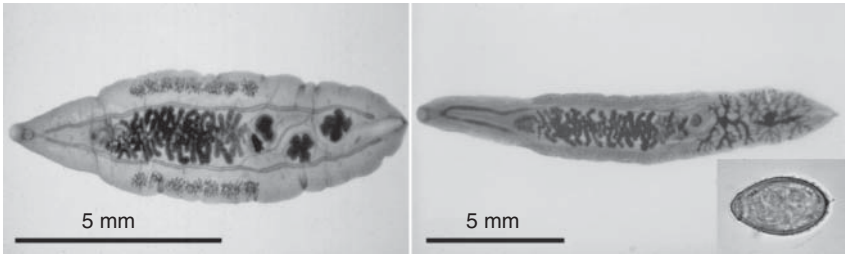
Table 3.4 Comparison of important species of Opisthorchiidae.

	Opisthorchis felineus	*Opisthorchis viverrini*	*Clonorchis sinensis*
Distribution	Russia, East Europe	Thailand, Laos, Vietnam, Cambodia, Malaysia	China, Japan, Korea, Taiwan, Thailand, Vietnam
Infected humans	not known	10 million	35 million
Life span in humans	not known	10 years	26 years (proven)
Adult worms, length (mm)	5–12	7–12	10–25
Width (mm)	Up to 3	2–3	3–5
Shape of testes	Clover-leaved with 4–5 lobes	As in *O. felineus*	Highly branched
Egg size (μm)	26–30 × 11–15	27 × 15	29 × 17
1. Intermediate host	*Bithynia leachi*	*Bithynia s. siamensis, B. s. goniomphalus, B. laevis, B. funiculata*	*Parafossarulus striatulus, Alocinma longicornis* (Hydrobiidae), *Bithynia fuchsianus*
2. Intermediate host	Mainly cyprinid fish	fish	fish

intestinal ceca pass to the distal end of the body. The eggs are yellow to brown in color and the operculum is bordered by a thickening. A long, tube-like organ is present in the miracidium, and is thought to be a gland of some kind. The lophocercous cercaria (Table 3.1) has two eyespots, a large oral sucker, short esophagus, small pharynx, and no gut. The ventral sucker is also small. There is a large excretory bladder with a conspicuous epithelial lining. The tail is provided with a dorsal fin-fold running up to the tip of the tail (Figure 3.19).

Pathology In humans, symptoms occur typically in heavy infections of 100 flukes upward. Infected individuals will suffer from intestinal inflammation with diarrhea, general morbidity, and changes of liver and pancreas function. In infections of long duration (several years), bile duct cancer may develop.

Other Important Opisthorchiids *O. viverrini*, the Southeast Asian liver fluke, and *C. sinensis*, the Chinese liver fluke are important species of Opisthorchiids (Figure 3.20). Their life cycles are the same as in *O. felineus*. The diseases caused by them, opisthorchiosis, can be very severe in infections with *O. viverrini*, which predispose infected people to cholangiocarcinoma, a cancer of the gallbladder and/or its ducts. *C. sinensis* causes obstruction of the bile duct (cholangitis), diarrhea, abdominal pain, jaundice, and ascites. For specific features of the three parasites, see Table 3.4



(a) (b)

Figure 3.20 Stained whole mounts of the cat liver fluke *Opisthorchis felineus* (a) and the Chinese liver fluke *O. sinensis* with egg (b). Note the very different shapes of the testes in the hind body and of the ovary situated anterior to them. The seminal receptacle is found adjacent and in front of the ovary (not clear in *O. sinensis*). (Images: Archive of the Department of Parasitology, University of Hohenheim.)

3.1.1.10 *Paragonimus westermani*

The oriental lung fluke (Table 3.2) of the family Paragonimidae inhabits crab-eating mammals and humans in East and Southeast Asia. More than 50 species occur worldwide, but not all of them become sexually mature in humans.

Paragonimus westermani exists as a diploid, triploid, and occasionally tetraploid species. In the diploid form (22 chromosomes), two adults are enclosed in one lung cyst and insemination is reciprocal. The triploid form (33 chromosomes) found in East Asia is sympatric with the diploid species, has an aberrant spermatogenesis, and thus propagates through parthenogenetically developed eggs. The single adult in a lung cyst is larger than the diploid worm and produces more eggs.

Development The life cycle (Figure 3.21) is dependent on freshwater. The first intermediate hosts in Malaysia, Thailand, and the Philippines are snails of the superfamily Cerithoidea with the family Thiaridae (*Thiara, Melanoides*), and the related *Semisulcospira libertina* in China, Japan, Korea, and Taiwan. Microcercous cercariae (see Table 3.1) develop in rediae. The cercariae, which are poor swimmers, because of their small, stub-like tail, enter crabs in the water bed. The encysted metacercariae are found in organs and muscles of the crabs. They have a very characteristic morphology (Figure 3.21f). The two arms of the winding intestine enclose the broad excretory bladder, which under transmitted light has a black appearance due to the presence of granules.

The final hosts are mammals, which feed on freshwater crabs. Humans become infected by eating infected crabs, raw or poorly preserved (pickled, salted) prawns, or shrimps. After excystation in the duodenum, the young worms bore through the intestinal wall and move from the body cavity through the diaphragm. Once in the pleural cavity they reach the lungs where as adults they become encased in host connective tissue, with an open connection to the airways. The shed eggs reach the airways and are transported by the ciliated epithelium of the lung into the bronchi. They are then coughed up and expelled with sputum or, after having been swallowed, excreted with feces. Furthermore, in humans, "ectopic" infections can

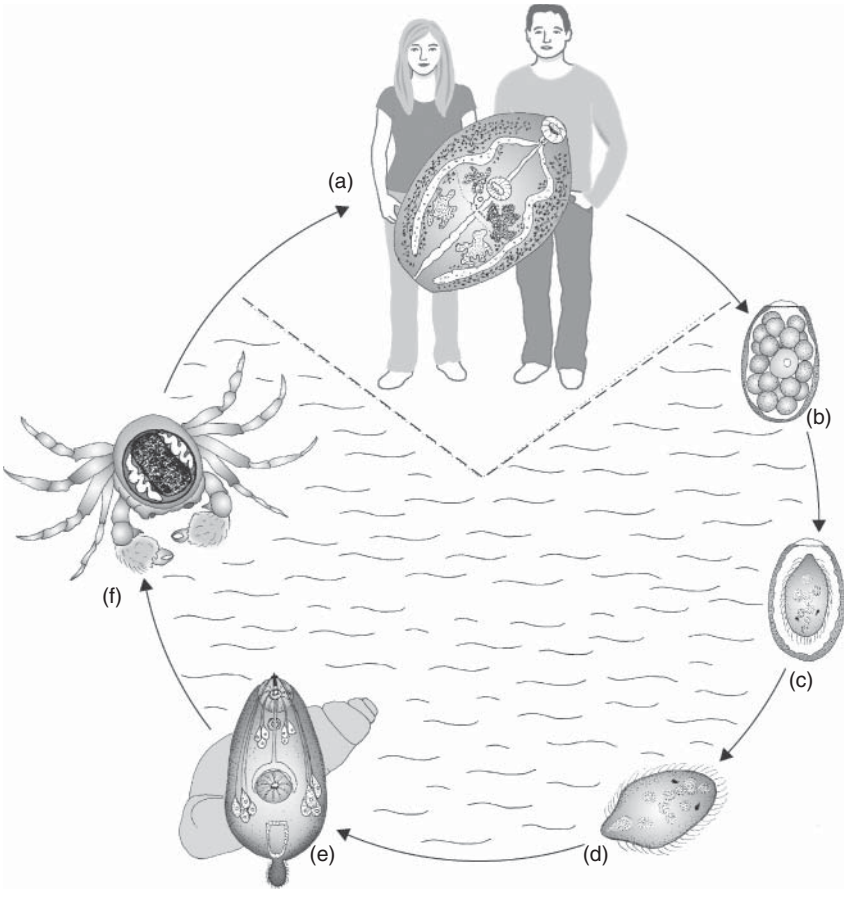


Figure 3.21 Life cycle of *Paragonimus westermani*. (a) Adult trematode in the lung of humans (see also Figure 3.5e). (b) Unembryonated egg. (c) Embryonated egg. (d) Miracidium. (e) Mircrocercous cercaria developing within rediae in the first-intermediate snail host *Semisulcospira* sp. (f) Enlarged metacercaria in a fresh water crab (black: excretory bladder, white and curled: intestinal ceca).

occur (Greek: *ek* = outside of, *tópos* = locality, abnormal position), where brain, heart, or spinal cord are infected.

Morphology The worms are thick and oval shaped (Figure 3.9e), measuring 8–12 mm in length. Their tegument is heavily covered with scale-like spines. In front of the deeply lobed testes in the rear part of the body, the lobed ovary is found on the right-hand side of the body and the tightly coiled uterus on the left-hand side. Between them and posteriorly to the ventral sucker, the genital opening and a long excretory bladder are located. These organs are enclosed by the two arms of the intestine, which are surrounded by conspicuous vitellaria,

which almost conceal the genital organs. The eggs appear light yellow, have a distinct operculum, are shed unembryonated, and measure 70–90 × 40–55 µm.

Pathology Pulmonary paragonimosis is a chronic disease associated with cough, bronchitis, blood-flecked brownish sputum, and shortness of breath. In heavy infections, it is also associated with fever and cachexia. The triploid species of *P. westermani* causes more severe lung symptoms than the diploid species. Another form of the disease, often more serious, is caused by ectopically located worms, which produce eggs in the pleura, abdomen, or brain. Abscesses and accumulation of ascites or fluid in the pleural cavity are often observed. Worms in the brain can result in epilepsy-like symptoms, encephalitis, meningitis, calcification, blindness, and paralysis. Ectopic infections and associated symptoms are also caused by *Paragonimus* species that do not normally become sexually mature in humans.

3.1.1.11 *Dicrocoelium dendriticum*

The lancet liver fluke or small liver fluke (in contrast to the large liver fluke *F. hepatica*) is a trematode with a fascinating biology. The members of the family Dicrocoeliidae, to which it belongs, inhabit bile duct, gallbladder, and pancreatic duct of reptiles, birds, and mammals. Almost all of them have terrestrial life cycles. This is why their eggs are shed embryonated and have to be *eaten* by the first intermediate host, which is a snail. Arthropods always serve as second intermediate hosts.

D. dendriticum (Syn.: *D. lanceolatum*) lives in the gallbladder of even-toed ungulates and occasionally other herbivorous mammals. Humans can become infected, but very rarely. The parasite, originating in Eurasia, has been nowadays found in many countries of the Old and the New World, usually in drier habitats.

Development When shed, the eggs contain a fully developed miracidium (Figure 3.22). The eggs are eaten by pulmonate, terrestrial, and xerophilic snails, which predominantly live on decaying plants. This indicates that they also feed on the droppings of herbivorous animals. In Middle Europe, the first intermediate hosts are *Zebrina detrita* (Figure 3.23) and species of the genus *Helicella*. In more humid regions of the United States, the favored host is *Cochlicopa lubrica.*

Cercariae develop from daughter sporocysts. They migrate to the snail's respiratory chamber, where commonly 100 or more mature cercariae stick together and are enveloped with solid mucus, forming a sphere of about 1 mm in diameter. These mucus balls are released one-by-one through the breathing hole of the snail until 5–10 of the white aggregates are assembled. Their appearance is similar to the snail's egg mass.

These mucus balls are apparently very attractive to certain ants of the genus *Formica,* living in the same habitat favored by the snail. The ants feed on the mucus balls, and in the crop, the cercariae throw off their tail. By rotating movements, they bore through the wall of the crop, which is sealed behind them. The sites through which the cercariae escape appear dark brown in color later on (Figure 3.22e).

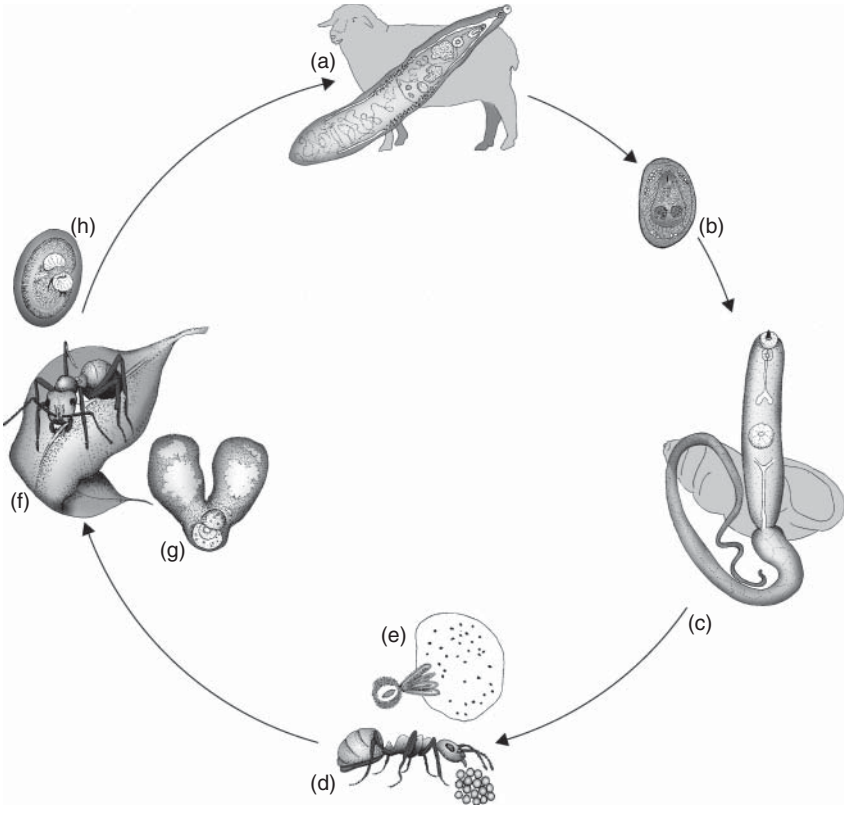


Figure 3.22 Life cycle (of *Dicrocoelium dendriticum*. (a) Adult trematode in the bile ducts of ruminants (see also Figure 3.9a). (b) Embryonated egg with fully developed miracidium. (c) Xiphidiocercous cercaria developing in sporocysts in the first-intermediate host *Zebrina detrita* (Pulmonata). (d) Second intermediate host *Formica* sp. eating from a slime ball released by the snail. (e) Ant's crop with melanized dots left behind from cercariae having penetrated its wall. (f) Ant clinging to a leaf with its mandibles. (g) "Brain worm" in the ant's subesophageal ganglion. (h) Infective metacercaria from the hindbody of the ant.

The cercariae, now in the hemocoel of the ant, migrate forward to the head. One of the cercariae invades the subesophageal ganglion innervating the mandibles of the ant and transforms to a metacercaria, the so-called "brainworm" (Figure 3.22g). It lacks a cyst wall and therefore can never become an adult worm in the definitive host. The remaining cercariae migrate backwards and settle in the hemocoel of the abdomen, where they encyst to become true metacercariae. Sometimes, their discarded mouth stylet can be seen within the thick hyaline cyst wall. After 6–8 weeks, the metacercariae become infective.

When the true metaceraciae in the gaster becomes infective, a modification of the ant's behavior begins. In the evening, the ant climbs to the tip of grass blades or low plants and attaches itself to the foliage by its mandibles. The spasm of the

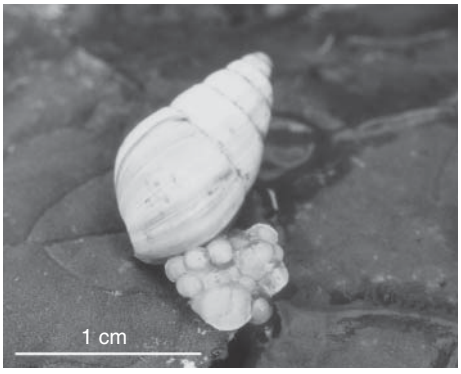


Figure 3.23 *Zebrina detrita* with slime ball of *Dicrocoelium denriticum*. (Image: Loos-Frank.)

mandibles lasts the whole night and ceases only in the morning hours with rising temperatures. The ant can then fulfill its daily tasks until the following evening, when it again climbs onto plants. As the behavioral modification exposes the ant to herbivorous mammals and prevents it from moving away, it increases the chances of being ingested during the night and early morning hours, when grazing is most common. After ingestion by the final host, and digestion of the cyst wall, the young worms migrate directly through the common bile duct into the intrahepatic bile ducts, where they become adults. The prepatent period is at least 8 weeks.

Ants of the genus Camponotus infected with the African lancet liver fluke species Dicrocoelium hospes also climb up plants – they do not bite into them, however, but simply sit there day and night.They are fed by passing nestmates and only leave their post temporarily when severely disturbed, which increases the chances of being eaten by a herbivore host.

Morphology The eggs of *D. dendriticum* are small (40 × 25 µm), thick-shelled, and dark brown in color with a distinct operculum. When shed, the eggs already contain a miracidium. It has a granulated apical gland in its proximal part and the distal part is occupied by two conspicuous masses, the germ balls. The cercaria (measuring approximately 560 × 130 µm) is gymnocephalous and xiphidiocercous (Table 3.1). There is the typical stylet found in this suborder. Between the small oral sucker and the larger ventral sucker is a tiny pharynx, a long esophagus, and two very short intestinal ceca. The excretory bladder is long and thin. The tail (~700 µm long) has a long tapering end. The adult worms measure 12 × 2 mm. The uterus comprises the largest part of the body. Untypically, the ovary lies behind the testes.

Epidemiology In Europe, the life cycle takes place most commonly in dry environments, mainly on the south side of limestone mountains, where the xerophilic snails and certain species of *Formica* live. The snails shed cercariae during spring

to early summer and again during autumn. Thus, the definitive host species are those domestic animals that mainly graze on poor, dry vegetation, which, in Europe, are predominantly sheep. Other hosts include cattle, goats, roe deer, red deer, horses, donkeys, hares, rabbits, and marmots.

Pathology As the young worm does not migrate through the liver, *D. dendriticum* is much less pathogenic than *F. hepatica*. Heavy infections may cause proliferation of the intrahepatic bile duct epithelium, emaciation, and liver cirrhosis. Sheep with a burden of 15 000 or more flukes can die. The presence of a few worms in an apparently healthy liver, however, can still lead to condemnation of the organ as unfit for human consumption. Interestingly, individuals that are known to have eaten such a contaminated liver have been observed to excrete *Dicrocoelium* eggs with their feces.

Further Reading

Arango Barrientos, M. and Carrasco, A.U. (2012) Paragonimiasis. *N. Engl. J. Med.*, **366**, 165.

Berriman, M., Haas, B.J., LoVerde, P.T. *et al.* (2009) The genome of the blood fluke *Schistosoma mansoni*. *Nature*, **460**, 352–358.

Colley, D.G., Bustinduy, A.L., Secor, W.E. *et al.* (2014) Human schistosomiasis. *Lancet*, **238**, 2253–2264.

Han, Z.G., Brindley, P.J., Wang, S.Y., and Chen, Z. (2009) Schistosoma genomics: new perspectives on schistosome biology and host-parasite interaction. *Annu. Rev. Genomics Hum. Genet.*, **10**, 211–240.

Hathaway, R.P. and Herlevich, J.C. (1976) A histochemical study of egg shell formation in the monogenetic trematode *Octomacrum lanceatum* Mueller, 1934. *Proc. Helm. Soc. Wash.*, **42**, 272–274.

Loker, E.S. and Brant, S.V. (2006) Diversification, dioecy and dimorphism in schistosomes. *Trends Parasitol.*, **22**, 521–528.

Olson, P.D., Cribb, T.H., Tkach, V.V., Bray, R.A., Littlewood, D.T. (2003) Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *Int. J. Parasitol* **33**, 733–55.

Park, J., Kim, K., Kang, S., Kim, W., Eom, K.S., and Littlewood, D.T.J. (2007) A common origin of complex life cycles in parasitic flatworms: evidence from the complete mitochondrial genome of *Microcotyle sebastis* (Monogenea: Platyhelminthes). *BMC Evol. Biol.*, **7**, 11.

Petney, T.N., Andrews, R.H., Saijunta, W. *et al.* (2013) The zoonotic, fishborne liver flukes Clonorchis sinensis, Opisthorchis felineus and Opisthorchis viverrini. *Int. J. Parasitol.*, **44**, 1031–1046.

Rollinson, R. and Chappel, L.H. (2001) *Flukes and Snails Re-visited*, Parasitology, vol. **123**, Supplement, Cambridge University Press.

Sripa, B., Brindley, J.P., Mulvenna, J., Laha, T., Smout, M.J., Mairiang, E., Bethony, J.M., and Loukas, A. (2012) The tumorigenic liver fluke *Opisthorchis viverrini*-multiple pathways to cancer. *Trends Parasitol.*, **28**, 395–407.

Tolan, R.W. (2011) Fascioliasis due to *Fasciola hepatica* and *Fasciola gigantica* infection: an update on this 'neglected' neglected tropical disease. *Lab. Med.*, **42**, 107–116.

Toledo, R. and Fried, B. (eds) (2014) *Digenetic Trematodes*, Springer, New York.

Test Questions

1. What are platyhelminths and which groups belong to them?
2. What are digenetic trematodes?

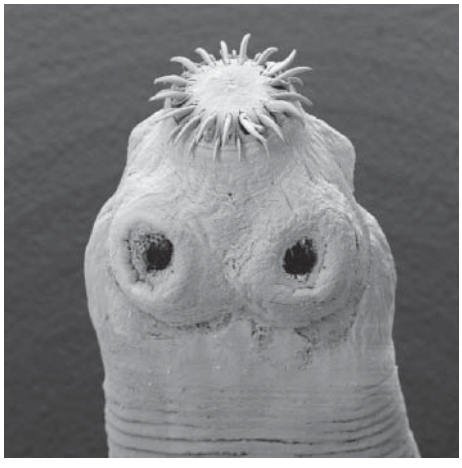
3. What are the main morphological features of trematodes?
4. What is characteristic for the life cycle of a digenean trematode?
5. Which stages occur in the life cycle of a digenean?
6. Which part of the life cycle of trematodes is most important for their propagation?
7. Which organ most often harbors adult trematodes?
8. Which is the second intermediate host of trematodes?
9. Which are the biotopes of *Fasciola hepatica*?
10. Does the cat liver fluke play a role for humans? If so, why?
11. How does *Dicrocoelium dendriticum* reach its final host?
12. Describe basic facts of *Paragonimus westermani*.
13. What are damages caused by *Diplostomum spathaceum* infection of fish?
14. What are the differences between Schistosomatidae and other trematodes (at least four answers)?
15. What is the most pathogenic stage of schistosomes?
16. Which are the most important schistosome species and where do they occur?
17. What is the localization of these species in the definitive host?
18. What are the intermediate hosts of the schistosomes?
19. Do Schistosome species play a role in temperate climates?

3.1.2 Cestoda

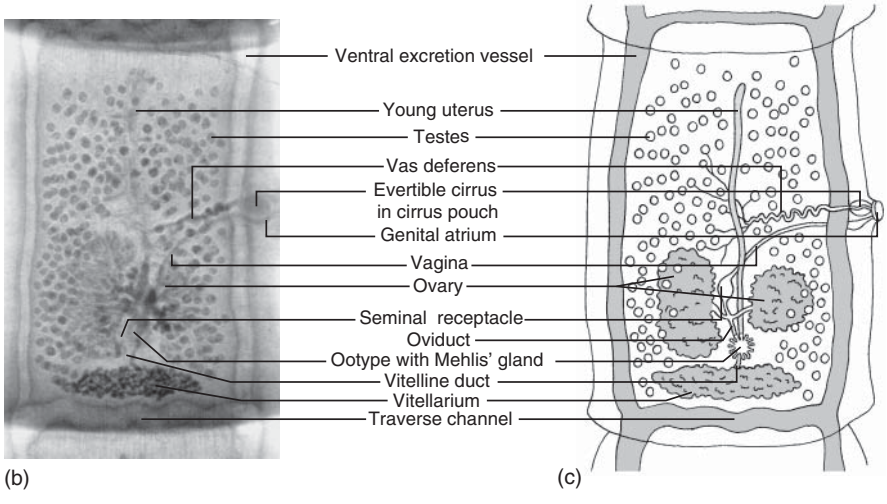
- Adult worms are intestinal parasites of all vertebrates.
- Typically polyzoic organisms, consisting of a scolex ("head") and many individual segments, the proglottids.
- Surface is a neodermis covered by microtriches (microvilli with electron-dense tips).
- Devoid of a mouth and intestine.
- Larva with six hooks, the hexacanth, or oncosphere.
- Metacestode with very variable morphology.
- Infection is acquired by ingestion of food.

The most important group of Cestoda is the subclass Eucestoda, the true cestodes or tapeworms, which will be dealt with here. Most adult tapeworms are parasites of cartilaginous fish (Chondrichthyes) or bony fish (Osteichthyes), but all members of higher groups as the Diphyllobothriidea, Cyclophyllidea and the family Mesocestoididae have vertebrates (excluding fish) as definitive hosts.

Damage by adult tapeworms, and thus the impact upon humans and domestic animals, is limited. As for most intestinal worms, pathology only occurs in heavy infections. However, larval stages of a single family of the Cyclophyllidea, the Taeniidae, can cause considerable problems.



(a)



(b) (c)

Figure 3.24 (a) Scolex of a tapeworm with two crowns of hooks and two of the four suckers. Note beginning of the proliferation zone in the lower part. (*Hydatigera taeniaeformis,* REM image: Courtesy of Eye of Science.) (b, c) Stained whole mount of a mature proglottid of *Taenia polyacantha*, containing male and female reproductive organs. (Image: Loos-Frank.)

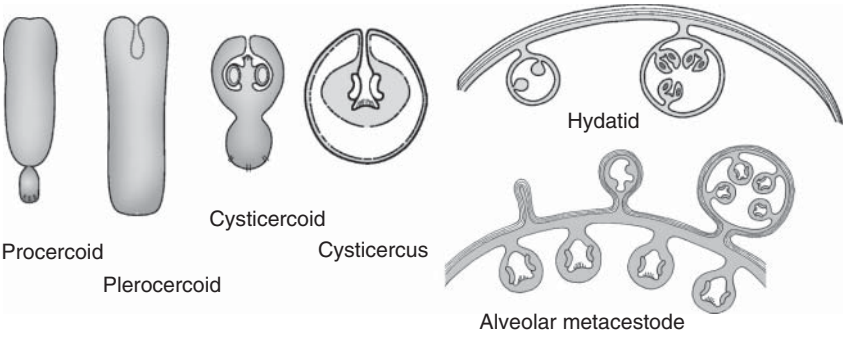


Figure 3.25 Types of metacestode stages of the Eucestoda (schematic).

3.1.2.1 **Development**

The life cycle of Eucestoda comprises three stages, which all arise directly from one another:

- The first larval stage, termed oncosphere (Greek: *ónkos* = hook, *sphaira* = sphere), also called a hexacanth larva (Greek: *hex* = six, *àcantha* = thorn),
- the second larval stage, termed metacestode (Greek: *méta* = after),
- and the adult worm.

The larval stages of the various taxa differ regarding their morphology and have thus been termed very differently, often they have even species-specific designations. For the main types of metacestodes, see Figure 3.25. Asexual proliferation of larval stages is only present in a few Cyclophyllidea. Intermediate and definitive hosts of the Eucestoda are infected by oral uptake of a larval stage. In general, two modes of development are possible:

- The oncosphere develops inside the eggshell within the uterus of the adult worm. It is shed with the feces of the final host and is taken up orally by the (only) intermediate host. It penetrates the intestinal wall and differentiates into a metacestode. When the infected intermediate host is ingested by the final host, the metacestode settles in the intestine and matures to become an adult tapeworm. This is the case in all Cyclophyllidea.
- The oncosphere develops from an unembryonated egg that has been released into water with the feces of a final host. In this case, the hatching larva is ciliated and is called a coracidium (see *Diphyllobothrium*). This larva has to be ingested by an invertebrate intermediate host, penetrates the intestinal wall, and differentiates into a metacestode called procercoid. When the first intermediate host is eaten by a predator, the procercoid develops into a metacestode called plerocercoid within the second intermediate host's body cavity or organs. This plerocercoid is infectious for the final host. This three-host type of life cycle is described for *Diphyllobothrium latum.*

When a host is infected by a high number of tapeworms, a phenomenon called "crowding" may occur: the worms and their biomass remain small and the number of proglottids generated per day as well as the number of eggs within them is decreased. There are several possible causes: (i) competition for nutrients, (ii) worm-derived cross-inhibition of growth, (iii) host immune control, or (iv) a combination of these factors.

3.1.2.2 Evolution and Origin of Life Cycles

The fact that fish are parasitized by many orders suggests an origin of the Eucestoda in these hosts. Phylogenetic analyses suggest that the parasites underwent a radiation within the ray-finned fish Actinopterygi 350–400 million years ago and from there acquired terrestrial vertebrate hosts via lobe-finned fish (Sarcopterygii).

Theories about the emergence of modern life cycles of Eucestoda diverge substantially. In cestodes, it is unclear whether the oncosphere, common to all Eucestoda, parasitized a vertebrate as future final host first, or an invertebrate as a future intermediate host first. As a tapeworm's chances of completing its life cycle via the food chain are low, the reproductive potential had to be enhanced considerably in one of the stages. This main reproductive stage is the adult worm within the vertebrate host, which produces thousands of eggs per day. This host offers sufficient space for the worm to reach a considerable size, while the much smaller intermediate hosts – such as small crustaceans, insects, and mites do not offer space for massive reproduction to occur. This is in stark contrast to trematodes, where the adults are typically rather small and can carry only a few eggs, while the main reproduction was shifted to the gastropod.

3.1.2.3 Morphology

The **eggs** of Eucestoda are round and consist of the egg cell and one to several yolk cells. The eggshell is of complex composition. During embryogenesis, several envelopes and layers are built, which partly develop from each other and can be lost again.

The **oncosphere** is round shaped. At its distal part, it carries six hooks arranged in pairs. The two middle ones are usually longer and more slender than the four outer ones. The larva has a U-shaped penetration gland with two lateral openings in the hook region, hook musculature, and cells from which the scolex is ultimately derived. Usually protonephridia are present, and calcareous granules are present as also can be observed in the parenchyma of metacestodes and adults.

In the intermediate host, the oncosphere penetrates the intestinal wall by means of enzymes of its penetration gland. In the body cavity, the transformation into the **metacestode** begins. Metacestodes are round, oval, or oblong larvae, and solid or contain a cavity, with a white appearance due to the calcareous granules in their parenchyma. As soon as they become infective, they have a fully developed scolex. The distal part has a tail-like appendage, called a cercomer (Greek: *kérkos* = tail, *méros* = part of). First, the infective metacestode still carries the six oncospheral hooks at this cercomer, which eventually are shed. When the infected

intermediate host is eaten by a final host, the metacestode sheds the material behind the scolex or absorbs it, and grows to become an adult tapeworm in the intestine.

Adult cestodes measure a few millimeters (some species of *Hymenolepis* and the genus *Echinococcus*) up to several meters (*Taenia*) or even 20 m (*Diphyllobothrium*). They are long, slender, and flattened dorsoventrally. Only members of one order, the Caryophyllidea, are monozoic, that is, have an unsegmented body with only one set of hermaphroditic genital organs. Members of all other orders are polyzoic: the genital organs are arranged serially and separated from one another by visible delineations, forming the proglottids. The proglottids collectively form the **strobila**, extremely narrow in its anterior part and broadening towards the posterior end. The anterior part carries the **scolex**, sometimes referred to as "head," but is just a widening of the body with holdfasts (hooks and suckers), but without a mouth or intestine. The holdfast organs of the scolex anchor the worm in the mucosa of the host's intestine. Depending on the specific order, there are suckers (round, muscular structures with a central indentation), tentacles (long, finger-like appendages) and bothria or bothridia (elongated structures with grooves or suckers). The surface of the scolex is rich in sensory cells. The effect of the suckers can be enhanced by hooks. These consist of a keratin-like substance and develop from specialized microtriches. The scolex hooks are important for morphological species identification, but drop off very quickly after the death of the worm. According to the number of their holdfast organs, tapeworms are called mono-, bi-, or tetrafossate (see Table 3.5).

Behind the scolex follows a narrow part, the neck or **proliferation zone**. All cell types needed to form a proglottid originate here.

The cestodes are **protandric hermaphrodites** (Greek: *protos* = the first, *andrós* = the male). The maturation of the male genital organs is completed in anterior proglottids, and that of the female organs in the posterior ones. The last part of the strobila consists of gravid proglottids, which visibly contain only the egg-filled uterus (Figure 3.26). In general, the last proglottids are shed singly in more or less regular intervals. Principally, the genital organs have the typical configuration of Platyhelminths. The cestodes, however, possess several testes in each proglottid. And there is a vagina for the introduction of sperm. A uterine opening is absent in the Cyclophyllidea.

Table 3.5 Some important taxa of the Eucestoda.

Taxon	Final hosts	Morphological characters
Diphyllobothriidea	Fish-eating reptiles, birds, terrestrial mammals	Two bothria, polyzoic
Mesocestoiidae[a)]	Carnivores or birds of prey	Four suckers, polyzoic
Cyclophyllidea	Vertebrates without fish	Four suckers, polyzoic

a) Systematic position of the family not yet known.

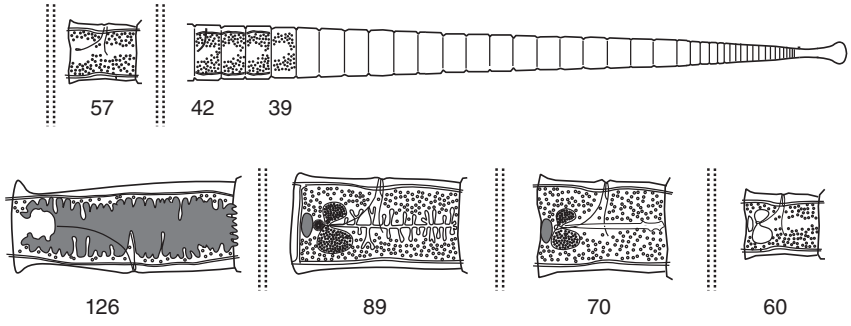


Figure 3.26 Formation of genital organs in a tapeworm, in this case of *Taenia crassiceps*. Organs become visible for the first time in the numbered proglottids as follows: 39, first testes; 42, vagina; 57, vagina and vas deferens; 60, two-lobed ovary, vitellarium posterior; 70, uterus; 89, branched uterus; 126, gravid uterus with eggs.

In the proglottids, the genital organs are surrounded by a sponge-like parenchyma. It is divided by bundles of longitudinal muscles into an outer layer, the cortex, and an inner layer, the medulla. The parenchyma contains masses of calcareous granules, concentrically layered structures of 10 to >30 µm, which consist mainly of calcium and magnesium carbonate together with a hydrated form of calcium phosphate. Possibly, they play a role in the detoxification of harmful compounds. In the living cestode, these granules render the visibility of inner organs impossible (they have to be dissolved and the worms to be stained for whole mounts). As the granules are highly refractive, tapeworms (and metacestodes) have a white appearance.

The **tegument** of cestodes, a neodermis, serves as protection against digestive enzymes and immune responses of the host, and is also the principal site of absorption and secretion. As an intestine is not present, all nutrients are absorbed through the surface of the worm. The basic structure of the tegument is similar to that of trematodes. The whole surface, however, even that of the suckers, is coated by microtriches, causing a considerable increase in the worm's surface area. The microtriches are highly specialized microvilli composed of a cap, base, baseplate, core, and covering glycocalyx.

The **nervous system** is quite difficult to visualize and consists of paired groups of ganglia in the scolex and two lateral nerve cords with connections in each proglottid. Sensory cells are concentrated especially in the region of the scolex and genital openings.

The **excretory system** is composed of protonephridia distributed over the whole parenchyma, visible only in the living, flattened worm. In the scolex, it is formed as a net. From there, it merges into four lateral channels in the strobila, the two ventral ones having a larger lumen than the two dorsal ones. The ventral channels are connected rope-ladder-like by a transverse channel at the distal part of the proglottid. In the Caryophyllidea and Pseudophyllidea, the whole excretory system is formed as a net, which lacks longitudinal channels.

3.1.2.4 Genome

A comparative genome analysis of the four tapeworm species *Echinococcus multilocularis, Echinococcus granulosus, Taenia solium*, and *Hymenolepis microstoma* revealed many similarities with, but also distinctive differences between genomes of tapeworms and digeneans. The *Echinococcus* species have a genome size of only about 115 Mb, organized in 18 chromosomes (2n). The repeat content is relatively low (~10%), probably allowing compression of the genome to one-third, as compared to trematodes. The number of putative protein-encoding genes corresponds to the Digenea (~10 500). Approximately 13% of protein-encoding genes are organized as polycistrons (see also Trypanosomes, Section 2.5.1). According to genome analyses, tapeworms lack the capacity to synthesize fatty acids and cholesterol *de novo*. Rather, they rely on scavenging these compounds from their host, using fatty acid transporters and tapeworm-specific fatty acid-binding proteins as well as the apolipoprotein Antigen B. Tapeworms have a strongly reduced capacity to synthesize amino acids, and use carbohydrates as a main energy source. Expansion of certain detoxification enzymes suggests that the worms are able to water-solubilize and excrete otherwise hydrophobic, toxic components.

3.1.2.5 Diphyllobothriidea

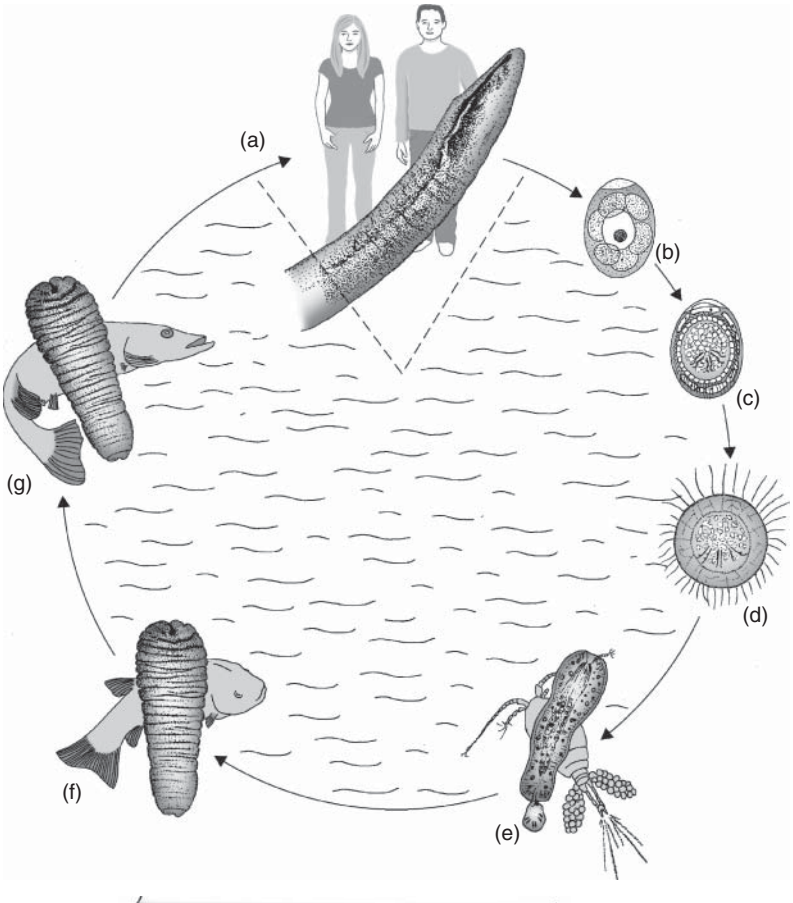
The order has been recently separated from the Pseudophyllidea based on new molecular data and differences in final host specificity, though the exact position within the cestode orders is still ambiguous. The Diphyllobothriidea occur mainly in fish-eating vertebrates such as birds, seals, whales, terrestrial carnivores, and humans.

Diphyllobothrium latum, the broad fish tapeworm, is not only famous because of its enormous length of 8–20 m, but is also infamous, because it occasionally leads to life-threatening anemia, due to its absorption of vitamin B12.

The development (Figure 3.27) of *D. latum* requires two intermediate hosts. The operculated eggs are shed unembryonated. Within 3–4 weeks a coracidium develops, which is a ciliated oncosphere with one pair of protonephridia. The coracidium bursts out of the egg through the operculum and swims in the water. If eaten by a copepod (*Diaptomus* or *Cyclops*), it grows in the body cavity becoming a procercoid of 0.5 mm length. This is a solid oblong larva, which has an undeveloped scolex in the retracted anterior end and a roundish cercomer, which still carries the six oncosphere hooks in the posterior end. The procercoid also contains several boring glands, which open at the anterior end of the body.

After the infected copepod has been taken up by a plankton-eating fish, the larva loses its cercomer when it bores through the intestinal wall and becomes a plerocercoid approximately 5 mm long, which now already possesses a scolex typical of the adult worm, but still in a retracted position.

Although the plerocercoid is infective for the final hosts after 2 months, the infected fish is usually predated by another fish. In this case, the plerocercoid bores through the intestinal wall of the predator, too, and survives in the organs of the body cavity without altering. Such transport hosts, in which no further


(a)
(b)
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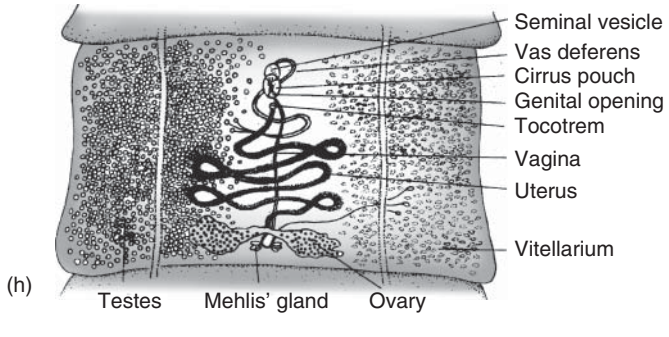

Seminal vesicle
Vas deferens
Cirrus pouch
Genital opening
Tocotrem
Vagina
Uterus
Vitellarium
(h)
Testes
Mehlis' gland
Ovary

Figure 3.27 Life cycle of *Diphyllobothrium latum*. (a) Anterior end of an adult worm. (b) Unembryonated operculated egg. (c) Embryonated egg containing a ciliated oncosphere, the coracidium. (d) Free-swimming coracidium. (e) Procercoid in the first intermediate host *Cyclops* (copepod). (f) Plerocercoid in the second intermediate host, a small fish. (g) Same larval form in paratenic host: pike. (h) Gravid proglottid.

development of the larva takes place, are called paratenic hosts. After the infected fish is eaten by a definitive host, the tapeworm usually settles in the jejunum and starts to grow very quickly. The first eggs are shed after 18 days post infection. Humans are usually inhabited by a single worm, which can live for many years.

Epidemiology As large predatory fish such as pike (*Esox lucius*), burbot (*Lota lota*), perch (*Perca fluviatilis*) or pope (*Gymnocephalus cernuus),* and more rarely the grayling (*Thymallus thymallus*) are preferred for consumption, these species play a role in the transmission to humans. The fish tapeworm occurs in regions, where musculature, liver, and roe are eaten raw or slightly salted. In the 1970s in Finland, more than 10% of human population were infected and even today 0.8% of the population in the East Siberian Lena basin are infected. As the worm grows approximately 5 cm/day, releases up to 1 million eggs per day, and can survive for 10 years, the production of eggs is high, so that water bodies can become contaminated very quickly. Besides humans, dogs and cats can also become infected.

Morphology The Broad Fish Tapeworm is 15–20 mm wide and has approximately 4000 proglottids. The scolex is finger-like and has a simple longitudinal groove each on the ventral and dorsal sides. The small follicles of testes and vitellaria are difficult to distinguish from one another. They are arranged in two broad lateral bands, leaving free only a narrow part in the middle of the proglottid (Figure 3.27h) with the bilobed ovary situated at the posterior margin. In front of it, the uterus leads in tight coils to the anterior part of the proglottid. Here, the seminal vesicle, the cirrus pouch, and the male genital opening are located together with the vaginal opening and the opening of the uterus, which is called a tocotrem (Greek: *tókos* = parturition, *tréma* = opening). Through this, the operculated, yellow-brown eggs, $60–66 \times 40–49$ µm in size, are shed. The emptied proglottids then fall off the strobila.

Pathology In most cases, humans are infected with one specimen only and usually do not show symptoms of infection, possibly for several years. In some people, however, faintness, giddiness, or diarrhea may occur. Only in exceptional cases, a life-threatening situation may arise: the fish tapeworm selectively absorbs vitamin B12. Yet, as long as it resides in the upper part of the duodenum (normal for cestodes), its presence does not harm the host, because the jejunum does not absorb B12. Only if the worm is located in the ileum – which happens in about 2% of cases – where absorption of the vitamin takes place, it deprives the host of such an amount of B12 that pernicious tapeworm anemia may develop with

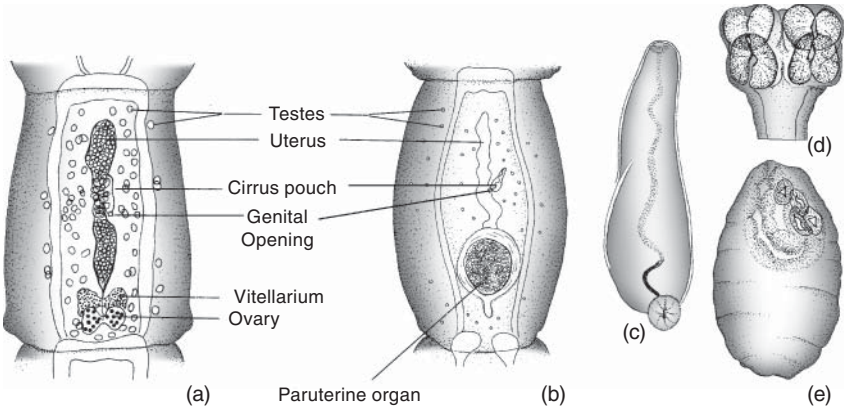


Figure 3.28 *Mesocestoides leptothylacus*. (a) Mature proglottid (vagina omitted). (b) Gravid proglottid. (c) Cirrus pouch with long thin cirrus, common genital opening at posterior end. (d) Scolex. (e) Tetrathyridium.

fatal outcome. Rapid therapy with vitamin B12 replacement is possible, but true recovery is only achieved by complete elimination of the parasite.

3.1.2.6 *Mesocestoides*

This is an enigmatic genus with species quite commonly seen in carnivores, for example, in foxes, and several species in birds of prey. Moreover, there are about 30 reported cases of human infection, most of them in East Asia. Its systematic position among the Eucestoda is not clear yet. The scolex (Figure 3.28d) has no hooks and the four suckers are extremely motile. The genital opening is ventromedian in the middle of the proglottid (Figure 3.28a). A large cirrus pouch is conspicuous (Figure 3.28c). In fully gravid proglottids, the last part of the uterus develops into a thick-walled capsule called the paruterine organ (Figure 3.28b), which contains all eggs and has a bright red appearance, visible to the naked eye in the discharged proglottids. Initially motionless on the shed feces for some minutes, the proglottids start to move with an extended slowly waving anterior end and migrate into the surroundings. They can be found adhered to grass blades. The larva infective for the final host is a so-called tetrathyridium (Figure 3.28e), an oblong structure with a deeply inverted scolex, which occurs in all vertebrate groups except fish. It is completely unknown, however, how the vertebrate intermediate host acquires its infection, as direct feeding of eggs does not yield tetrathyridea. Therefore, it is likely that a further intermediate host is required to complete the life cycle.

3.1.2.7 **Cyclophyllidea**

- Highly developed and large order of Eucestoda.
- Parasites of vertebrates excluding fish.

- Four suckers.
- Vitellarium posterior to ovary.
- Genital openings nearly always lateral.
- Unoperculated eggs, containing an oncosphere when shed.
- Always one intermediate host.
- Metacestode as cysticercoid in invertebrates, as cysticercus in vertebrates.

This order is the largest among all Eucestoda. Its name originates from the four round suckers. Between them, a conical muscular extensible and retractable muscular structure, the rostellum, is sometimes present. The scolex hooks are situated on the rostellum or between the suckers. The proglottids contain one, sometimes two paired hermaphroditic sets of genitalia. The joint genital opening is situated on the lateral margin of the proglottid. There are at least three, but usually numerous testes present. The compact vitellarium is always positioned behind the ovary at the posterior margin of the proglottid. There is no uterine pore. Instead, the eggs are freed from the released proglottids when they decay in the field.

The cyclophyllidean metacestodes exhibit extremely different shapes and, in the past, have been given many different names. In the following text, they are reduced to two basic types: (i) the **cysticercoid** (Figure 3.24) of invertebrate intermediate hosts, which has a scolex *retracted* into a cavity, and a tail-like ceromer with six oncospheral hooks, (ii) the **cysticercus** (Figure 3.24), found in mammals only, where the scolex is *invaginated* into a fluid-filled bladder. Species of only three of 14 families of the Cyclophyllidea – Anoplocephalidae, Hymenolepididae, and Taeniidae – are dealt with in the following.

3.1.2.8 *Moniezia expansa*

This tapeworm of sheep may become pathogenic in lambs (digestive problems, diarrhea), but worms are eventually expelled after several months, rendering the host immune. The parasite belongs to the family Anoplocephalidae (Greek: *an* = without, hoplon = weapon, *cephalé* = head), the scolex of which does not bear hooks, but prominent suckers. Anoplocephalidae are parasites of terrestrial mammals, especially of lagomorphs and ungulates. Intermediate hosts are free-living mites, *Oribatidae* (moss or beetle mites), which live amongst bark, leaf litter, and roots.

Morphologically, the genus *Moniezia* is characterized by very long worms (up to 10 m), bearing very short and broad segments with two sets of lateral genital organs. A net-like uterus is spread between them, which later becomes a transverse sac. The distal border of the gravid proglottids is furnished with rows of the so-called interproglottid glands of unknown function. The eggs (Figure 3.41e) are somewhat triangular and rather large. They contain an embryophore, formed as the so-called pyriform apparatus (= pear-shaped), which carries the very small oncosphere. Development of the oncosphere can occur when the thick eggshell is broken open by the mite intermediate host and the pyriform apparatus is swallowed. The oncosphere then penetrates the intestinal wall and becomes a

cysticercoid within the haemocoel. This larva carries oncospheral hooks at the tip of a long tail, and is infective for sheep only after 15–18 weeks. The prepatent time in the final host is 25–40 days.

3.1.2.9 *Hymenolepis diminuta*

The Hymenolepididae are an extremely genus- and species-rich family in birds (especially geese and waders) as well as mammals (insectivores, bats, and rodents), containing relatively small to tiny tapeworms. The name of the family refers to polar filaments looking like thin membranes (Greek: *hymen* = membrane, *lepís, lepídos* = shell*)* between eggshell and oncosphere. Morphologically, the Hymenolepididae are characterized by a rostellum, usually with very small hooks, few testes, and unilateral genital pores. The intermediate hosts are always insects, in which cysticercoids develop.

Hymenolepis diminuta, the rat tapeworm, is frequently used as a model tapeworm in the laboratory because of its easy maintenance. It is a relatively large species. In infections with single-specimen worms may reach 0.6 m in length. Their rostellum is rudimentary and without hooks (Figure 3.29a). In the laboratory, the tiny flour beetle (*Tribolium confusum*) is used as an intermediate host. The eggshell is perforated by the beetle's mandibles releasing the oncosphere, which bores through the intestinal wall to reach the hemocoel, where it develops into a cysticercoid. Its slender cercomer is about twice as long as the body (300 × 150 µm) enclosing the scolex. In young larvae, oncospheral hooks are still visible on the posterior tip of the cercomer. The cysticeroids are infective after 2–3 weeks. At that time, the beetles lose their photophobia and motility so that rats can take them up more easily. Oviposition in the duodenum of the rat starts between day 13 and 21 post infecion. The eggs (Figure 3.29b) measure 60–70 µm. They do not possess the polar filaments typical of the family. As in most hymenolepidids, there are only three testes but unusually they have a bipartite seminal vesicle (Figure 3.30). The genital openings are located laterally. Humans are rarely infected.

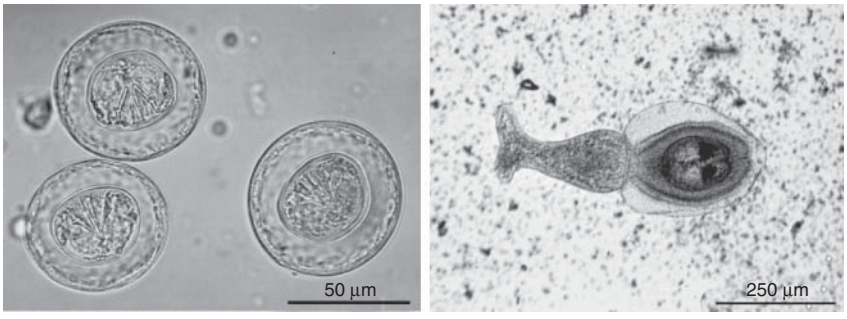


Figure 3.29 *Hymenolepis diminuta*, the rat tapeworm. (a) Eggs. (b) Cysticercoid from the beetle *Tenebrio molitor*. (Image: Archive of the Department of Parasitology, University of Hohenheim.)

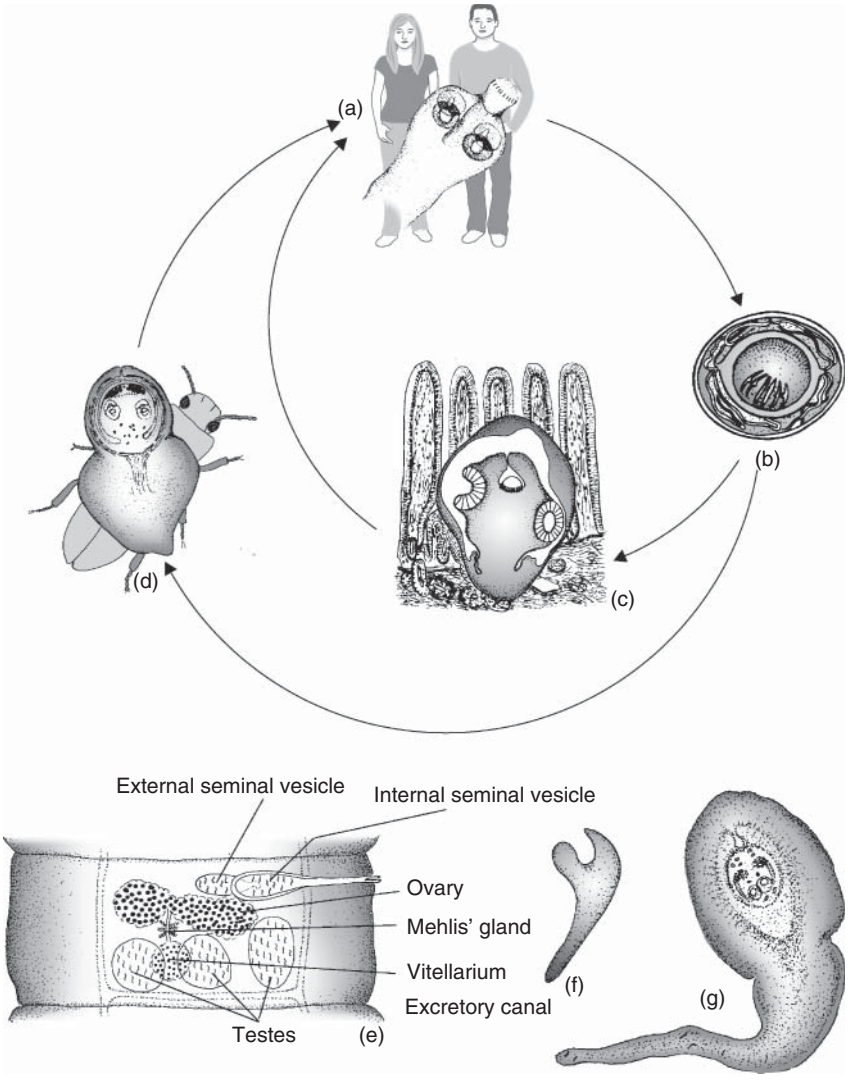
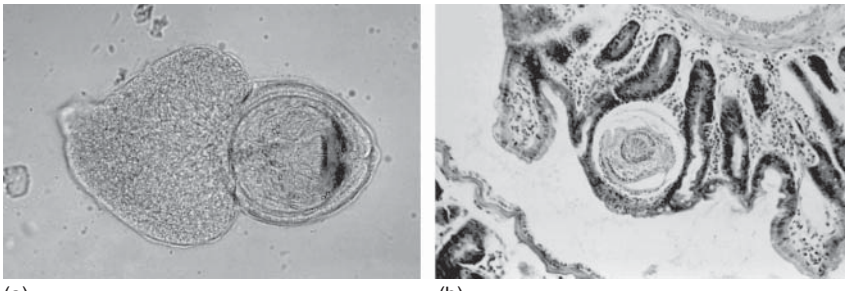


Figure 3.30 Life cycle of *Rodentolepis nana*. (a) Scolex of the adult tapeworm in the human small intestine (note the presence of scolex hooks). (b) Egg containing oncosphere and polar filaments. (c) Cysticercoid in intestinal villi. (d) Cysticercoid in hemocoele of a beetle. (e) Mature proglottid of *R. nana*. (f) Scolex hook of *R. nana*. (g) Cysticercoid of *Hymenolepis diminuta* from a beetle.

3.1.2.10 ***Rodentolepis nana* (*Hymenolepis nana*)**

The dwarf tapeworm (Latin: *nanus* = dwarf) of humans and other primates is, like *H. diminuta*, a model organism for tapeworm infections in mammals, since it is easily kept in the laboratory in mice and grain beetles. The genus *Rodentolepis* has a retractable rostellum bearing a ring of 18 very small equal hooks of $16 \times 8\,\mu m$



(a) (b)

Figure 3.31 *Rodentolepis nana*. (a) Cysticercoid from a beetle. (b) Cysticercoid in the duodenal villious of a mouse. (Images: Archive of the Department of Parasitology, University of Hohenheim.)

size (Figure 3.30a and f). Its broad-oval eggs, 50 × 40 µm in size, are equipped with polar filaments (Figures 3.30b). *Rodentolepis nana* reaches a length of only 50–60 mm and a width of 0.5–1 mm.

R. nana is exceptional in having two different developmental options (Figure 3.30). In the infection mode normal for hymenolepidids, eggs are ingested by grain beetles or other insects. Cysticercoids develop within them in 3 weeks. The cysticercoid has a thick mantle and heart-shaped cercomer, slightly larger than the "cyst" surrounding the scolex (Figures 3.30d and 3.31). If humans or mice ingest beetles together with a contaminated meal, the wall of the larva is dissolved. The scolex settles in the duodenum of the final host and starts to build up a strobila. In mice, the young worms migrate into the lower part of the ileum after 3–4 days, where they become sexually mature and start to shed eggs on day 7.

The second mode of infection, more common than the first one, is an autoinfection without the involvement of an insect: if a final host (human or mouse) swallows eggs, these enter a villus and become thin-walled cysticercoids without a cercomer (Figure 3.30c) in the upper part of the intestine. Upon rupture of the villus, the cysticercoids return to the intestinal lumen, evaginate their scoleces, attach to the intestinal mucosa, and develop into adults that reside in the ileal portion of the small intestine. Eggs produced by these worms can also give rise to thin-walled cysticercoids, without being released via feces. Thus, high numbers of parasites can build up from a single infective stage, which is a rare exception in helminths. These autoinfections make the dwarf tapeworm difficult to control and *R. nana* infections are often seen in children in poor hygienic conditions. Here, infections can induce nausea, vomiting, diarrhea, abdominal pain, weight loss, and nonspecific systemic symptoms.

Rodents can become immune to a challenge egg infection for at least 3 months following settling of a single oncosphere in the duodenal wall. It is not known if the same protective immunity develops in humans.

Figure 3.32 *Taenia crassiceps,* double crown of isolated scolex hooks. Large hooks are approximately 180 µm long. (Image: Archive of the Department of Parasitology, University of Hohenheim.)

3.1.2.11 **Taeniidae**

The family Taeniidae contains the most highly developed and important adult and larval cestodes of humans. Adult Taeniidae (Figure 3.24) inhabit in particular mammals and in almost all cases, their intermediate hosts are mammals too. The family occurs worldwide. Its most obvious feature is a double crown of scolex hooks of different shape and size, identical in adults and metacestodes. The metacestode is a cysticercus. When found in meat, it is known commonly as beef or pork measles (Figure 3.32).

The family contains the monophyletic genus *Echinococcus* and a group of presently three genera: (i) *Taenia* with the majority of roughly 40 species, (ii) *Hydatigera* with *Hydatigera taeniaeformis* and two other species, and (iii) *Versteria* (after the distinguished South African helminthologist Anna Verster 1931–1994) with several species from mustelids. These three genera are sometimes grouped as Taeniinae to distinguish the entity from the genus *Echinococcus*.

The final hosts of the family Taeniidae are terrestrial carnivores, that is, Canidae, Felidae, Mustelidae, Ursidae, Hyaenidae, Procyonidae, and Viverridae. Exceptions to this are the three *Taenia* species of humans: *Taenia saginata*, *T. solium*, and *Taenia asiatica*.

Intermediate hosts of the Taeniidae are those – usually plant-eating – mammals, which are part of the food chain of carnivores. Asexual reproduction of larvae does sometimes occur in the subfamily Taeniidae, but is present throughout the genus *Echinococcus.*

Development In most species, gravid proglottids, freed in the gut from the strobila, leave the anus actively. They stay active for a short time with movements squeezing most of the eggs out of the proglottid. In less-motile proglottids, the eggs remain inside. The eggs can survive for a long time and are highly resistant to environmental influences. Only heat and prolonged drought destroy them quickly.

Table 3.6 Some species of the Taeniidae.

Species	Definitive hosts	Intermediate hosts	Location of metacestode	Asexual multiplication of metacestode	Old name of metacestode
Genus *Taenia*					
T. solium	Humans	Pig (humans)	Musculature	No	Cysticercus cellulosae, Cysticercus ocularis
T. saginata	Humans	Cattle	Musculature	No	Cysticercus bovis, Cysticercus inermis
T. asiatica	Humans	Pig	Musculature	No	—
T. multiceps	(Wolf) dog	Sheep	Brain	Yes	Coenurus cerebralis
T. ovis	(Wolf) dog	Sheep, goat	Musculature	No	—
T. serialis	Dog, fox	Hare, rabbit	Mesenteries	Yes	Coenurus serialis
T. crassiceps	Fox, dog	Rodents	Subcutaneous connective tissue, thoracic cavity	Yes	—
Genus *Hydatigera*					
H. taeniaeformis[a)]	Cat	Rodents	Liver	No	Strobilocercus

a) Consists of two cryptic species.

They are taken up by the intermediate host by ingestion, usually together with food plants. The oncosphere bores through the wall of the small intestine, is carried off with the blood stream, and settles in species-specific organs such as the liver, musculature, body cavity, brain, or connective tissue (Table 3.6), where it becomes a cysticercus. In approximately one-third of the Taeniidae, the cysticeri reproduce asexually. The host specificity regarding the intermediate hosts is usually rather broad.

Morphology Taeniidae measure from roughly 15 cm up to many meters in length (*T. saginata*). Their scolex is devoid of a rostellum. A ring of scolex hooks between the four suckers is composed of alternately arranged hooks of two different sizes and shapes. Their number, size, and shape are important characteristics for identification. The male genital organs (Figure 3.25) consist of the testes (70–1200, depending on the species) distributed among the longitudinal excretory vessels, the vas deferens leading from the middle to the lateral part of the proglottid, ending with a cirrus pouch with a cirrus at the lateral margin of the proglottid. The female organs comprise a bipartite ovary, behind

which is a compact vitellarium, and a vagina parallel and posterior to the vas deferens (Figure 33 l and g), stretching to the lateral margin. The vagina may have a sphincter (Figure 3.33h). The common genital openings are situated alternately at the margins. The uterus is a median tube in young proglottids and branches in maturing segments (Figure 3.33i and n). In gravid proglottids, its anterior branches penetrate beyond the border of the anterior proglottid, leaving holes when the mature segment is detached. Through these holes, the eggs are squeezed out by the proglottid's movements. The number of eggs in each segment is 10 000 – 100 000 depending on the species. They are round to oval in shape, spherical, and measure 25 – 35 µm. They are protected by an "embryophore" composed of blocks of brown segments of keratin-like material. Through the influence of enzymes in the stomach and duodenum, the embryophore breaks open and releases the oncosphere. The eggs of all taeniids are very similar, which renders species-specific diagnosis based on morphology impossible.

The cysticercus has no cercomer. It is a transparent bladder into which the scolex complete with hooks inverted. The scolex is visible to the naked eye as a white spot. From this basic type of cysticercus, as seen in *T. saginata* and *T. solium*, the larvae of many of the species differ markedly (Figure 3.25), so much so that they were originally given distinct species names (Table 3.6).

Moreover, adult members of the Taeniidae are not easily identified by traditional morphological methods, resulting in 130 competing names generated for approximately 45 species. This complexity is now being addressed using molecular techniques.

Immunobiology Adult tapeworms were considered to be of low immunogenicity, although it is now well known that they are readily recognized by the immune system of the infected host. As their pathogenicity is limited and they are easily eliminated by drugs, they have not received the level of attention that other parasites have received. Metacestodes dwelling in host tissues can only survive with the aid of pronounced mechanisms of immune evasion. For example, they produce a multiplicity of factors, which inhibit components of the complement system. Moreover, the tegument of metacestodes contains scavenger proteins such as glutathione-S-transferases (GTS) and superoxide dismutases (SODs), which are able to inactivate host effector molecules. In addition, the chemotaxis of phagocytes and the activation of lymphocytes are inhibited. The larval stages in the intermediate host induce a strong protective response, which limits superinfection. For instance, an infection with only one oncosphere of *H. taeniaeformis* conveys complete protection against subsequent infections. This efficient immunity is elicited by complement-fixing antibodies against proteins on the surface membrane of the oncosphere. On the basis of this robust immune mechanism, vaccinations with recombinant oncosphere antigens of agriculturally important taeniids have successfully been tested, but have not yet been commercialized.

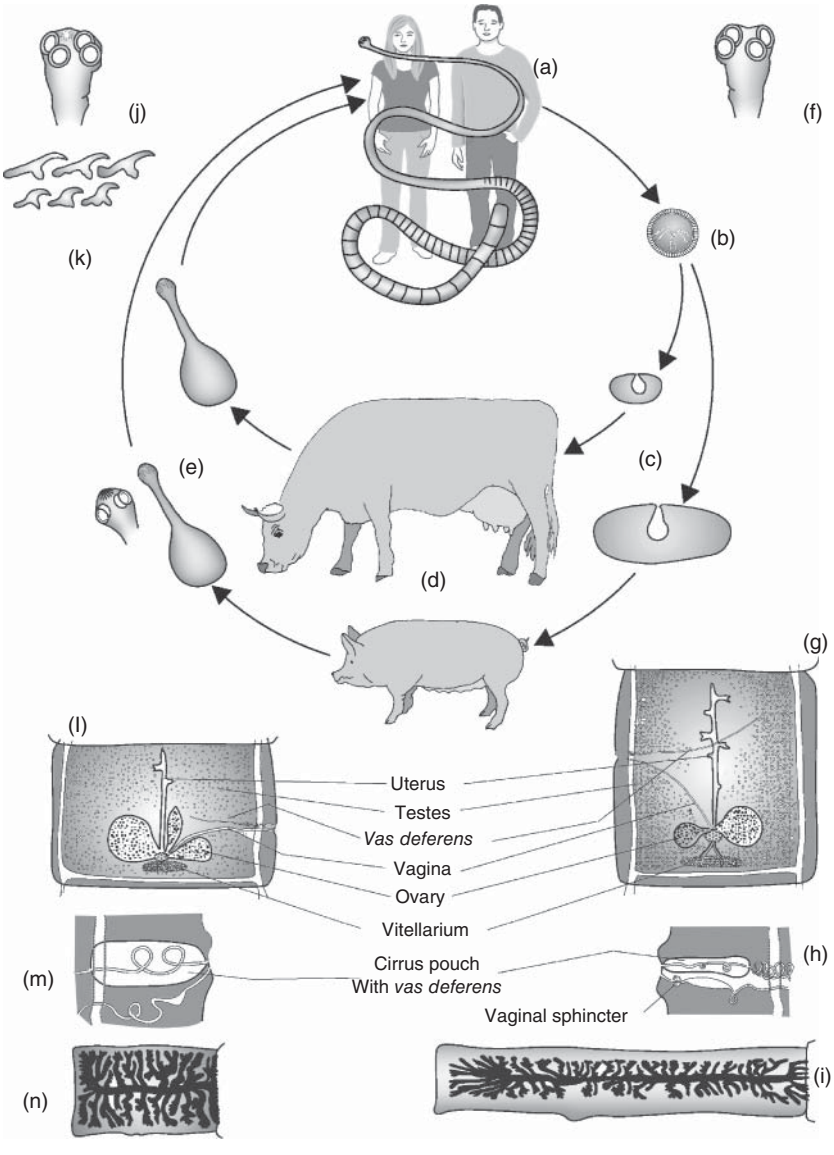


Figure 3.33 Life cycles of *Taenia saginata* (inner circle) *and Taenia solium* (outer circle). (a) Adult worm in the small intestine of humans. (b) Egg. (c) Cysticercus (invaginated). (d) Intermediate host. (e) Cysticercus (evaginated). Morphology of *T. saginata*. (f) Scolex (no hooks!). (g) Mature proglottid. (h) Genital opening (on left side). (i) Gravid proglottid. Morphology of *T. solium*: (j) Scolex. (k) Scolex hooks. (l) Mature proglottid (note: the additional ovary lobe). (m) Genital opening (on right side). (n) Gravid proglottid.

3.1.2.12 *Taenia saginata*

The beef tapeworm (Figure 3.33, right side) is one of the three species infecting humans but not carnivores. In fact, the name beef tapeworm is a misnomer, since only the larval tapeworm is found in beef. *T. saginata* has a cosmopolitan distribution together with its intermediate host. In cattle, it causes relatively few health problems, but is of major concern for meat inspection.

T. saginata is the only species of the genus without scolex hooks. Although the hooks are present in the young cysticercus, they disappear soon afterward. The differences between *T. saginata* and *T. solium* are given in Table 3.7.

T. saginata is present in up to 0.5% of the human population even in countries with strict sanitation policies. Humans usually harbor only one tapeworm per individual, but show relatively few symptoms although loss of appetite (rarely increased appetite), headache, fatigue, diffuse abdominal pain, diarrhea, or constipation may be noted. After a prepatent period of 10 weeks, the tapeworm sheds six proglottids per day, each of which contains 80 000 – 100 000 eggs. A single specimen can produce up to 1 million eggs/day. As proglottids are very motile and squeeze out the eggs gradually, these are dispersed loosely and are ingested by cattle only singly. Infection occurs, when incompletely treated sewage is discharged into rivers, which contaminate neighboring pastures or when calves in fattening units lick the eggs off the hands of infected personnel. Under normal circumstances, the number of ingested eggs is small and the resulting infection is not intensive, due to the dispersion of eggs. In a large beef carcass, the cysticerci are easily overlooked during meat inspection and experts doubt that *T. saginata* will ever be fully eliminated. Prevalence in cattle is estimated at between 0.7%

Table 3.7 Morphological differences between *Taenia saginata* and *Taenia solium*.

Adult	***Taenia saginata***	***Taenia solium***
Name	Beef tapeworm	Pork tapeworm
Scolex	Without hooks	With hooks
Full length	>10 m	3 – 4 m
Size of gravid proglottids	18 – 20 × 4 – 7 mm	9 – 12 × 6 – 7 mm
Proglottids length: width	6 : 1	3 : 1
Shape of ovary	Bipartite	Bipartite with small extra lobe at the polar[a)] lobe
Number of uterus branches	2 × 20 – 30	2 × 7 – 12
Vagina	With sphincter	Without sphincter
Metacestode		
Size	7 – 9 mm	6 – 15 mm
Appearance	Yellow-white, firm	Whitish, transparent
Intermediate hosts	Cattle only	Pig (man), experimentally other mammals, too
Old terms	*Cysticercus bovis*, *Cysticercus inermis*	*Cysticercus cellulosae*, *Cysticercus ocularis*

a) Polar = at the side of the genital opening.

and 1.5%. The cysticerci become infective after 10 weeks and preferentially settle in the masseter muscle, tongue, diaphragm, and heart of the intermediate host. They elicit relatively little symptoms in terms of host reaction, even when dead or calcified.

3.1.2.13 *Taenia solium*

The pork tapeworm is the second species to infect humans (Figure 3.33, left side). In most countries, in which thorough meat inspection is carried out and where pigs are raised in farms, but not near human dwellings, the tapeworm has been eliminated. Reasons for this include the fact that detached proglottids exhibit poor mobility, are passed intact with the feces, and are taken up with all their eggs by the coprophagous pig, so that the numerous pea-sized cysticerci developing in the pig are easily found in meat inspection.

T. solium is of importance as there is no marked species specificity for the intermediate host. In addition to the pig (and the golden hamster which can be infected experimentally), humans can harbor cysticerci. However, in humans, they do not only inhabit the musculature as they do in pigs, hardly evoking symptoms, but settle in various organs. In 60% of people with cysticercosis, the brain is infected. Depending on the location of the "*cysticercus cellulosae*" such as in the meninges or in the parenchyma of the brain and whether the larvae are living, dead or calcified, the consequences can be meningitis or increased intracranial pressure, both leading to serious neurological symptoms. Patients may suffer from epilepsy-like seizures, headache, vomiting, or other neurological complications and, if infected when young, individuals can show cognitive impairment. Following infection of the eye with the "*cysticercus ocularis*" impaired vision or, at worst, blindness can develop. Cysticercosis is often prevalent in people who are already harboring an adult tapeworm themselves. Human cysticercosis is frequent in countries where pigs are kept in close contact with humans, where slaughtering is done without veterinary control measures, where sewage is not purified and sanitation is poor. Current estimates of cysticercosis worldwide are approximately 50 million cases. In Mexico, for example, postmortem analysis of inhabitants of Mexico City showed a prevalence of 1.4–3.6%, with many not knowing that they had been infected.

3.1.2.14 *Taenia asiatica*

This species is prevalent in people in East Asian regions, and was discovered only in 1990. Molecular analyses showed such a close relationship between the Asian species and *T. saginata* that even hybridization could occur. The cysticerci of *T. asiatica*, however, do not infect cattle, but only pigs. Their larvae possess two types of scolex hooks, an outer circle with at least 200 hooks, measuring less than 7 µm, and an inner circle of less than 95 hooks up to 11 µm. In contrast to *T. solium,* humans are never infected by cysticerci.

According to Hoberg (2006), *T. saginata*, *T. asiatica*, and *T. solium,* in common with all other *Taenia* species, once had carnivores as definitive hosts. Only when the ancestors of *Homo sapiens* in Africa began to hunt antilopes did they become

exposed to cysticerci in such herbivores and became hosts to adult tapeworms in the intestine. Only when humans invaded Europe and Asia, between 780 000 and 1.7 million years ago did *T. saginata* and *T. asiatica* become separated, and only when cattle and pigs became domesticated some 10 000 years ago did they develop into intermediate hosts of human taenias.

3.1.2.15 *Hydatigera taeniaeformis*

This feline tapeworm is highly prevalent in free-roaming cats that catch and eat mice or voles. A great number of rodents living in or near human settlements carry the larvae present as large yellowish cysts attached to the liver surface and surrounded by connective tissue. About 20 mm in diameter, they contain a 100-mm-long larva. It has a segmented appearance similar to an adult tapeworm, and is called a strobilocercus. In infected cats, gravid proglottids can be seen on the hairs around the anus. Cats do not readily develop immunity, and therefore can become infected again after clearance with anthelmintics.

3.1.2.16 *Echinococcus*

Adult worms of the monophyletic genus *Echinococcus* measure only a few millimeters. These parasites are of great importance, because the metacestodes proliferate asexually and infect mammals including humans inducing severe disease, either as cystic, alveolar, or polycystic echinococcosis (Table 3.8). The biology of *Echinococcus* is the same as in most taeniids: final hosts are carnivores and intermediate hosts are herbivorous prey mammals. Asexual proliferation in the intermediate host is obligatory. Unusually, adult worms measure maximally 7 mm in length and have six proglottids at the most. The arrangement of the genital organs is basically that of the genus *Taenia,* but there are generally no more than 60 testes present. The number of species and strains has increased greatly since the introduction of molecular methods of analysis (Table 3.8).

3.1.2.17 *Echinococcus granulosus*

The "Hydatid Worm" is now known to be a species complex. Therefore, its correct name should be *E. granulosus s.l.* (Latin: *sensu latu* = in the broad sense). Its larvae cause cystic echinococcosis (also: unilocular echinococcosis or cystic hydatid disease).

Metacestodes established in the liver, but also in the lungs and other organs, are unilocular (meaning with one chamber) hydatid cysts (Greek: *hydátinos* = abounding in water) (Figure 3.34). They are fluid-filled and may grow to the size of 15 cm in diameter. They consist of an outer lamellar layer and thin inner germinal layer. The germinal layer produces invaginations into the cyst cavity, forming brood capsules (daughter cysts, Figure 3.34) that contain protoscolices, which are released into the cyst fluid and give rise to secondary and tertiary brood capsules that can contain up to 30 protoscoleces. Brood capsules and protoscoleces are termed hydatid sand. Pathology arises from the enormous growth of the hydatids in the liver (Figure 3.35). Pressure upon the bile duct and portal vein endanger the drainage of bile or induce ascites. When the hydatids

Table 3.8 Important species and strains of the genus *Echinococcus*.

Species (and strains)	Final hosts	Intermediate host(s)	Human infections	Hydatid features	Geographical distribution
Echinococcus granulosus s. str., strains G1–G3	Dog	Sheep, goat, and cattle	Common	Unilocular	Worldwide in regions with extensive sheep breeding
Echinococcus canadensis strains G6/G7	Dog	Pig, camel, cattle, goat, sheep	Uncommon	Unilocular	Worldwide
Echinococcus canadensis strains G8, G10	Wolf	Moose, wapiti	Uncommon	Unilocular	Northern arctic and boreal
Echinococcus equinus	Dog	Horse	Unknown	Unilocular	Worldwide
Echinococcus ortleppi	Dog	Cattle	Uncommon	Unilocular	Worldwide
Echinococcus felidis	Lion	Unknown	Unknown	Unknown	Africa
Echinococcus oligarthrus	Wild felids	Agouti	Uncommon	Unilocular	Neotropical
Echinococcus vogeli	Bush dog	Paca	Uncommon	Polycystic	Neotropical
Echinococcus multilocularis	Red fox, arctic fox	Arvicoline rodents	Common	Alveolar	Holarctic

Source: After Nakao et al. (2013b).

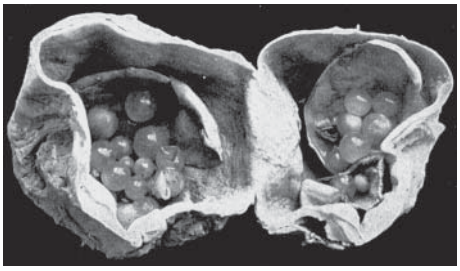
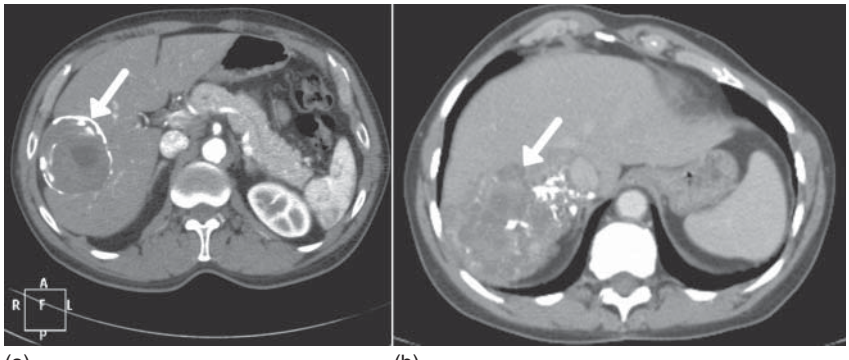


Figure 3.34 *Echinococcus granulosus*, hydatid with secondary and tertiary brood capsules in a human liver. (Image: Piekarski (1954) *Lehrbuch der Parasitologie*. Springer Verlag, Heidelberg.)

are localized in the lungs, severe shortness of breath can develop. In other organs, the growth of larvae at restricted sites may cause problems, for example, inside joints, bones, or in the vertebral column.

The hydatid cysts can, however, be easily removed surgically, as they are clearly delineated from host tissue by surrounding connective tissue. However, if a cyst ruptures, the whole operation area can be flooded with hydatid sand and fluid containing parasite tissue. Due to the regenerative capacity of the germinal layer cells and of protoscolices, the spilled material may generate new hydatids as a



(a) (b)

Figure 3.35 Sonography of human livers with *metacestodes of Echinococcus*: (a) bladder-like hydatid (arrow) of *Echinococcus granulosus*. (b) compact larval mass (arrow) of *Echinococcus multilocularis*. (Images: Courtesy of P. Kern.)

secondary infection. In order to avoid this complication, hydatids are injected with alcohol to kill the metacestode before surgical removal.

The final hosts of the *E. granulosus* complex are carnivores (wolf, dog, dingo, jackal, fox, hyenas, and cats). Thousands of small tapeworms live in their intestine. They are up to 5 mm long and possess only three proglottids. The terminal proglottid measures more than 50% of the whole length of the worm. On average, there are 44 testes. The gravid uterus is elongated and has small lateral bulges. There are 24–32 scolex hooks, the large ones measuring 37 µm, the small ones 29 µm. The intermediate hosts are usually farm animals.

To date, the *granulosus* complex has been believed to contain 11 strains and three valid species (Table 3.8). Strains G1, G2, and G3 form the species *E. granulosus s.str*. (*sensu stricto* = in the strict sense). G1, the sheep strain, has the widest distribution of all. Humans are often infected, because in areas with extensive sheep breeding, herding dogs gain easy and uncontrolled access to the entrails of dead animals and become infected by eating hydatid material. Tapeworm eggs that are passed with dog feces and unintentionally taken up orally are a source of infection for humans.

3.1.2.18 *Echinococcus multilocularis*

This species causes alveolar echinococcosis, a disease less common than hydatid echinococcosis, but more serious and more difficult to treat. The final hosts are mainly foxes (several *Vulpes* species), including the arctic fox, but also dogs, wolves, coyotes, jackels, racoon dogs, lynx, and wild and domestic cats. Intermediate hosts are rodents (mainly voles) and very rarely shrews (Fig. 3.37). As in all *Echinococcus* species, epidemiologically, humans can acquire the metacestode by ingesting oncosphere-containing eggs, which leads to a dead end infection (Figure 3.37).

The metacestodes are multiple, budding blisters, usually in the liver, without an outer capsule, but limited by a thin laminar layer. The germinal layer proliferates externally by forming long solid protrusions, which then develop into

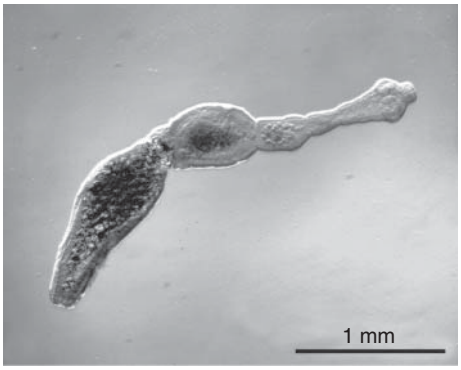


Figure 3.36 *Echinococcus multilocularis*, adult from fox intestine. (Image: Archive of the Department of Parasitology, University of Hohenheim.)

multilocular (= with many chambers) structures, usually 1–10 mm in diameter, containing hundreds of protoscolices. They are able to spread to other organs and tissues nearby. Eventually a large white mass develops, which infiltrates the whole organ and in rodents almost completely displaces the liver parenchyma. In humans, disease symptoms are often recognized only after two or three decades.

The adult worm is up to 3 mm long and has three to five proglottids. The last proglottid measures less than 50% of the whole worm length and has 22 testes on average (Figure 3.36). The longitudinal uterus has a characteristic swelling of its anterior part. There are 12–34 scolex hooks measuring 31 µm (large hooks) and 27 µm (small hooks).

E. multilocularis is distributed in the Northern Hemisphere of the Old World from Eurasia to Japan. In Europe, it currently spreads to the North. In the New World, it occurs in Canada, Alaska, and the North Central US, from Montana to Central Ohio, but has recently been introduced to other parts of the United States by commercial fox hunting.

Alveolar echinococcosis is a serious disease, as it is next to impossible to remove the invasive and metastasizing larval mass completely through surgery unless it is detected in a very early stage of infection. Chemotherapy with anthelmintics can stop growth of the larval mass, but usually does not kill the parasite, which makes continued treatment necessary.

3.1.2.19 *Echinococcus vogeli* and *Echinococcus oligarthrus*

Both species cause the third form of disease, the polycystic echinococcosis and occur in Central and South America (Table 3.8). In *Echinococcus vogeli,* the fluid-filled cysts occur singly or as aggregates in the liver, sometimes in the lungs and other organs and can have multiple chambers. Clinical signs resemble alveolar echinococcosis. Metacestodes of *E. oligarthrus* have an appearance similar to *E. vogeli*, but develop in muscles, subcutaneous tissues, heart, and lungs and have only rarely been found in humans. *E. vogeli* and *E. oligarthrus* are closely related and form a sister group to all other *Echinococcus* species.

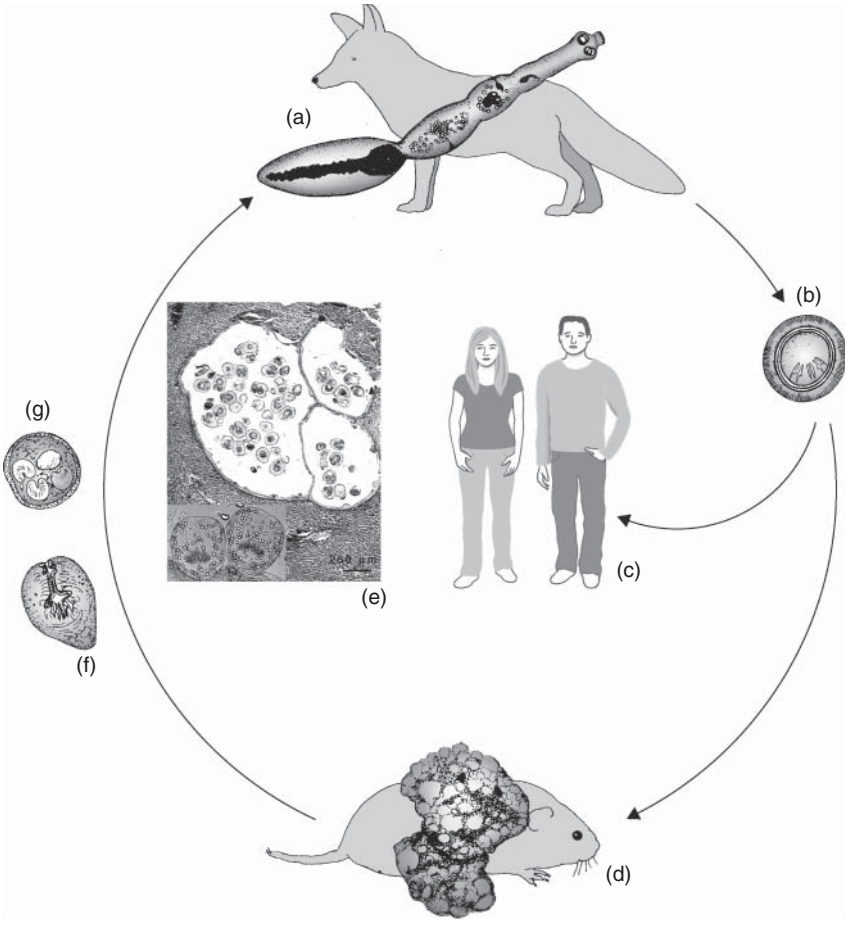


Figure 3.37 Life cycle of *Echinococcus multilocularis*. (a) Adult worm in the intestine of a fox. (b) Egg with oncosphere. (c) Humans as accidental intermediate hosts. (d) Larval mass in the true intermediate host, the common vole. (e) Section of vole liver with chambers containg protoscolices. (f, g) Protoscolices from a vole liver.

Further Reading

Eckert, J., Deplazes, P. (2004) Biological, clinical and epidemiological aspects of echinococcosis, a zoonosis of increasing concern. *Clin. Microbiol. Rev.* **17**, 107–135.

Garcia, H.H., Rodriguez, S., Friedland, J.S., and Cysticercosis Working Group in Peru (2014) Immunology of *Taenia solium* taeniasis and human cysticercosis. *Parasite Immunol.*, **36**, 388–396.

Hoberg, E.P. (2006) Phylogeny of *Taenia*: Species definitions and origins of human parasites. *Parasitol. Int.*, **55**, Suppl: S23–30.

Knapp, J., Nakao, M., Yanagida, T., Okamoto, M., Saarma, U., Lavikainen, A., and Ito, A. (2011) Phylogenetic relationships within *Echinococcus* and *Taenia* tapeworms (Cestoda: Taeniidae): an inference from nuclear protein-coding

genes. *Mol. Phylogenet. Evol.*, **61**, 628–638.
Loos-Frank, B. (2000) An update of Verster's 1969 "Taxonomic revision of the genus *Taenia* Linnaeus (Cestoda)" in table format. *Syst. Parasitol.*, **45**, 155–183.
Macnish, M.G., Morgan, U.M., Behnke, J.M., and Thompson, R.C. (2002) Failure to infect laboratory rodent hosts with human isolates of *Rodentolepis* (= *Hymenolepis*) *nana*. *J. Helminthol.*, **76**, 37–43.
McManus, D.P. (2013) Current status of the genetics and molecular taxonomy of *Echinococcus* species. *Parasitology*, **140**, 1617–1623.
Nakao, M., Lavikainen, A., Iwaki, T. *et al.* (2013a) Molecular phylogeny of the genus *Taenia* (Cestoda: Taeniidae): proposals for the resurrection of *Hydatigera* Lamarck, 1816 and the creation of a new genus *Versteria*. *Int. J. Parasitol.*, **43**, 427–437.
Nakao, M., Lavikainen, A., Yanagida, T., and Ito, A. (2013b) Phylogenetic systematics of the genus *Echinococcus* (Cestoda: Taeniidae). *Int. J. Parasitol.*, **43**, 1017–1029.
Olson, P.D. and Tkach, V.V. (2005) Advances and trends in the molecular systematics of the parasitic Plathyhelminthes. *Adv. Parasitol.*, **58**, 165–243.
Tsai, I.J., Zarowiecki, M., Holroyd, N. *et al.* (2013) The genomes of four tapeworm species reveal adaptations to parasitism. *Nature*, **496**, 57–63.

Test Questions

1. Which morphological features characterize an adult tapeworm?
2. Which is the tapeworm stage usually most important for propagation?
3. Which stages occur in the life cycle of *Diphyllobothrium latum* and where do they live?
4. Why can *D. latum* become dangerous for humans?
5. Which stages occur in the life cycle of cyclophyllideans?
6. What is the dwarf tapeworm (name and family)?
7. What are the two different developmental possibilities in the dwarf tapeworm?
8. What are the genera of the Taeniidae family?
9. What are the final hosts of the Taeniidae?
10. What are the intermediate hosts of the Taeniidae?
11. Which species in the Taeniidae family do reproduce asexually?
12. What does the name "beef tapeworm" tell you?
13. Which *Taenia* species occur in humans?
14. Which of them is dangerous for humans and why?
15. Why is *Echinococcus* dangerous for humans?
16. Name some species of *Echinococcus*.

3.2 Acanthocephala

The thorn- or spine-headed worms (Greek: *acanthos* = thorn, *kephalé* = head) are a phylum of obligatory parasites of vertebrates. Arthropods act as the intermediate hosts. There are about 1150 documented species, more than half of them inhabiting fish, mostly of freshwater. In aquaculture, they can become economically important pathogens. Acanthocephala are placed in the relatively

recently described group Syndermata (Figure 3.1). The Syndermata contain in addition to the Acanthocephala, the Rotifera (tiny free-living aquatic animals). The name Syndermata refers to the syncytial epidermis, which, among other features is common to both groups.

Acanthocephala use aquatic or terrestrial arthropods as intermediate hosts. Only where arthropods are not part of the food chain of the final host, will paratenic hosts become involved (Figure 3.38).

The adult parasites are worm-shaped, have no intestinal tract, and are dioecious. Their most conspicuous feature is the retractable proboscis armed with spines (Figure 3.39c, 3.40) that serves as holdfast and anchors the parasite in the intestinal wall of the host like a dowel. Females have free-floating ovaries

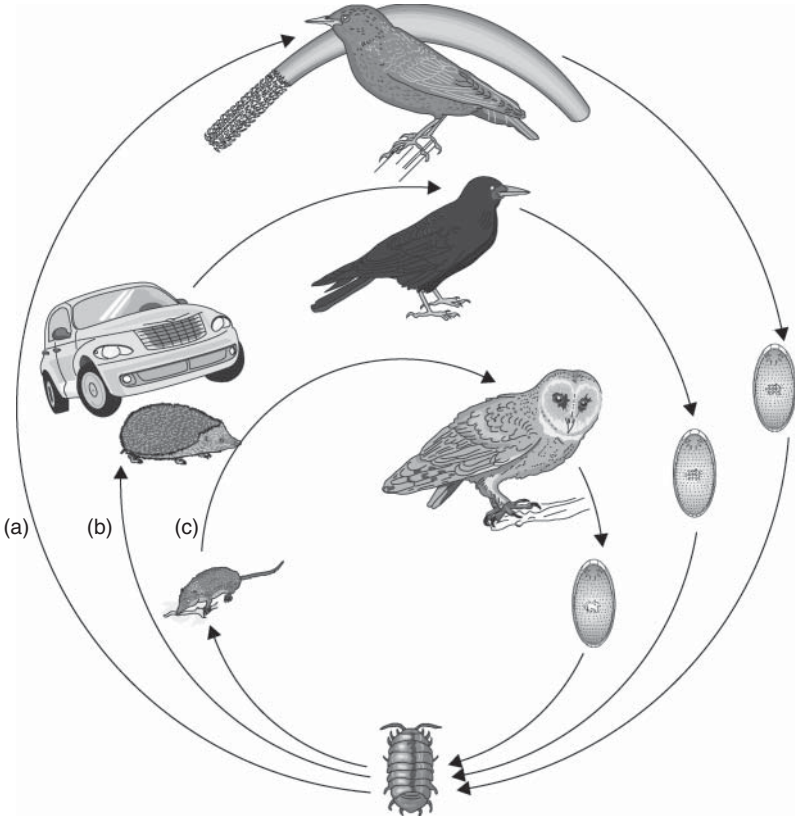


Figure 3.38 Life cycle of *Plagiorhynchus cylindraceus* with three possible routes of transmission. (a, outer circle) Short two-host life cycle with the final host starling and the intermediate host pill bug *Armadillidium vulgare* (Crustacea, Isopoda). (b, middle circle) "postcyclic" transmission with hedgehog killed by traffic and carrion-eating bird as final hosts. (c, inner circle) three-host life cycle with shrew as paratenic host and barn owl as final host. For further explanations see text. (After Skuballa *et al.* (2010). *Parasit. Res.* **106**, 431–438.)

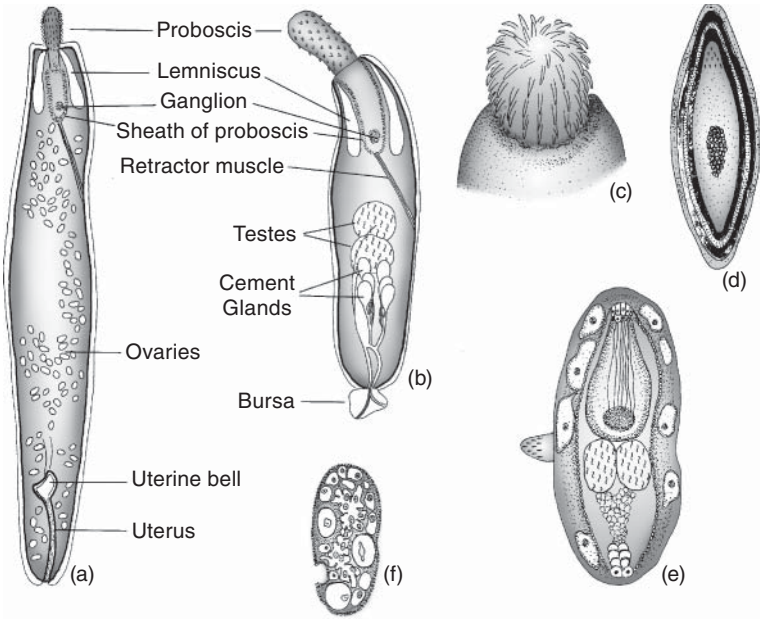


Figure 3.39 Acanthocephala: (a) Diagram of a female with floating ovaries (eggs not depicted). (b) Diagram of a male Palaeoacanthocephalan. (c) Proboscis of a thorny-headed worm. (d) Egg with acanthor larva. (e) Acanthella (on the outer right side: apex of the acanthor). (f) Ovary from the body cavity of a female worm.

(Figure 3.39f) in which eggs (Figure 3.39d) develop and within these, the first larval stage called acanthor matures. The eggs with the larvae inside are released and subsequently ingested by an arthropod (Table 3.9). Once they reach the gut, the acanthor hatches and penetrates the gut wall of the intermediate host. The larva, now called an acanthella, continues to grow and develops a complete set of organs. The parasite now undergoes a developmental change unparalleled in the animal kingdom, namely the rotation of the larva's longitudinal axis through 90° (Figure 3.39e). The proboscis is fully developed and found invaginated at the end of the growth process. The acanthella is now termed a cystacanth, and is infective for the final host. The passage from intermediate to final host may be facilitated by manipulation of the behavior of the latter (see Section 1.7.3).

In addition, paratenic hosts can be involved. The cystacanth present in such hosts in the body cavity or organs stays unaltered. Paratenic hosts, most often small vertebrates, are important where arthropods are not included in the final host's diet. *Centrorhynchus* species that infect birds of prey, and *Macracanthorhynchus* species that infect canids and felids, reach their final hosts via small lizards, snakes, or amphibians. In *Corynosoma* species that infect seals and piscivorous birds, the paratenic hosts are fish infected by crustaceans. A phenomenon called postcyclic parasitism is found, when an infected final host is taken up by a carnivore; in this case, the adult acanthocephalan can continue to live in the

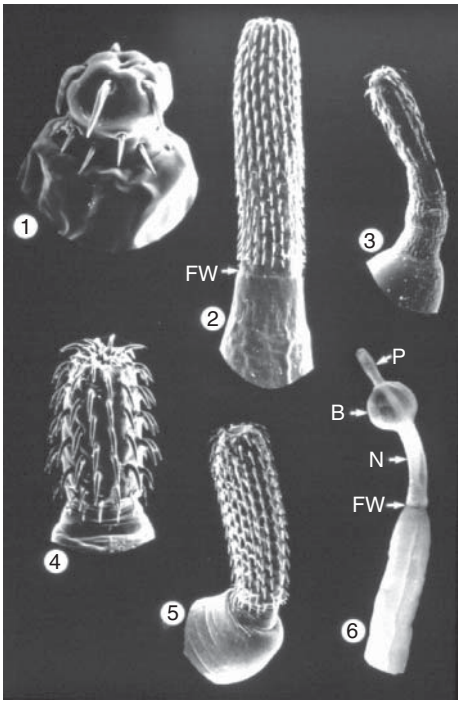


Figure 3.40 Acanthocephala. Presoma with proboscis of various species. (1) *Neoechinorhynchus rutili*, (2) *Paratenuisentis ambiguus*, (3) *Acanthocephalus anguillae*, (4) *A. lucii*, (5) *Echinorhynchus truttae*, (6) *Pomphorhynchus laevis*. B, Bulbus; FW, fibrous wall between the tegument of presoma and metasoma; N, neck; P, proboscis. (SEM image: Courtesy of H. Taraschewski.)

intestine of the carnivore. Figure 3.38 shows the example of *Plagiorhynchus cylindraceus*, where all three possibilities of acanthocephalan development are realized.

- A short life cycle with a starling as final host and the common pill-bug *Armadillidium vulgare* (crustaceans, isopods) as intermediate host (Figure 3.38a).
- A three-host cycle with a shrew as paratenic host, in which the cystacanth is encysted in the mesenteries and becomes sexually mature in the barn owl (Figure 3.38c).
- A postcyclic transmission in which worms present in the gut of a killed animal (hedgehog in this case) resettle in a final host, a carrion-eating bird like a crow or magpie (Figure 3.38b).

Acanthocephala are rigid and elongate. The living worms have a slightly flat shape and appear white, yellow, or orange. Females are larger (up to 6.5 cm) than males. *Macracanthorhynchus hirudinaceus*, however, measures 70 cm in length. The body is divided into two sections, the presoma and the metasoma. The **presoma** consists of the protrusible proboscis, covered with horizontal rows of hooks,

Table 3.9 Overview of the four classes of Acanthocephala (selected).

Class	Final hosts	Intermediate hosts	Egg shape	Species, host (intermediate host)
Archiacanthocephala	Terrestrial birds and mammals	Terrestrial insects, rarely Myriapoda, often paratenic hosts	Oval, thick-shelled	*Moniliformis moniliformis*, rodents (cockroaches) *Macracanthorhynchus hirudinaceus*, pig (beetles) *Prosthenorchis elegans*, New World monkeys (cockroaches)
Polyacanthocephala	South American caimans	not known	Oval with radial sculptures	Caimans (paratenic hosts: Cichlidae)
Eoacanthocephala	Fish (amphibia, reptiles)	Aquatic insects, small crustaceans: ostracods	Oval to oblong with polar thickening of second membrane	*Neoechinorhynchus rutili*, freshwater fish (ostracods)
Palaeacanthocephala	All vertebrates	Small crustaceans: isopods, amphipods	Variable	*Pomphorhynchus laevis*, fish, (gammarids) *Polymorphus minutus*, aquatic birds, gammarids *Filicollis anatis*, ducks (*Asellus aquaticus*)

followed by a neck and a sac-like proboscis sheath, into which the proboscis can be withdrawn (Figure 3.39a,b). It separates the two cavities of presoma and metasoma from each other. The prosoma contains a cerebral ganglion and invagination muscles. Paired invaginations of the presoma tegument are the lemnisci (Greek: *lēmniskos = band of fibres).* They serve to hydraulically protrude the proboscis, and possibly also store lipids, which are shed from the presomal tegument as protection against host defenses. The **metasoma** is the part perceived as the body of the parasite. Essentially, it contains the genital organs. There is no compact ovary, but many single ovaries freely float in the body cavity. The eggs exit the body in a unique manner via a goblet-like organ, the uterine bell, situated in the distal part of the body. At the base of this, the mature eggs are separated from the immature ones, swept into the uterus, and released, whereas immature eggs return to the body cavity (Figure 3.40).

In the male, there are two testes (rarely one), and one or several cement glands.

A polymerized secretion of these glands seals the vagina after insemination until the eggs are released. The body wall is composed of a three-layered syncytial tegument. A gut does not exist and nutrition in form of lipids and lipophilic

compounds is absorbed by the presomal tegument and by perforated hooks of the proboscis in the Eoacanthocephala and Archiacanthocephala. Carbohydrates and amino acids from the lumen of the host's gut are absorbed by the metasoma. An excretory system is only present in the Archiacanthocephala. It consists of groups of protonephridia and excretory channels, which unite with a male *vas deferens* or the uterus.

The eggs, released with the feces of the host, are oval or spindle-shaped (Figure 3.39f). They contain an embryo, the acanthor, whose surface is covered with tiny spines and enveloped by at least four membranes generated by the embryo.

Adult acanthocephalans fix themselves to the gut wall with their proboscis either superficially and change their position repeatedly, or they penetrate deeply into the gut wall or even perforate it and stay attached. These can give a papular appearance to the outer gut wall. Such species may, in unsuitable final hosts, even perforate the gut wall, settle in the body cavity, and live there for some time. Occasionally, intestinal obstruction occurs in infections with large numbers of parasites. In the past, for example, serious disease or death was frequently the result in captive monkeys, caused by *Prosthenorchis* or *Onicola* infection. This has been nowadays successfully controlled by careful elimination of cockroaches, the intermediate hosts.

Further Reading

Herlyn, H., Piskurek, O., Schmiz, J. *et al.* (2003) The syndermatan phylogeny and the evolution of acanthocephalan endoparasitism as inferred from 18S rDNA sequences. *Mol. Phylogenet. Evol.*, **26**, 155–164.

Lasek-Nesselquist, E. (2012) A mitogenomic re-evaluation of the bdelloid phylogeny and relationships among the Syndermata. *PLoS One*, **7** (8), e43554.

Skuballa, J., Taraschewski, H., Petney, T.N., Pfäffle, M. and Smales, N.R. (2010) The avian acanthocephalan Plagiorhynchus cylindraceu (Palaeacanthocephala) parasitizing the European hedgehog (Erinaceus europaeus) in Europe and New Zealand. *Parasit. Res.* **106**, 431–437.

Verweyen, L., Klimpel, S., and Palm, H.W. (2011) Molecular phylogeny of the Acanthocephala (class Palaeacanthocephala) with a paraphyletic assemblage of the orders Polymorphida and Echinorhynchida. *PLoS One*, **6** (12), e28285.

Weber, M., Wey-Fabrizius, A.R., Podsiadlowski, L. *et al.* (2013) Phylogenetic analyses of endoparasitic Acanthocephala based on mitochondrial genomes suggest secondary loss of sensory organs. *Mol. Phylogenet. Evol.*, **66**, 182–189.

Test Questions

1. What are the morphological characteristics of Acanthocephala?
2. Which type of animals are the final hosts of Acanthocephala?
3. Which organ is infected by them?
4. How are the parasites anchored in the host tissue?
5. Which intermediate hosts do they use?
6. What damage does Acanthocephala infection cause?

3.3 Nematoda

- A complex three-layered cuticle
- Processes of the muscles extended toward the nerves (and not the other way round as in other animals
- A cloaca in the male
- The absence of circular muscles
- The absence of protonephridia
- Spermatozoa without flagella

Roundworms are an extremely successful phylum in the animal kingdom. With the exception of hot springs, they have conquered all ecosystems on earth as either free-living organisms or pathogens of plants and animals. Probably more than 1 million nematode species exist, but only about 25 000 have been described. Approximately 50% of the species inhabit marine environments and 25% live in soil. About 10% of the described species are plant pests and 15% are parasites of animals. They do not occur as ectoparasites. This is probably due to the absence of body appendages that would allow anchorage on skin or fur. In fact, the nematodes have a remarkably uniform rotund and spindle shape. Nematoda are placed in the relatively new taxon Ecdysozoa, molting animals, including among others the arthropods.

Essential insights into developmental biology were gained through nematodes. Thus, at the end of the nineteenth century, Theodor Boveri, a German biologist, discovered reductive cell division in the eggs of the horse roundworm *Parascaris equorum*, distinguished between somatic and generative cells and found the x-chromosome and the centromere. The nematode, however, which provided most of the modern knowledge, is not a parasite but the small, free-living *Caenorhabditis elegans*. It is easily grown on bacterial cultures and serves as model for developmental, molecular, and genetic studies throughout the world. Its genome was the first metazoan to be completely sequenced and has a size of approximately 100 Mb with about 21.800 protein-encoding genes organized in 12 chromosomes (2n). Approximately 70% of genes are trans-spliced, meaning a primary transcript is fused to a 5′ leader sequence of 22 bp before being translated. There are two different spliced leaders, SL1 and SL2. Approximately 15% of genes are present in polycistrons, that is, they are regulated by a common promoter. Gene silencing by RNAi allows downregulation of gene expression in *C. elegans*, but has so far not efficiently worked in parasitic nematodes.

The **systematics** of nematodes have been altered extensively by molecular data. The new phylogeny contains three classes: the nonparasitic Enoplea, the Dorylaimea and a large group, the Chromadorea.

3.3.1 Development

Nematodes lay their eggs (Figure 3.41 f–k) at the one- to eight-cell stages or containing the first juvenile stage known as larva. Strictly speaking, the term larva is not accurate, as there is no fundamental difference to an adult worm and no clear metamorphosis takes place. Nematodes go through four larval stages, L1–L4. What is sometimes called the L5 stage is not a larval stage, but a juvenile adult. To pass from one larval stage to the next, the animals go through a molting process called ecdysis. A larva that has not shed its cuticle by the time it progresses to the next stage is called a sheathed larva (Figure 3.49, 3.56). In Chromadorea, (see Table 3.10), the L3 is the stage that is infective to the final host, whereas in the vertebrate parasitic Dorylaimea, the L1 is the infective stage

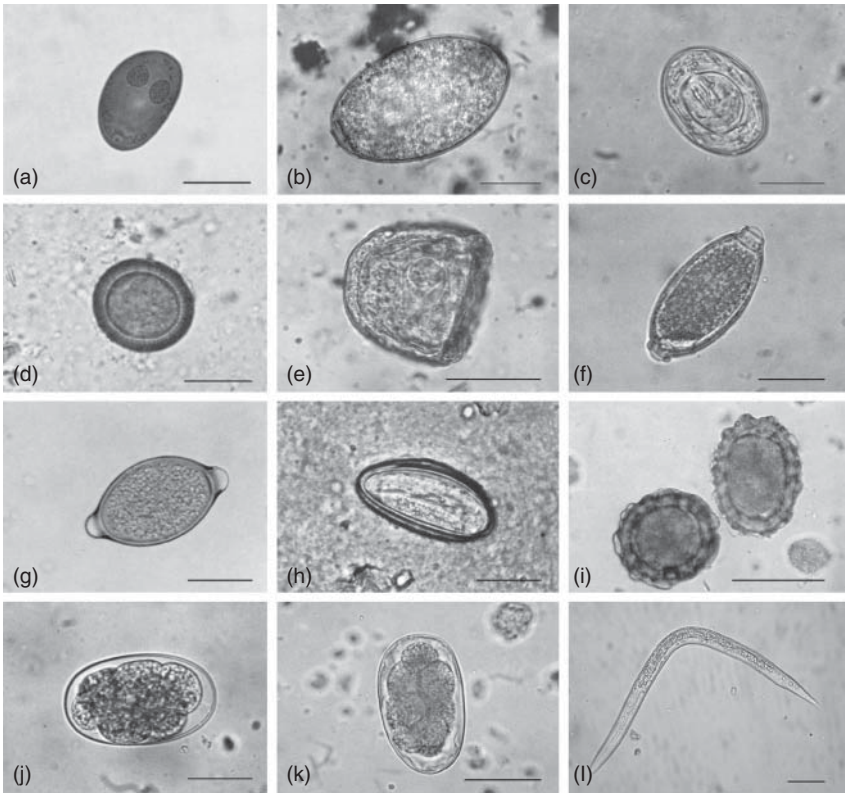


Figure 3.41 Eggs and larva of parasitic worms to be detected in feces. (a) *Dicrocoelium dendtriticum*, (b) *Fasciola hepatica*, (c) *Hymenolepis nana*, (d) *Taenia* sp., (e) *Moniezia expansa,* (f) *Capillaria aerophila,* (g) *Trichuris suis*, (h) *Enterobius vermicularis*, (i) *Ascaris lumbricoides*, (j) *Ancylostoma duodenale*, (k) *Haemonchus contortus*, (l) L1 of *Dictyocaulus viviparus*. (By courtesy of Janssen Animal Health, b2340 Beerse, Belgium. Image (l): Courtesy of Thomas Schnieder.)

Table 3.10 Systematics of the Nematoda according to Zhang, Z.-Q. (ed.) (2013) Animal biodiversity: an outline of higher-level classification and survey of taxonomic richness (2013 Addenda). *Zootaxa*, **3703**, 1–82 (only parasites mentioned in this book).

Enoplea (class)	Ancestral nematodes, no animal parasites
Dorylaimea (class)	
Mermithida (order)	Endoparasitic larva in arthropods. Not in here
Trichocephalida (order)	
Trichinelloidea (superfamily)	
Capillariidae (family)	*Calodium hepaticum*
Trichinellidae (family)	*Trichinella spiralis*
Trichuridae (family)	*Trichuris trichiura*
Chromadorea (class)	
Rhabditica (superorder)	
Panagrolaimida (order)	
Strongyloidoidea (superfamily)	*Strongyloides stercoralis*
Strongyloidea (superfamily)	
Ancylostomatidae (family)	*Ancylostoma duodenale, Necator americanus*
Metastrongylidae (family)	*Angiostrongylus cantonensis*
Trichostongylidae (family)	*Haemonchus contortus, Dictyocaulus viviparus*
Spirurida (order)	
Ascaridina (suborder)	
Ascaridoidea (superfamily)	
Ascaridae (family)	*Ascaris lumbricoides, A. suum, Toxocara canis*
Anisakidae (family)	*Anisakis, Anisakis simplex*
Dracunculina (suborder)	
Dracunculoidea (superfamily)	*Dracunculus medinensis*
Oxyurina (suborder)	
Oxyuroidea (superfamily)	
Oxyuridae (family)	*Enterobius vermicularis, Oxyuris equi*
Sprirurina (suborder)	
Filarioidea (superfamily)	
Filariidae (family)	Animal parasites
Onchocercidae (family)	*Onchocerca volvulus, Wuchereria bancrofti, Bugia malayi, Loa loa, Dirofilaria immitis*

with a stylet on its head. Most nematodes are oviparous. Only *Trichinella* and the filariae of the family Onchocercidae are viviparous.

Hosts are invaded through several routes:

- By ingestion of embryonated eggs (e.g., *Ascaris*), developed larvae (*Haemonchus*) or an intermediate host (e.g., *Dracunculus*)
- By percutaneous larval invasion (e.g., *Ancylostoma*)
- By larval injection through bloodsucking arthropods (e.g., *Wuchereria*)
- Only rarely by prenatal larval infection of the fetus (e.g., *Toxocara*)
- By ingestion of nonobligatory paratenic hosts, in which L3 stages remain unchanged and accumulate, facilitating the infection of the end host (*Anisakis*).

Very often, the route of infection is convoluted with larvae traveling through several organ systems before reaching their final location (e.g., *Ascaris*).

3.3.2 Morphology

Parasitic nematodes are usually colorless. They measure from 1.5 mm (*Trichinella spiralis*) up to 1 m (*Dioctophyme renale*) or even 9 m (*Placentonema gigantissima* in the placenta of sperm whales) in length. Nematodes are long, slender, rotund, unsegmented, and bilaterally symmetrical. The anterior oral opening is connected with the posterior anus by a straight unbranched intestine (Figure 3.42a, b). The body cavity is a fluid-filled pseudocoelom (meaning that it is not lined by mesoderm) under high hydrostatic pressure. There is no circulatory system. Two excretory channels run along each side of the body, emptied by an excretory pore near the head. Gender dimorphism is usually present: females are generally larger than males. Eutely (constant number of cells of the organism) is the rule and indicates that no repair is possible after injury.

The body wall of nematodes is composed of cuticle, epidermis, and a layer of longitudinal muscles beneath. The cuticle is usually three-layered and collagen-rich. It is secreted by the epidermis, has a tough and flexible consistency, and is permeable to water, certain ions, nonelectrolytes, and certain organic substances. The epidermis may have a ring or longitudinal ridge structure as well as bristles or other distinctive features. Skin duplications in the cervical and caudal areas are diagnostically relevant features and aptly named cervical (Figure 3.42f) or caudal alae (Latin: ala = wing). The epidermis is a thin syncytial layer. It protrudes ventrally, dorsally, and laterally into the pseudocoelom, forming four strands that run along the whole body (Figure 3.42c). The lateral strands contain the excretion channels, while the main nerve cords are embedded within the dorsal and ventral epidermal strands. Between the epidermal strands are quadrants of somatic muscle, consisting of a single layer of longitudinal muscle cells. Each muscle cell has an extroverted contractile section with longitudinal muscle filaments and an introverted noncontractile section with processes that connect to the dorsal motoneurons in the dorsal half and with the ventral nerve cord in the ventral half (Figure 3.42c). It is not the muscles that are innervated, but they establish the connection to the nerves. Apart from the head, where muscle cells connect to cerebral neurons to permit circular movement, the rest of the body can only produce undulating dorsoventral movements, due to the longitudinal muscles. Cuticle, pseudocoelomatic fluid (hemolymph), and the muscles function together as a hydrostatic skeleton.

The **digestive system** has three parts:

- The oral opening and pharynx (sometimes called esophagus), both lined with cuticle and thus subject to molting
- The endodermal intestine
- The rectum, lined with cuticle.

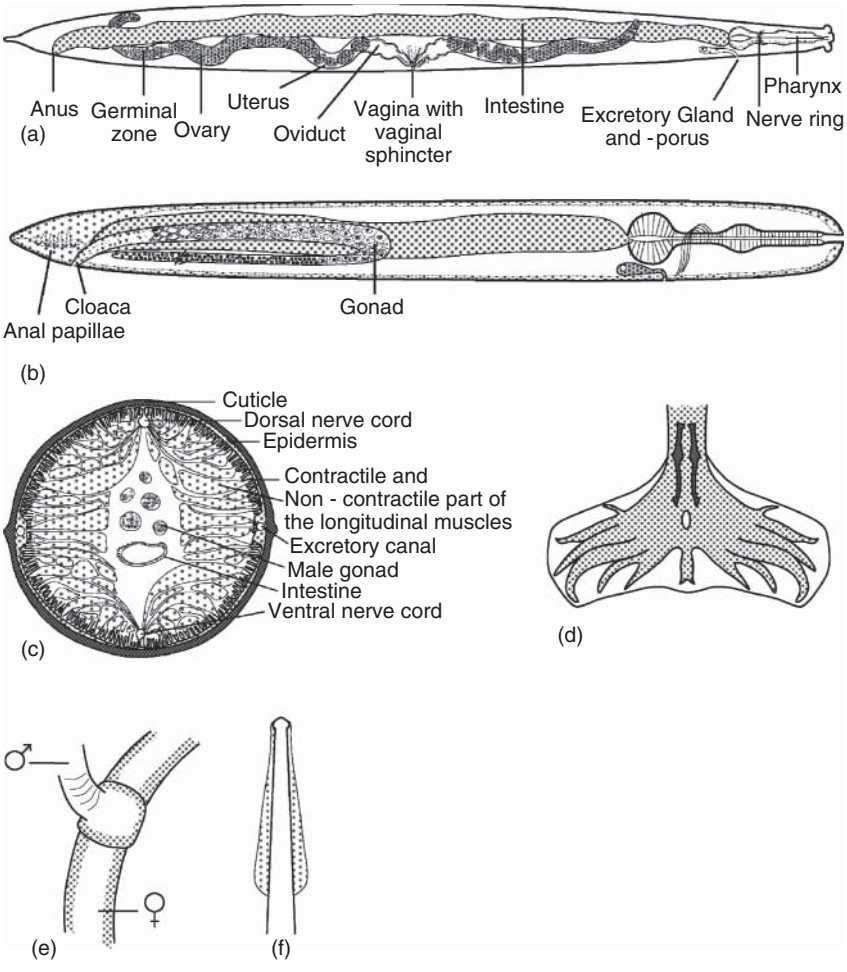


Figure 3.42 Structures of nematodes. (a) Scheme of a didelphic female. (b) Scheme of a male. (c) Cross section of an *Ascaris* male. (d) Copulatory bursa of *Trichostrongylus* (two copulatory spicules). (e) Position of the copulatory bursa of Strongyloidea (the male copulatory bursa envelops the female body at her genital opening). (f) Cervical alae of *Toxocara cati*.

The oral opening is surrounded by three or six lips (*Ascaris* for instance, Figure 3.51b) or bilaterally symmetrical (Filaroidea, Figure 3.59f). The buccal cavity may be small or large. The pharynx is usually a muscular sucking organ with a triradiate lumen, sometimes with a valve at its distal end, which prevents regurgitation. The intestine is a simple tube of epithelial cells, lined with a brush border of microvilli at the luminal side. The rectum of the male receives from the testis and ejects the sperm, and is thus considered a cloaca.

Of the **nervous system,** only the circumpharyngeal commissure is visible (Figure 3.42). From this, a ventral nerve cord draws backward, and extensions on the opposite side join to form the dorsal cord. Sensory endings with modified cilia called papillae serve as chemo- or mechanoreceptors concentrated in head and tail region. Pairs of them are particularly conspicuous at the posterior end of male nematodes. Also present are the pairs of lateral amphids, complex sensory organs just behind the oral opening. Phasmids, similar to amphids, are not present in all species.

The excretory system is actually an excretory/secretory system. In Chromadorea, it consists of narrow channels embedded in the two lateral epidermal strands. A median passageway connects the channels to two subventral gland cells and a ventral excretion pore that is level with the pharynx. The channels have two extensions toward the anterior end, forming a H shape. In the absence of such front extensions, the channels form an inverted "U" shape. Asymmetrical channel formations are also known. The channels are lined with microvilli. Unlike protonephrids in platyhelminths, the tubular system has no filtration purposes, but has osmoregulatory functions and facilitates the excretion of nitrogen-containing products of the metabolism.

The **gonads** of nematodes are long, sturdy looped tubes, narrow at one end, and gradually widening (Figure 3.42a, b). Most females have two gonads and are called didelphic (Greek: *delphys* = uterus). Those with one gonad are monodelphic. The ovary merges into an oviduct, uterus, and muscular vagina. The vagina opens into the ventral vulva (the exact location of which may vary, depending upon species). The male usually has a single gonad, starting with a testis. Then follows a seminal vesicle, the *vas deferens*, and the muscular ejaculatory duct. This opens into the cloaca together with the intestine. The males possess secondary copulation structures. For instance, many Chromadorea have two rod-shaped or filiform spicules (Figure 3.42d), which can be everted and thrust into the female's vulva to hold it open and secure the anchorage of the sexual partners. Only one spicule is present in many oxyurids. The distal end of all males of the Strongyloidea forms the *bursa copulatrix*, a thin fan- or bell-shaped dorsal expansion of the cuticle reinforced by bursal rays. The lateral parts of the bursa are bent ventrally to hold the female during copulation (Figure 3.42e). Nematode spermatozoa lack a flagellum and an acrosome, contain many mitochondria and membranous organelles and display ameboid movements.

Nematode **eggs** are easily distinguished from eggs of other parasitic worms. Their shape is elliptical and the two poles are symmetric (except in rare cases where an operculum is formed. The shell may be thick or thin. Usually eggs are very durable and resistant. In *Trichuris* and *Capillaria,* the eggshell contains openings at both ends, which are blocked by a plug of chitinous microfibrils (Figure 3.41f and g).

3.3.3 Dorylaimea

The class Dorylaimea (sometime called Dorylaimida) contains the important order Trchocephalida, with its families Capillariidae, Trichinellidae, and Trichuridae.

Characteristics of the order are:

- The L1 infects the vertebrate host.
- A stylet within the wall of the esophagus, the odontostyl.
- Presence of a secretory organ, the stichosome.
- Presence of two testes and only one ovary.
- Absence of particular sensory organs, the phasmids.
- Thread-like tension receptors (metanemes) in or near the lateral epidermis cords.

The Capillariidae, sometimes called hair worms, are extremely long and thin nematodes, many of them parasites of fowl with earthworms as intermediate hosts. *Calodium hepaticum*, formerly *Capillaria hepatica,* a parasite of rodents and other mammals, has to infect a first host, where the worms become adult in the liver. They produce eggs which remain in the liver. The eggs are liberated and become infective, when the host dies and decomposes, or is eaten by cannibalism or a predator and eggs are discharged with its droppings. In humans, severe disease can to occur in connection with eggs produced in urban rats.

3.3.3.1 *Trichinella spiralis*

The family Trichinellidae contains many human parasite species of the genus Trichinella. The medical importance of *T. spiralis* (Figure 3.43) was significant in the nineteenth century. In Germany, for instance, there were approximately 15 000 cases of trichinellosis between 1860 and 1890, with a mortality of 6%. This number dropped to zero during the subsequent 50 years, when, at the beginning of the twentieth century under the influence of Rudolf Virchow, meat inspection was established. Nowadays, however, trichinellosis has appeared to be on the advance again. Between 1991 and 2000, there were >20 000 cases in Europe. One of the causes was a breakdown of health care systems in East European countries after the political changes in the last few decades. In the United States, the prevalence of infection in commercial production has been reduced from an estimated 1.41% in 1900, down to 00.13% animal cases in 1995.

The genus *Trichinella* is highly unusual, because all infected host individuals are final hosts that harbor adult worms in their intestine and then become intermediate hosts bearing L1 stages in their muscles, ready to infect another final host. *Trichinella* is viviparous.

Ever since the discovery of *T. spiralis* at postmortems in human musculature in 1835, it has been considered as the only species. However, in 1972, Russian scientists discovered unencysted muscle-stage larvae of a new species, *Trichinella pseudospiralis,* in birds and mammals. They conducted elaborate

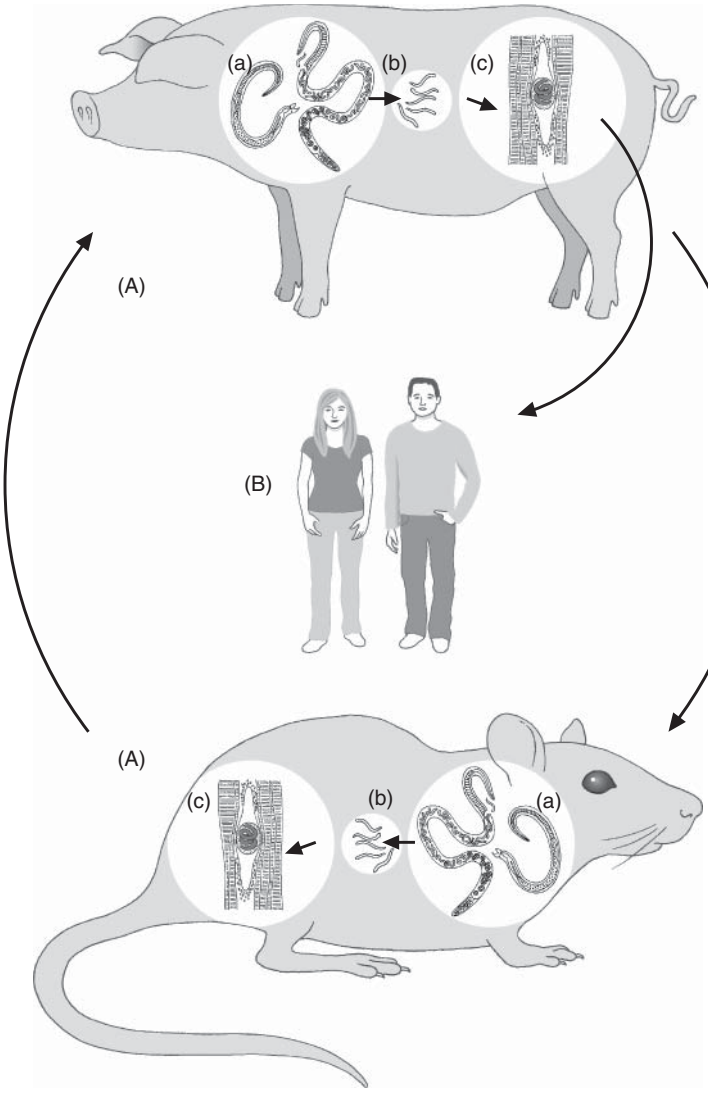


Figure 3.43 Life cycle of *Trichinella spiralis*. (A) (above) Pig, (A) (below) Rat. (a) male worm. (b) female worm, giving birth to (b) larvae, which settle (c) in skeletal muscle. Cycle repeats if pig eats rat, if rat eats pig slaughtering waste, or humans (B) eat infected pork. All stages develop also in man, who is, though, a dead-end host.

cross-breeding experiments and isolated two other new species, *Trichinella nativa* and *Trichinella nelsoni*. Meanwhile, eight valid species (three of them with unencapsulated muscle-stage larvae) and three genotypes are known from many parts of the world (Table 3.11) with the consequence that all earlier publications on *T. spiralis* have to be assessed with caution, as they might refer to other species. Differences between the species were demonstrated in parameters such as larval

Table 3.11 Species of the genus *Trichinella* (genotypes T6, T8, and T9 omitted). Human infections are possible with all species, but have not been demonstrated for *T. zimbawensis. T. native is* highly pathogenic for humans.

Species	Geno-type	Hosts	Type of cycle	Geographical distribution	Cyst wall present
T. spiralis	T1	Pig, carnivores, rats (rarely birds)	Urban and sylvatic	Cosmopolitan	+
T. nativa	T2 + T6	Carnivores, bear, walrus, rodents	Sylvatic	Arctic, subarctic (holarctic)	+
T. britovi	T3	Carnivores, also horse, rodents, insectivores	Sylvatic	Temperate regions down to West Africa	+
T. murelli	T3	Carnivores, bear, horse	Sylvatic	Nearctic temperate regions	+
T. nelsoni	T7	Carnivores, hyena, lion, warthog	Sylvatic	Africa	+
T. pseudospiralis	T4	Birds and mammals	Sylvatic	Cosmopolitan	–
T. papuae	T10	Mammals, reptiles	Sylvatic	Papua New Guinea	–
T. zimbabwensis	T11	Mammals, reptiles, man?	Sylvatic	Africa (Zimbabwe)	–

production *in vitro,* duration of development of the capsule of the muscle larva, and resistance of the muscle larva to freezing and isoenzyme patterns. Nowadays, species are identified using molecular biological methods applied to individual larvae in muscle, as a mixed infection cannot be excluded. The following account pertains to *T. spiralis* if not otherwise noted.

Development The host is infected by oral uptake of contaminated meat with encapsulated L1. Digestion of the capsule-like cyst in the stomach releases the larva. It passes into the small intestine, where it invades several neighboring cells of the mucosal epithelium, which are believed to fuse to form a syncytium. Within 30 h four molts, rapid growth, and copulation take place. The female gives birth to approximately 1500 L1 within 1–6 weeks. The L1 enter the subepithelial connective tissues, and are carried from there via lymph or blood via the right-hand side of the heart, through the lung capillaries and systemic circulation into all organs of the body.

The L1 invades a muscle cell of the skeletal muscles, especially those with high motility potential (diaphragm, eye, tongue, extremities). Until day 20, it increases its volume by approximately 40% each day. The parasite transforms the host cell into the so-called "nurse cell-parasite complex," developing a collagen capsule

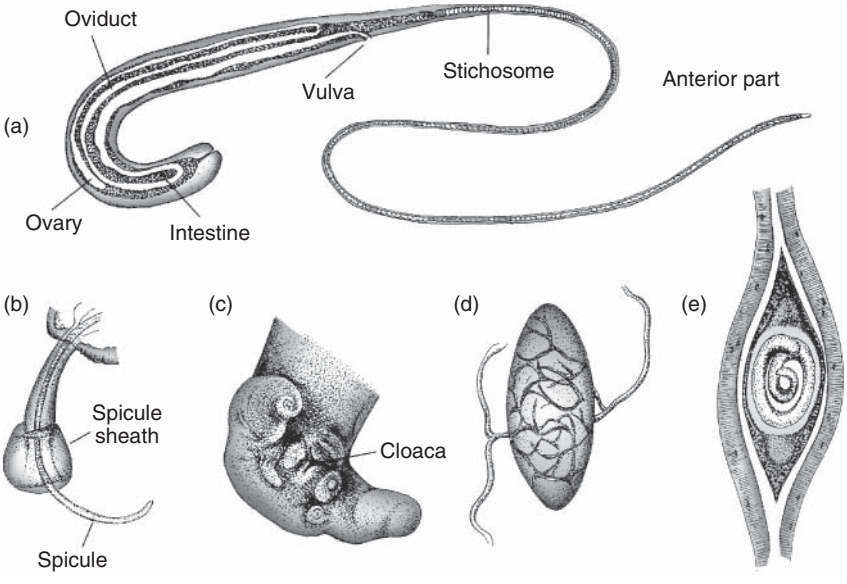


Figure 3.44 *Trichinelloidea*. (a) *Trichuris trichiura*, female, (b) *T. trichiura*, posterior end of male, (c) *Trichinella spiralis,* posterior end of male, (d) muscle larva of *T. spiralis*, nurse cell complex surrounded by blood vessels, (e) *T. spiralis*, cross section of muscle larva.

surrounded by a network of blood vessels (Figure 3.44d). Such a capsule does not exist in *T. pseudospiralis*, *Trichinella papuae*, and *Trichinella zimbabwensis*. After 4–6 weeks, the nurse cell attains a spindle shape and measures 180–950 µm. The larva coils up in the capsule and enlarges to 1 mm in length by 8 weeks (Figure 3.44e). It is now infective and, in some host species, remains infective lifelong. In humans, calcification of the capsule starts to develop from the fifth month onward. It then looks white and is visible by the naked eye. If this is the case, both larva and nurse cell die.

Morphology Morphologically, *Trichinella* is characterized by the structure of the pharynx, the long posterior part of which is called the stichosome. This is a narrow tube consisting of myofilaments and epithelial cells secreting cuticle. Also present are one to three rows of large gland cells, the stichocytes (Greek: *stichos* = row). They open by pores into the lumen. The stichosome is believed to play a role in the invasion of the host cell. Another characteristic is a head stylet in the L1.

The females measure 4 mm and males 1.5 mm in length. The stichosome occupies more than two-thirds of the whole body length. The *bursa copulatrix* of the male consists of two fleshy extensions (Figure 3.44c). A spiculum is lacking.

Genome The genome of *T. spiralis* has a size of only 64 Mb and is thus considerably smaller than that of *C. elegans* (100.3 Mb). The repeat content is estimated at 18%. There are approximately 15 800 protein-encoding genes organized in

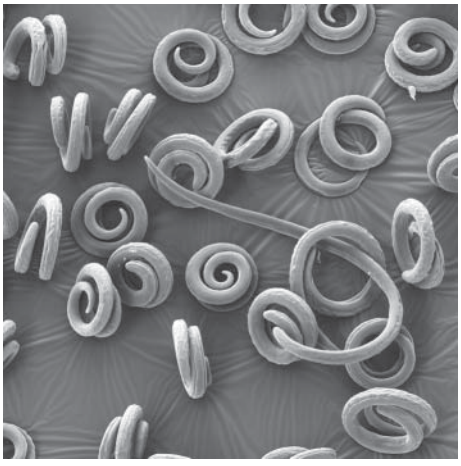


Figure 3.45 *Trichinella spiralis*, L1 digested from infected muscle tissue. (EM image: Courtesy of Eye of Science.)

2n = 12 for females and 2n = 11 for male worms. Polycistrons and trans-splicing of primary RNA seem to exist, but *T. spiralis* lacks the canonical sequences for the two spliced leader sequences found in other nematodes.

Epidemiology Trichinellosis is a zoonosis that requires the presence of carnivorous and scavenging animals. Two types of life cycles exist. The **urban cycle** involves humans and livestock. Pigs, rats, and (in China) dogs are the meat source. The nematode in the domestic cycle is *T. spiralis*. The second type, the **sylvatic cycle**, involves wild animals, that is, free-living carnivores, including pinnipeds. Whereas trichinellosis in the urban cycle could be largely eliminated by strict control mechanisms, animals in the sylvatic cycle pose a threat to humans where no adequate inspection of game is in place and meat is eaten raw or not sufficiently cooked. This applies in particular to wild boar and bear ham. One mystery remained unsolved until today: In 1975, horse meat was identified as the source of epidemic *Trichinella* infections in France and Italy, some of which were fatal. These outbreaks involved *T. spiralis, Trichinella britovi,* and *Trichinella murrelli*. How could plant-eaters get infected by *Trichinella*? It turned out that all infected horses originated from either Eastern Europe or the United States, and it is now believed that infected carcasses were used as feed additives for horses. Since that time, the directions for meat inspections have become more strict and especially include horses.

Pathology *Trichinella* infection goes through several phases. The first, enteral, phase is caused by adult worms in the intestine. If it is a strong infection, abdominal pain, vomiting, vertigo, and diarrhea may occur during the first week. However, the link between these symptoms and the consumption of *Trichinella*-infected meat is usually only made retrospectively. The enteral phase

is followed by a parenteral phase that sets in when L1 stages (Figure 3.45) invade the muscles. Symptoms are edema of the eyelids, lower jaw, or ankles, suggestive of an allergic response. Following this, aching skeletal muscles, fever, weakness, and dyspnea appear and sometimes also myocarditis, dysfunction of the central nervous system, and eosinophilia of up to 80% occurs. Unless infection has a fatal outcome, the symptoms may persist for 1 year and can then disappear without consequence. A positive diagnosis is usually made by muscle biopsy and serology.

Immunobiology In laboratory animals, intestinal *Trichinella* infections clearly induce strong adaptive immunity, reducing the impact and duration of challenge infections. In challenge infections of pigs, fertility of the female worms is reduced by 75% compared to controls, and hardly any cysts are found in the muscles. Rodent studies in the laboratory have demonstrated that expulsion of the worms from a primary (first) infection is mediated by intestinal inflammation involving the action of mast cells and mucus production from goblet cells present in the intestinal epithelium. The production of these is governed by cytokines produced from $CD4^+$ T helper cells and specialized populations of innate cells. The net result is that the intestine becomes unsuitable for worm reproduction and survival, and worms are then lost from the gut via enhanced peristalsis. The expulsion of parasites from subsequent infections can differ from the type seen after a primary infection in host species, at least, experimentally. For example, in the rat, expulsion can occur via the so-called rapid expulsion mechanism. This involves IgE-sensitized mast cells in the intestinal mucosa. Upon stimulation by worm antigens, they release inflammatory mediators, resulting in the attraction of effector cells and increased mucus secretion and peristaltic movement. Within hours, the live worms become enveloped in mucus and expelled.

3.3.3.2 *Trichuris trichiura*

The whipworm of humans *T. trichiura*, is one of the most common intestinal parasites of the tropics and subtropics. About 900 million people are infected by this parasite. The adult worms live in the epithelia of the large intestine, particularly the cecum. When more than 100 worms are present, hemorrhages, diarrhea, and sometimes intestinal prolaps can develop.

The worms have, a long thread-like anterior end and a thick, short posterior end (Figure 3.44a) which is in contrast to the name (Greek: *thrix*, *trich.s* = hair, our = tail). The male has a bell-shaped spiculum sheath with one spiculum (Figure 3.44b). The eggs are lemon-shaped and brown. They are excreted with the feces and contain the infectious L1 after development for 3–4 weeks at appropriate temperatures and humidity. Both poles are closed with a plug of gelatinous-looking material (Figure 3.42i). These plugs are important in the hatching process, as in the murine parasite *T. muris,* their contact with bacteria in the large intestine activates the hatching process. Egg-laying starts about 2–3 months after infection.

Other species of the genus are ***Trichuris vulpis*** in dog, ***Trichuris ovis*** in sheep, and ***Trichuris suis*** in swine, although more than 70 different species have been

documented in a variety of mammals. The porcine whipworm ***T. suis*** has generated considerable interest, as it is being tested as a possible therapy for a variety of inflammatory and autoimmune diseases, including inflammatory bowel diseases, such as Crohn's disease. Repeated doses of live eggs are administered orally to patients. It is believed that infective larvae do not reach maturity in humans, but downregulate inflammation. This approach is based on the observation that such infections are usually present naturally as chronic infections and infected people harboring worms live without serious pathology. It was hypothesized that to survive, the parasites have evolved mechanisms to regulate the immune response that could clear the parasites and cause pathology, that is, inflammation. Moreover, **T. muris**, the mouse whipworm is used extensively in the laboratory in studies of immune responses to intestinal nematodes. It is clear from these studies that long-lived infection is indeed associated with regulation of damaging host pathology. A reference genome of *T. muris* (85 Mb) and draft genomes of *T. trichiura* (75 Mb) and *T. suis* (80 Mb) were produced. Depending on species, between 9000 and 15 000 protein-encoding genes are predicted. Many proteases are present in all species reflecting their mode of life burrowing into intestinal epithelia. In addition, many homologies are found with the genome of closely related *T. spiralis*, which occupies a similar niche within the intestine.

3.3.4 Chromadorea

The class Chromadorea contains the overwhelming majority of the parasitic nematodes of vertebrates. Characteristics of the Chromadorea are:

- The L3 infects the vertebrate host
- Presence of one testis
- Presence of particular sensory organs in the tail region, the phasmids
- Location of amphids (sensory, innervated invaginations of cuticle) on the lips
- Absence of caudal glands

3.3.4.1 *Strongyloides stercoralis*

The threadworm of humans occurs in the tropics and subtropics. It causes problems in immunodeficient individuals or patients undergoing immunosuppressive therapy. Under certain circumstances, infection may be lethal. The parasite inhabits the mucosa of the upper small intestine. The species belongs to one of the two superfamilies of the mainly plant-parasitic order Panagrolaimida (mainly free-living or plant-parasitic nematodes), and the superfamiliy Strongyloidoidea (see Table 3.10).

Development *S. stercoralis* has no intermediate host. The life cycle is characterized by an alternation of generations and the ability to cause autoinfection (Figure 3.46). **The parasitic phase** starts when filariform L3 from contaminated soil invade the body, usually the feet. The larvae are carried via circulation to the

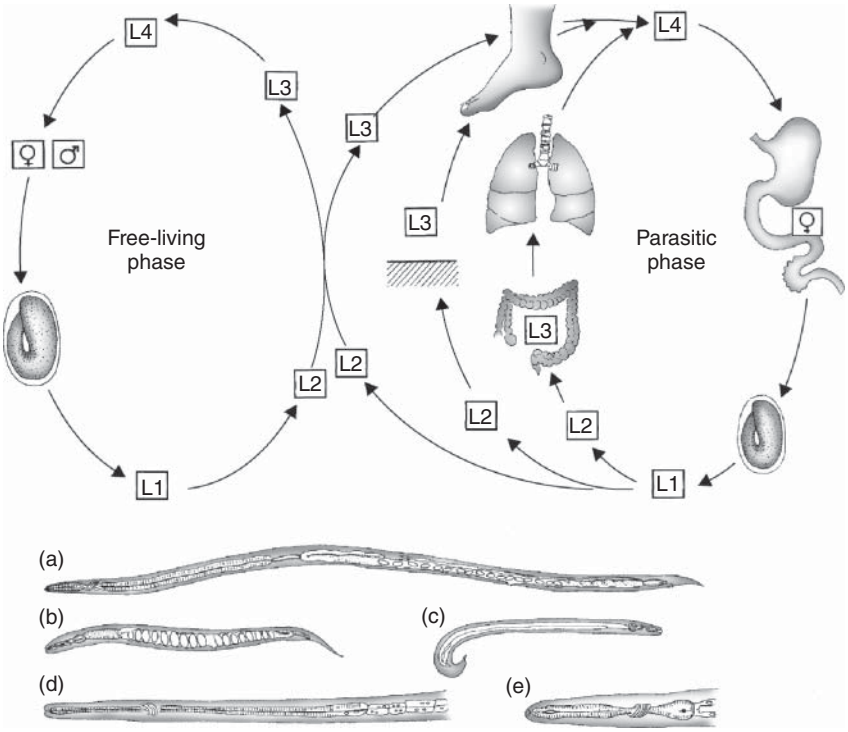


Figure 3.46 Life cycle of *Strongyloides stercoralis* (L in cycle = larval stages). (a) Parasitic female. (b) Free-living female. (c) Free-living male. (d) Anterior end of filariform larva with typical esophagus. (e) Anterior end of rhabditiform larva with typical esophagus.

lungs and trachea, penetrate the pulmonary aveoli, and are coughed up and swallowed. In the duodenum and jejunum, they molt twice and give rise to parthenogenetic females. Rhabditiform L1 hatch from the eggs in the intestine and are excreted with the feces. Eggs are rarely found in the feces. In addition, a small percentage of the L1 can develop to the L3 stage in the gut. These larvae are the origin of autoinfection, as they invade the mucosa of the colon or perianal skin, eventually reach the intestine after another body migration and become adult females. In this way, a small population of worms can be sustained in the body for years as chronic infection without overt pathology. In immunocompromised persons, a situation called hyperinfection may occur, giving rise to dramatically increasing numbers of infective larvae born in the intestine, which disseminate widely in the body. Such hyperinfections may have a lethal outcome if not diagnosed early and treated. The **free-living phase** starts with first-stage larvae hatching in the gut and excreted with the stool. They can follow two pathways: either molt twice and become infective female third-stage larvae (filariform L3, Figure 3.46d) or molt four times, thereby going through the stage of a rhabditiform L3 (see Figure 3.46e) and become free-living adult males and females, which can propagate again in the open.

Morphology The parthenogenic females from the gut are 2.1–2.7 mm long. Their buccal capsule is small and their pharynx is filariform (Figure 3.46a). Free-living females are about 1 mm long, the males slightly shorter (Figure 3.46b,c). Both have a rhabditiform pharynx. Both types of females are didelphic, with the vulva of the parasitic female two-thirds along the body length and that of the free-living female at the midpoint of the body. The infective filariform L3 is 490–630 µm long, the rhabditiform L1 passed in the stool is 180–240 µm long (Figure 3.46d,e). Eggs of the parasitic females are thin-shelled and embryonated when shed. They measure 54 × 32 µm.

Pathology In a heavy infection, pneumonia-like symptoms with dry cough may occur during the passage of larvae through the lungs. Intestinal symptoms are usually not present or are only slight in the chronic phase. Anal pruritus, though, may occur, when L1 invades the perianal skin.

The major concern is hyperinfection. This does not necessarily occur as often assumed in individuals that are immunocompromised due to HIV infection, for example. Rather it appears that immunosuppressive therapies with corticosteroids are responsible for disseminated strongyloidosis, as these substances are similar to molt-regulating substances and allow a faster development of the larvae hatching in the gut than under normal conditions. The large number of females and the larval migration through the body during a hyperinfection induces watery diarrhea, digestive problems, edema, heavy pneumonia, occasionally meningitis, and even death, as available therapies are rarely successful.

3.3.4.2 *Ancylostoma duodenale* and *Necator americanus*

The hookworms of humans (Figure 3.47) are of great importance in the tropics and subtropics. They inhabit the small intestine. About 900 million humans are currently infected, 50 000–60 000 per year die of the disease they cause. *A. duodenale*, commonly known as the Old World hookworm, prevails in subtropical countries of the Old World (Middle East, North Africa, India, and formerly Southern Europe) and *N. americanus* predominates in tropical regions (the Americas, sub-Saharan Africa, Southeast Asia, China, and Indonesia). The two species belong to the order Panagrolaimida and their second superfamily Strongyloidea (Table 3.10) (not to be confused with Strongyloidoidea) and the family Ancylostomatidae. Their members are called "bursa nematodes" because of the male copulatory apparatus, the conspicuous *bursa copulatrix* (Figure 3.47).

Development Eggs are shed at the two to eight cell stage and embryonate in the feces. L1 and L2 feed on bacteria in the excreted feces and soil. The infective L3 that develops is enveloped by the sheath of the second-stage larva. It migrates actively into the upper layers of the soil, invades the host percutaneously, usually in the feet, sheds its sheath, and enters subcutaneous blood and lymph vessels. It travels via circulation to the lungs, where the third molt occurs. It burrows through the alveoli, is coughed up and swallowed, and reaches the small intestine, where

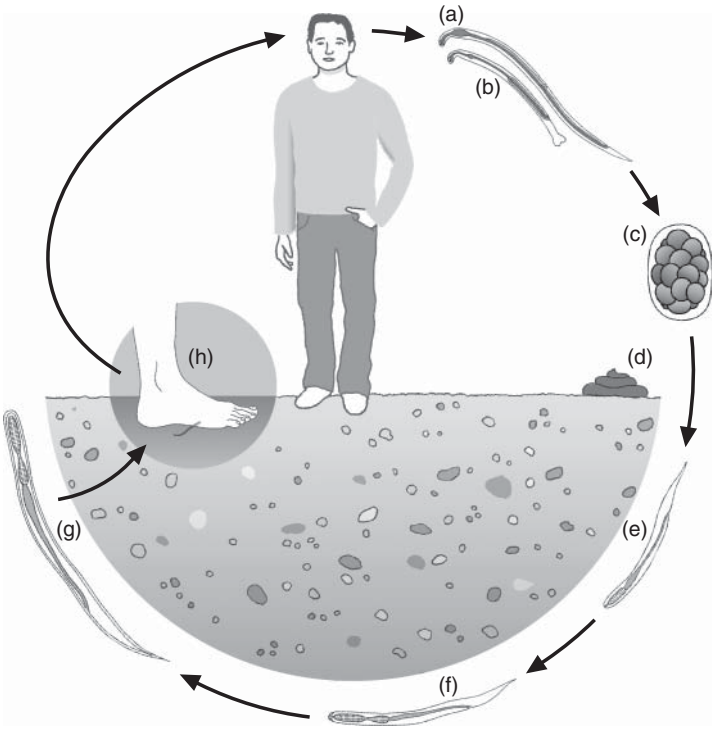


Figure 3.47 Life cycle of *Necator americanus*. (a) female, (b) male, (c) young egg, to develop in human feces, (d) L1, (e) L2, (f) in soil and feces. Sheathed L3 (g) invades the skin (h). Migration in human body not shown.

it molts to become an adult. The prepatent period is 3–4 weeks. Infection with *A. duodenale* can also occur by oral uptake of larvae. In this case, a body migration does not occur. *N. americanus* can live for 15 years. The production of eggs is higher in *A. duodenale* (25 000 eggs/day) than in *N. americanus* (10 000 eggs/day).

Morphology The worms are curved at the anterior end to give them the shape of a hook. They have a large mouth cavity with a skewed opening armed with teeth or cutting plates (Figure 3.48a,b, Table 3.12). The eggs are thin-shelled as in all Strongyloidea. The copulatory bursa has short ribs and is relatively small. The two spicules are long and thin (Figure 3.48c).

Genome The genome of *Necator americanus* is comparatively large and comprises 244 Mb with a relatively small proportion of repeat sequences (23.5%). It encodes for about 19 200 proteins, approximately 15% of which are trans-spliced. Approximately one-third of the putative proteins were predicted to be secreted. The expression of transcripts was very different in the free-living versus the parasitic phase. Free-living L3 expressed a high proportion of genes also found in

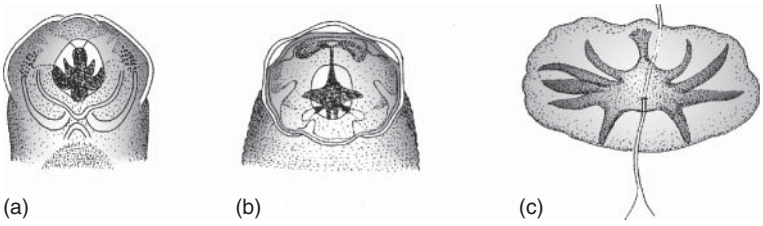


Figure 3.48 Hookworms (Ancylostomatidae). (a) Mouth capsule of *Ancylostoma duodenale*. (b) Mouth capsule of *Necator americanus*. (c) Copulatory bursa of *A. duodenale* with spicules.

Table 3.12 *Ancylostoma* and *Necator*.

	Ancylostoma duodenale	***Necator americanus***
Distribution	Subtropical: South Europe, northern Africa, Middle and Far East	Tropical: Southern USA, Latin America, Africa south of the Sahara, India, Southeast Asia, Oceania
Female length	10–13 mm	9–11 mm
Male length	8–11 mm	7–9 mm
Mouth capsule	Two pairs of ventral teeth on the anterior margin directed inward	Two dorsal cutting plates directed downward
Vagina	In posterior third	Shortly in front of middle
Eggs size	50–80 × 36–42 µm	64–75 × 36–40 µm
Prepatent period	38–74 (53) days	44–46 days
Larval development (temperature)	At 22–26 °C	At 31–44 °C

other nematodes, reflecting the lifestyle shared with many other nematodes. The proportion of hookworm-specific genes was much higher in the adult parasitic stage, revealing the adaptations to parasitism. Conspicuous among those genes was the high number of proteases, supposedly involved in migration and digestion of blood. Protease inhibitors were also numerous, supposedly required to protect the parasite against digestive and immunologically relevant proteases. In addition, hookworms express an array of secreted putative immunomodulators, some of which have been discussed as possible leads for the development of anti-inflammatory drugs.

Pathology The third-stage larvae invading the skin cause inflammatory redness and an itchy rash. The L4 and the adults in the intestine feed as bloodsuckers by drawing an intestinal villus into their mouth, rupturing it with their teeth, or cutting plates and sucking the blood. As soon as no blood is leaking out any more, the worm moves to a new villus. The blood loss caused by 100 adult *A. duodenale* amounts to tens of mls/day, that of *N. americanus* to somewhat less. In addition, the multiple attachment points on the villi are prone to secondary hemorrhages.

Infections with 500–1000 worms cause iron deficiency anemias. Infected people suffer from fatigue, dyspnea, low body temperature, palpitations, depression, apathy, and temporary loss of consciousness. Death by heart failure can occur. Children may suffer from stunted growth and impaired cognitive performance. Mortality is more pronounced in this group. Adaptive immunity is very slow to develop and difficult to determine, although some studies of *N. americanus* have shown an association of elevated IgE antibody levels and reduced worm fecundity. The public health importance of hookworms stimulated international efforts to develop a vaccine that targets proteolytic enzymes of the parasites involved in digesting host blood.

Cutaneous larva migrans or **creeping eruption** is a skin disease in humans caused by the larvae of the genus in dogs and cats, *Ancylostoma braziliense, Ancylostoma ceylanicum, Ancylostoma tubaeforme,* and (more rarely) *Ancylostoma caninum*. The larvae can invade the skin of humans, but cannot develop into adults. The disease, also known as ground itch, is an intense itching eruption, which may become very painful and, if scratched, may develop a secondary bacterial infection. The itching ceases when the parasites die. Creeping eruption, especially by *A. braziliense*, is one of the most common tropically acquired dermatoses.

Epidemiology The L3 larvae need warm, humus-rich soil for their development. They do not tolerate direct sunlight, water, and urine. Ideal conditions exist where defecation takes place near dwellings and working environments, where fertilization with untreated excrement is undertaken and where people walk barefoot. Children in such areas are particularly exposed to infection. Interestingly, *A. duodenale* was prevalent in coal mines of Central Europe in the past, where the necessary warm temperatures were present in the mines. In this case, the local name for the parasite was "pit worm" (Grubenwurm). The well-known disease named mining anemia came to public attention during the construction of the Gotthard Road Tunnel (1872–1880), where many workers became ill or even died from the disease. Autopsies performed by the Italian parasitologist Edoardo Perroncito identified *A. duodenale* as the cause of the disease. His book "La malattia dei minatori" was published in 1910.

3.3.4.3 *Angiostrongylus cantonensis*

A second family of the Strongyloidea (Table 3.10), the Metastrongylidae, are lung-dwelling parasites of animals. *A. cantonensis* causes a disease of growing importance called eosinophilic meningitis or encephalitic angiostrongylosis in humans in Southeast Asia and the Pacific Basin. The final hosts of *A. cantonensis* are rats (*Rattus rattus, Rattus norvegicus*), with the intermediate hosts being snails and slugs, in which infective third-stage larvae develop. Once taken up by a final host, the L3 migrate through the gut wall and blood vessels into the brain and then into the pulmonary artery, where they become adults (="rat lungworm"). Eggs hatch in the lung and the first-stage larvae are transported via

the trachea to the intestine and are passed in the rodent feces. When taken up by the intermediate host, they develop to the infective L3.

Humans are infected by eating raw or undercooked intermediate hosts. These are various land snails, such as *Achatina fulica*, the giant African snail, and fresh water snails such as apple snails (*Pila* species) or slugs.

In humans as accidental hosts, the migration through the body ends in the brain, where the larvae, depending on their number and location (meninges or deeper tissues of the brain), cause severe damage that can lead to death or permanent brain or nerve damage. The disease is currently spreading because of globalization of the trade of snails as a delicacy. In addition, diagnosis is difficult and often inconclusive.

Females of *A. cantonensis* are 17 – 34 mm long, with a conspicuous spiraled red gut with white uterine tubules. Males measure 15 – 25 mm and have a small bursa copulatrix with short rays. The L3 are 425 – 524 µm long.

3.3.4.4 *Haemonchus contortus*

A third family of the superfamily Strongyloidea are the Trichostrongylidae with many parasites of veterinary importance. The red stomach worm *Haemonchus contortus*, also called wire worm or barber's pole worm, is a parasite mainly of sheep and goats, more rarely of cattle (Figure 3.49). It has been suggested that no flock exists completely free from this parasite. It is one of the several species, which, in domestic ruminants, causes parasitic gastroenteritis. *H. contortus* is the nematode in which the phenomenon of hypobiosis has been studied most extensively. Further genera of the family (with several species) are *Ostertagia, Trichostrongylus, Telodorsagia, Cooperia*, and *Nematodirus*.

Development Eggs of *H. contortus* are shed by the host in the morula stage (Figures 3.41k and 3.49f) and embryonate in the feces. The first two larval stages (rhabditiform) feed on bacteria in the excreted feces. The sheathed, filariform, infective L3 arising from the second molt after 5 days is very active, leaves the feces, and crawls upward on the blades of wet grass. When taken up orally by a suitable host, it sheds its sheath. In the crypts of the abomasum, it molts to become a bloodsucking L4. This phase is called histiotropic (Greek: *histós* = tissue, *trópos* = direction). Afterward, the larva molts in the lumen of the abomasum. The adults are also bloodsucking. The prepatent period is between 12 and 24 days.

In addition to this normal course of infection, *H. contortus* has the ability to enter a stage of arrested or inhibited development, known as hypobiosis. From late summer onward, increasing numbers of L4 remain in their histiotrophic phase. As soon as the short-lived adults in the abomasum are eliminated, no fecal eggs are detected any more and the host appears parasite-free. The inhibited larvae do not show any metabolic activity and are resistant to anthelmintics. In temperate climates, hypobiosis has been observed from September onward. It appears that lower temperatures induce hypobiosis in infective L3, as conditioning of L3 by low temperatures results in arrested development. Only in spring is

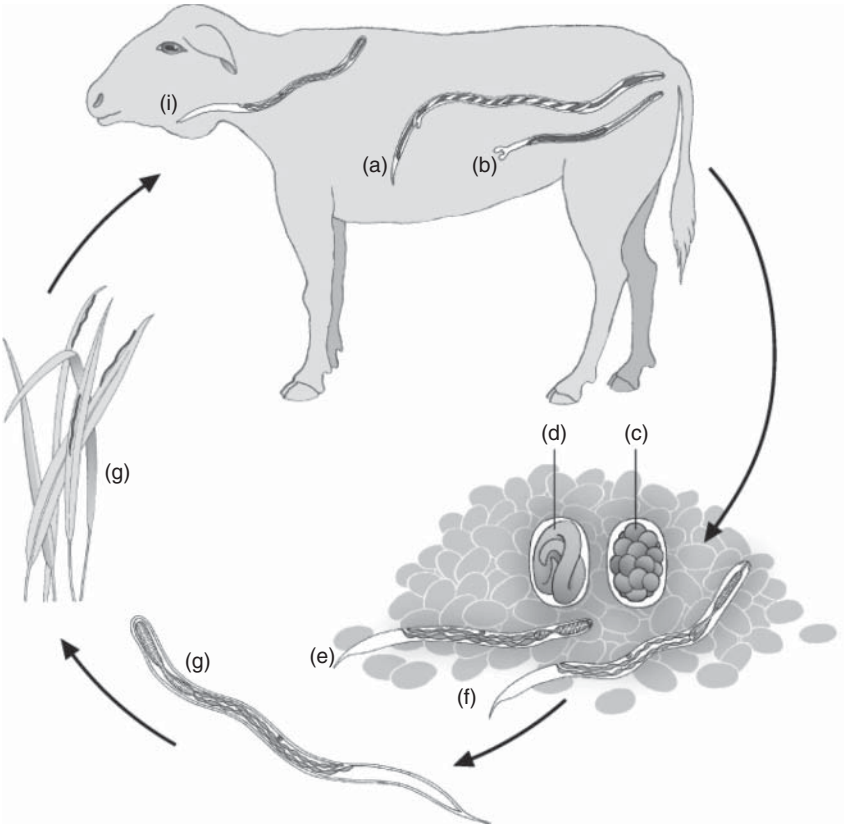


Figure 3.49 Life cycle of *Haemonchus contortus*. Female (a) and male (b) in abomasum of sheep. Young (c) and fully developed (d) egg in feces. L1 (e) and L2 (f) in feces. Sheathed L3 (g) leaves feces and crawls onto plants (g). When eaten by sheep it sheds its sheath to become an L4 (i).

development resumed. The number of eggs then increases dramatically in what is called "spring rise." In pregnant ewes, this happens around the time of the birth ("periparturient rise") and during lactation facilitating the infection of lambs. Hypobiosis has also a genetic basis. If infected hosts are transferred from one climate to another, contaminating "clean" pastures with eggs, the "old" periods of hypobiosis prevail in spite of the new temperature regime.

The true mechanisms of hypobiosis and its termination are still not entirely understood. The host immune system could be involved or it could have its origin in the parasite, which aims to coordinate the seasonal appearance of its progeny with beneficial environmental conditions, very much like the diapause of insects. This theory is supported by the fact that in arid regions, hypobiosis starts before the dry season and eggs are only shed at the beginning of the rainfall period. Hypobiosis is also seen in most trichostrongylids and occurs in a variety of host species.

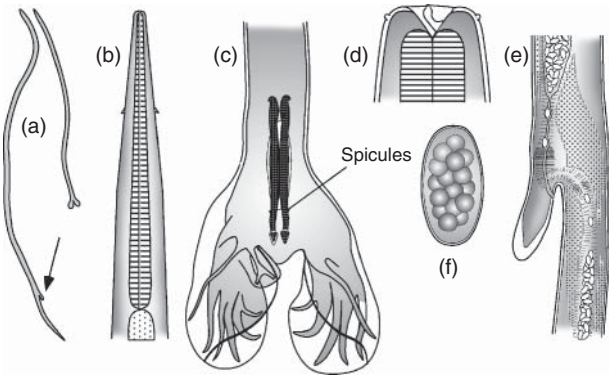


Figure 3.50 *Haemonchus contortus*. (a) Male and female at equal magnification (arrow: valvular flap). (b) Anterior end with cervical papillae. (c) Copulatory bursa with spicules. (d) Mouth capsule with hook and muscular pharynx. (e) Female genital opening with vulvar process (arrow). (f) Egg.

Morphology *H. contortus* is the easiest to identify among the trichostrongylids by its reddish appearance when alive. As usual in nematodes, the females are longer than males (Figure 3.50a). In the female, 20–30 mm long, the blood-filled red intestine and the white reproductive organs are wound around each other (barber's pole). About half a millimeter behind the anterior end, a pair of conspicuous cervical papillae is visible (Figure 3.50b). Within the very small oral opening, there is a tiny hook (Figure 3.50d). The vulva of the female in the posterior quarter of the body is covered by a prominent protrusion, termed a vulvar process (Figure 3.50e). The copulatory bursa of the 18- to 21-mm-long male is asymmetrical, because the small dorsal lobe, usually in the middle, is located laterally (Figure 3.50c). The two spicules are relatively short, structures measuring 490–540 µm with a barb at the distal end. The eggs are thin-shelled and measure 80 × 45 µm (Figure 3.50f, 3.41k).

Genome The genome of *Haemonchus contortus* is 320 Mb in size and thus more than three times larger than the *C. elegans* genome. The phylogenetic position within the group of parasites most closely related to *C. elegans* makes comparisons interesting: In line with a widespread conservation of synteny between the two species, the number of protein-encoding genes in *H. contortus* and *C. elegans* is similar (23619 vs 21733 genes), resulting in a strongly reduced gene density per Mb in *H. contortus*. While about 7% of the parasite genome are protein-coding, the proportion in the free-living nematode is 30%. The protein-encoding genes of the parasitic nematode contain more and longer introns. While 29% of the *H. contortus* genome consist of repetitive elements, this is only approximately 16% in *C. elegans*. Comparative analyses also show a dramatic expansion of important parasite gene families, related, for example, to blood feeding. These facts are in strong contrast to the popular view that parasites generally have reduced genomes, but the reasons for the expansion of this parasite genome are unclear.

Epidemiology Infection of lambs with high worm burdens occurs mainly in spring, due to the massive contamination of pastures with eggs as a consequence of hypobiosis and spring rise. Another crucial factor is the high rate of reproduction. One female worm can produce 5000 – 10 000 eggs per day.

Pathology The acid-producing gland cells of the host's abomasum are destroyed by the L4 during the histiotropic and hypobiotic phase. The consequence of this is an elevated pH, a decreased conversion of pepsinogen into pepsin, disturbed protein digestion, increased growth of bacteria, inflammation leading to indigestion, and decreased food intake. The bloodsucking activity of the L4 and the adults causes further problems and blood loss may amount to 50 µl per worm per day. As a consequence, lambs can develop life-threatening anemia. The natural elimination of the adult worms is associated with watery diarrhea. Lambs do acquire immunity but only after 6 months of age. This protects them during the forthcoming grazing periods. Such immunity can be induced by vaccination with purified worm glycoproteins, but, to date, the production of recombinant vaccines has not been successful.

Control Benzimidazoles (e.g., Albendazole© and Mebendazole©) and macrocyclic lactones (e.g., Ivermectin©) have been effective in treating these infections for decades, but are losing their potency because resistance has developed. Therefore, complete elimination of these infections will probably never be achieved. At the moment, a decrease of *H. contortus* can be accomplished by appropriate grazing management (i.e., not allowing lambs to graze on contaminated pastures). Alternative approaches are being considered. For example, the nematophagous fungus *Duddingtonia flagrans* can agglutinate live nematode larvae and kill them. In addition, the administration of tanniferous fodder of plants such as esparcet (sainfoin) *Onobrychis viciifolia* has also been shown to have antiparasitic activity.

3.3.4.5 *Dictyocaulus viviparus*

Unlike other trichostrongylids, this species inhabits the lungs of cattle. In this infection, parasite eggs having passed from the bronchia to the trachea are coughed up and swallowed. From these eggs, the L1 hatch while still in the intestine and develop to L3 within the droppings. The L3 are dispersed across the pasture by the fungus *Pilobulus* that spreads its own spores by an explosive mechanism, a mechanism increasing the chances to reach the coprophobic cattle host. Apart from chemotherapy, protection against *D. viviparus* can be achieved by the use of an irradiated live L3 vaccine. With this approach, protective immunity is induced by the parasite that dies before its pathological stages develop, leading to significant protection against new infection.

3.3.4.6 *Ascaris lumbricoides*

The first of four suborders of the order Spirurida (Table 3.10) is the Ascaridina. Their family Ascaridae contains, next to the genus *Toxocara,* the Giant Roundworm *Ascaris lumbricoides*. This is one of the best-known parasites and

occurs worldwide, but preferentially in the tropics and subtropics. Its impressive size – the females are 30–40 cm long – and occasional appearance in the stool have always generated interest. Indeed, the parasite is mentioned in the Papyrus Ebers (~1540 B.C.) and has been described regularly from archeological studies in humans across the continents and ages. It is of no regard for status either, with the recent discovery of the body of the English king Richard III showing ascaris eggs in the monarch's gut. In fact, ascariosis occurs in all areas with sufficient soil humidity. Of the >1 billion people currently believed to be infected, for approximately 60 000 per year, the infection is lethal.

Development The development of the zygote within the egg only begins when the egg has been shed with the feces and sufficient oxygen is available (Figure 3.51). Embryonation leads to the development of the first larval stage, which molts twice within the egg to become an infective third-stage larva. This process takes 8–10 days in tropical environments and 16–18 days in temperate regions. After ingestion of the egg, the L3 perforates the wall of the duodenum, and is transported by the blood stream into the liver, where it molts to the L4 stage and grows. Via the right ventricle it is carried with venous blood into the lungs, where it penetrates the wall of the alveolar capillaries and is detectable in the bronchial mucus 12 days post infection. With the mucus, the larvae are transported up the trachea and then swallowed. In the small intestine, they molt to adults. Eggs are shed with the stool from 60 to 75 days post infection. The worms have a life span of 6–18 months.

Epidemiology Embryonated eggs may stay infective for 2–4 years, if kept moist and protected from ultraviolet light, that is, in soil or under dense vegetation. They are extremely resistant to strong chemicals and low temperatures, but are destroyed by desiccation. Eggs accumulate in the soil especially in regions where feces are not properly disposed. In addition, vegetables fertilized with human feces and eaten raw contribute to human infection. Naturally, children playing on the ground are most at risk and most heavily infected, although ascariosis often occurs as family infections.

Morphology The large worms (Figure 3.51a), when alive have a slightly pink appearance, which indeed, as suggested by the Latin name, makes them look similar to an earthworm (*Lumbricus terrestris*). The mouth is surrounded by three lips (Figure 3.51b). The lateral excretory channels are visible with the naked eye as whitish lines. The females are 20–35 cm long and 3–6 mm thick. Their vagina is situated in the proximal third of the body. The two ovaries, 10 times as long as the worm, contain about 27 million eggs. Approximately 200 000 of them are shed per day. The eggs are broad-oval, thick-shelled, brown due to phenolic compounds, and carry an operculum only visible with scanning electron microscopy. Two types of eggs appear in the stool: fertilized eggs measure about 60 × 45 µm, and on their surface exhibit knobbly deposits of sticky mucopolysaccharides generated by the uterus. Unfertilized eggs are longer and slightly more

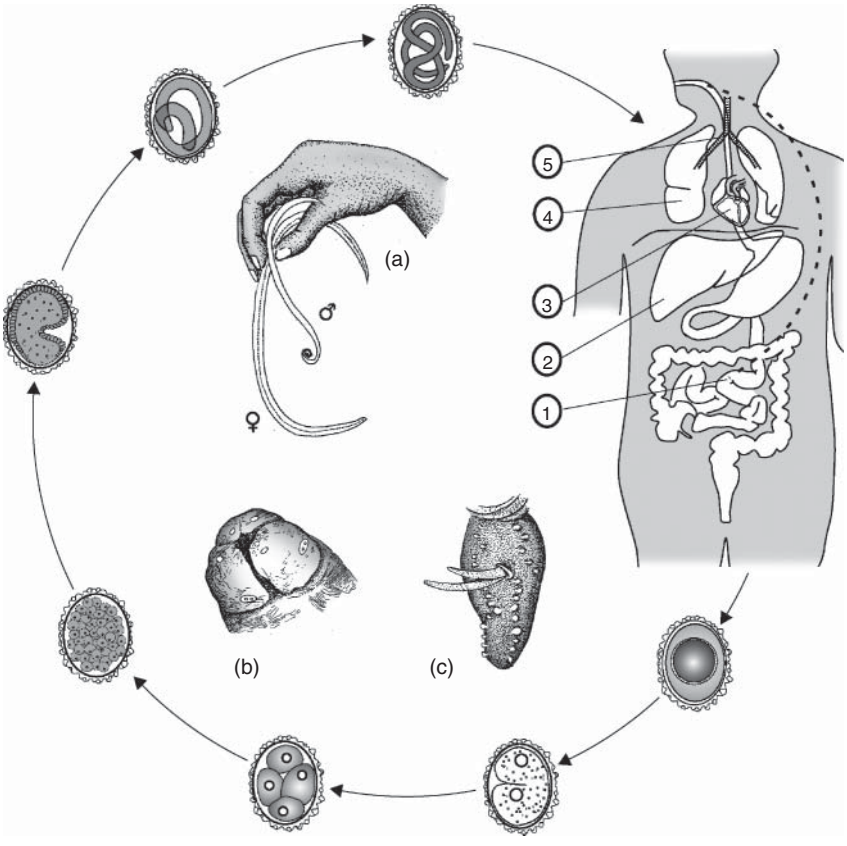


Figure 3.51 Life cycle of *Ascaris lumbricoides*. Circle: in eggs, released by humans, larval stages develop in the external environment to the infective L3, which then has to be ingested. Within humans: (1) L3 hatches in the small intestine. (2) Following penetration larvae are carried by venous blood via intestinal wall and portal vein to the liver, where they molt to L4. (3) Transportation via vena cava to the right ventricle and (4) to the lung. (5) L4 penetrate the alveolar walls, are coughed up into the trachea, swallowed and carried to the small intestine, where the last moult occurs. (a) Hand with adult male and female. (b) Head region with three lips and oral opening. (c) Distal end of male with the two spicules. Combined after several authors.

slender (88 – 94 × 44 µm). Males are 15 – 35 cm long and somewhat thinner than females. Their tail end is coiled ventrally. Sometimes two spicules can be seen, which are 2 mm long (Figure 3.51c).

Pathology Migrating larvae can cause temporary allergic reactions such as rashes of the skin. During passage through the lung, an "*Ascaris*-pneumonia" arises, which is characterized by fever, cough, heavy production of mucus, eosinophil infiltrations, and asthma-like attacks. The adult worms may also cause colic, dizziness, and vomiting. The worms interfere with optimal utilization of

nutrition, especially lactose. Acute complications can occur by blockage of the lumen of the gut or bile ducts, and even the pancreas, by adult worms due to their large size. The parasites may also break through the wall of the intestine. In persons already sensitized, strong allergic reactions can occur upon subsequent contact, as *Ascaris* is known to contain potent allergens. Occasionally, worms can migrate backward into the stomach and react to gastric acid with violent movements, resulting in intense malaise, or even regurgitation of adult parasites.

3.3.4.7 *Ascaris suum*

The large roundworm of pigs is of considerable economic importance, as it occurs in a high percentage of pigs kept in both indoor and outdoor housing, even when thorough anthelmintic treatment is given. The biology, morphology, and pathology are essentially the same as in *A. lumbricoides*. In young pigs, the infection causes developmental retardation through indigestion and absorption of nutrients and vitamins. *A. suum* is considered a sibling species of *A. lumbricoides*. Experimental cross-infections with *A. lumbricoides* are possible, and such natural infections, although being rare, have been verified by molecular methods. High levels of homology between complete *A. suum* and *A. lumbricoides* mitochondrial genomes support a very close relationship between the species and some authors consider *A. lumbricoides* and *A. suum* as one species..

Genome The genome of *Ascaris suum* has a size of 273 Mb and encodes for about 18 500 proteins. Despite its large size, it has a relatively low amount of repetitive sequences (4.4%). As in other ascarids, the genome organization is very peculiar, in that the number of chromosomes differs between germ line cells and somatic cells. The germ line genome of *A. suum* comprises 19 autosomes and 5X-chromosomes ($2n = 38A + 10X$ in females, $2n = 38A + 5X$ in males). A tightly regulated process of chromatin diminution during differentiation to somatic cells leads to a loss of heterochromatin, which eliminates about 25% of germ line DNA, with a concomitant increase of chromosome numbers and new formation of telomers. Comparisons between various ascarid species have shown that the germ line-specific genome is much less conserved than the somatic one, suggesting that chromatin diminution may allow a balanced expression of genes needed for normal development in somatic cells, while allowing rapid and profound evolutionary changes in the germ line.

3.3.4.8 *Toxocara canis*

The dog roundworm *T. canis* is also a member of the Ascaridae family and has a worldwide distribution in canids. In addition, the so-called paratenic hosts – various vertebrates including humans – may be infected, in which, by definition, no further development takes place. This is of medical importance, because in humans the larvae may cause damage especially to the eyes. *T. canis* most commonly has a one-host cycle similar to *Ascaris*. Furthermore, the

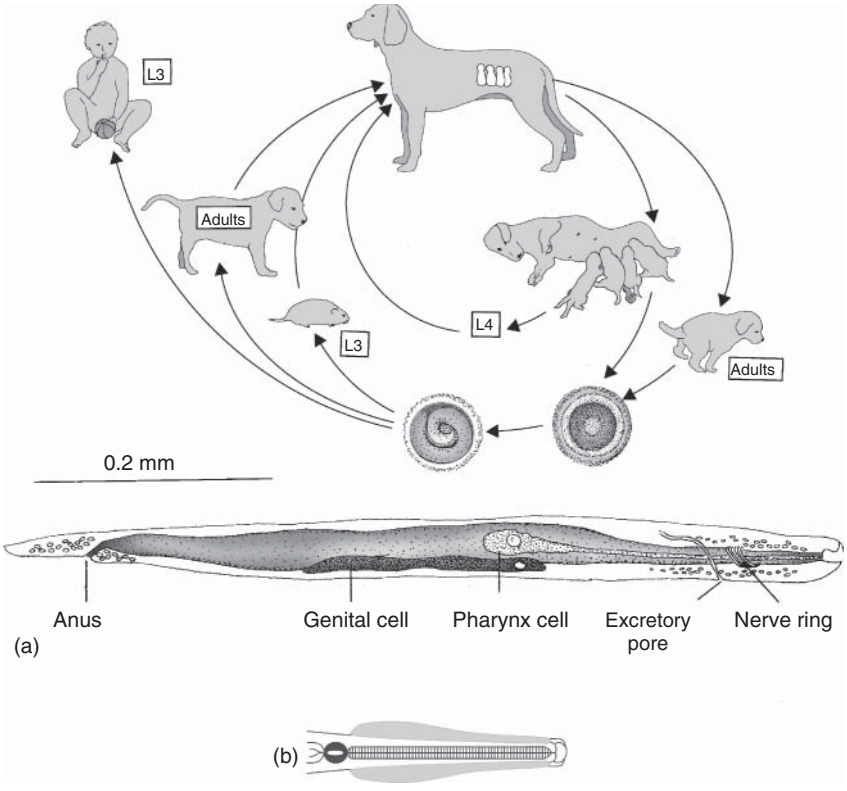


Figure 3.52 Life cycle of *Toxocara canis* (rectangular frames in cycle refer to worm stages shown in boxes). (a) Infective L3. (b) Head region of adult with cervical alae, shaded in grey.

migration patterns of the larvae through the body and the site of mating are the same. However, the following features are specific to *Toxocara* (Figure 3.52):

- In general, only pups under the age of 5 months harbour adult worms.
- In older animals, the larvae may migrate as far as the lungs, but are then swept back into the heart and carried through the body by the bloodstream. They enter striated muscle, kidney, liver, and the central nervous system, where granulomas form around them. These inhibit the further growth of such "somatic" larvae, but the larvae remain viable for several years.
- In infected pregnant bitches, the somatic larvae are reactivated 6 weeks into the pregnancy and migrate through the placenta into the liver of the fetuses. Once the pups are born, they continue their usual migration route through lungs and trachea into the small intestine, where they start laying eggs in 3-week old puppies.
- Puppies can also be infected through their mother's milk (lactogenic infection).
- The mother, in turn, can be infected while grooming her young through L4 stages excreted by them with their feces.

- *T. canis* can also be transmitted through ingestion of paratenic hosts (several mammals as, for instance, rodents), in which no development to the adult stage is achieved. The larvae, after passage through the intestinal wall, migrate into various organs of the body, where they stay alive and infective for years. This also happens in humans. The migrating larvae give rise to the disease known as *larva migrans* (see Pathology).

Morphology Females measure 6.5–10 cm, males 4–6 cm in length. The proximal end of *T. canis* has lateral cervical alae 2–2.5 mm long (Figure 3.52b). Males have two flat spicules. Eggs measure 75–90 µm and have a net-like surface.

Pathology Dogs may stay symptomless, but may develop similar symptoms as those seen in *A. lumbricoides* and *A. suum* infections. Massive infections can be fatal in puppies. Humans and several other mammals can serve as paratenic hosts, the L3 halting its development, but becoming a *larva migrans visceralis* in various organs, although damage in humans only tends to develop when more than 100 larvae are involved. A more serious and more frequent disease is caused by the *larva migrans ocularis*. Infection of the eye may result in retinal granulomas, retinal detachment, and endophthalmitis with vision loss.

Epidemiology Estimations suggest that 15% of adult dogs and up to 100% of pups can become infected. If puppies are not treated early with anthelmintics, they account for considerable contamination of the environment, as one female of *T. canis* produces 25 000–85 000 eggs per day. It is therefore strongly recommended that puppies be treated with anthelmintics every fortnight until they are 12 weeks old. It usually takes 10–15 days for the shed eggs to become infective L3 larvae. Therefore, infection from eggs found in the fur of dogs and from "fresh" feces is extremely unlikely. However, eggs in feces deposited, for instance, in the sand of playgrounds and not removed promptly, may easily initiate infection in children.

3.3.4.9 *Anisakis simplex* and *Anisakis* spp.

A second family of the suborder Ascaridina is the Anisakidae. The herring worms have some significance for food hygiene, as humans can be dead-end intermediate hosts when eating dishes with certain raw fish. Adult worms inhabit marine mammals such as whales, seals or dolphins. Eggs embryonate in the seawater and release a second-stage larva. If this is eaten by shrimp-like crustaceans, mostly euphausids, also known as krill, they develop to the L3. Final hosts can be infected directly by swallowing the crustaceans, but also fish acting as paratenic hosts may be involved. In this case, the third-stage larvae are encapsulated in several organs without further development.

If humans eat raw, undercooked, lightly pickled, or insufficiently salted infected fish, the larvae develop to the fourth stage, but hardly ever become adult. The 2- to 3-cm-long larvae are difficult to detect. Infections can be found in all krill-eating

fish, in Europe mostly herring (for instance fermented herring or maatjes) and in the Americas bluefin, or yellow fin tuna, maguro, and other fish used for sushi.

Following ingestion of contaminated fish, nausea and vomiting may occur within a few hours and the larvae may be regurgitated. If the larvae penetrate the stomach or the gut wall, severe intestinal symptoms can follow 1 or 2 weeks later, sometimes mistaken for Crohn's disease. In response to the larvae, IgE may be produced and an allergic reaction develops, including urticaria or even anaphylaxis. If intestinal perforation occurs, immediate surgery is necessary.

Blast-freezing of infected fish for several hours, as part of the food-processing process kills the larvae. Anthelmintic treatment of infected individuals is sometimes used, but often the infection resolves without further intervention.

Modern genetic techniques have identified several more *Anisakis* species, including ***Anisakis physeteris***, ***Anisakis typica,*** and ***Anisakis pegreffii***. Another member of the family Ansakidae may infect humans in the same way as *Anisakis* species, namely ***Pseudoterranova decipiens***, the codworm.

3.3.4.10 *Dracunculus medinensis*

Although dracunculiosis may be the first tropical disease to have been entirely eradicated, its cause, the Guinea worm *Dracunculus medinensis* is such an interesting parasite that, also in future, no textbook can avoid to report on it. Despite its past popularity, little is known about its phylogeny. It is either not listed in the order Spirurida or among them is ranged near the families Thelaziidae and Physalopteridae.

The infective L3 and the L4 of *D. medinensis* migrate through the human body until they reach sexual maturity. In their final destination, the male worms, only 30–40 mm long, die after they have fertilized the females. The females are very long and thread-like, measuring 0.8–1 m. The viviparous female lives in subcutaneous tissue, mainly in the extremities (Figure 3.54a). A year after the infection, the female induces a blister in the host's skin, just above the vulva in the head region of the female. The blister contains thousands of L1 stages (Figure 3.53a) and bursts open on contact with water. The blister then turns into an ulcer that keeps releasing larvae whenever it is immersed in water.

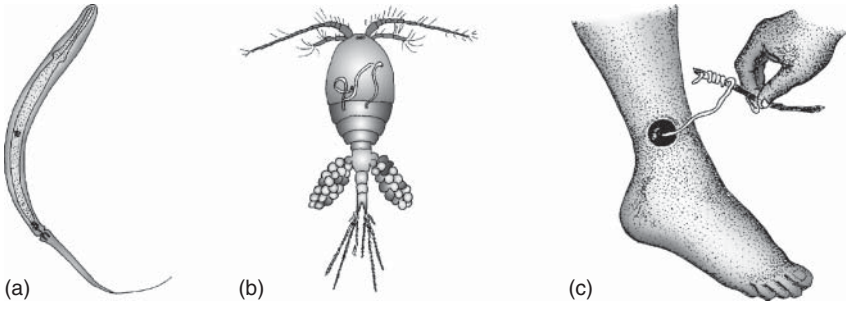
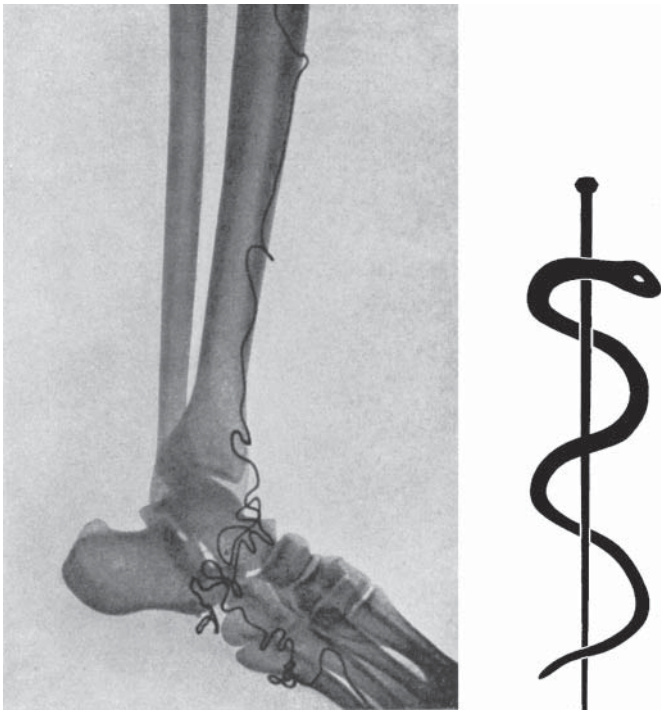


Figure 3.53 *Dracunculus medinensis*. (a) L1 released into water. (b) L3 in the body cavity of a copepode. (c) Extraction of a female worm.

The larvae are ingested by small crustaceans (copepods) and move into their hemocele, where they molt twice within a fortnight. If crustaceans containing the infective L3 (Figure 3.53b) are ingested with water, human infection starts and the larvae migrate through the stomach and gut wall into the body.

Dracunculiosis is a debilitating disease, which can lead to chronic inflammation of joints. There is little evidence of effective acquired immunity, so that people can be reinfected several times during their life. Moreover, there is no test (blood smears or skin biopsy) to detect the presence of the worm before the blister appears. Only at this time do allergic reactions appear, such as redness, minor fever, and rash together with severe itching, including nausea, vomiting, diarrhea, and dizziness. The symptoms begin to diminish with the rupture of the blister, and the worm usually can be manually forced out by slowly winding it onto a stick over several weeks to months (Figure 3.53c). It has been suggested that the long, slender worm wound around a stick is the origin of the physicians' icon, the caduceus or Aesculapian staff (Figure 3.54b).

Recently dogs have been confirmed as an animal reservoir for the adult worms in parts of Africa. The life cycle is strictly tied to the dry season when people in rural areas without well-regulated tap water supply are dependent on natural ponds.



(a) (b)

Figure 3.54 *Dracunculus medinensis*. (a) X-ray of female in human leg. (From Piekarski (1954) *Lehrbuch der Parasitologie*. Spinger-Verlag Heidelberg.) (b) Aesculapian staff.

Prevention is relatively easily implemented by providing a safe water supply. The Guinea worm was widely spread over Africa and India. When the Carter Center began leading a campaign to eradicate the parasite in 1986, there were an estimated 3.5 million cases in 21 countries in Africa and Asia. In 2014, India is certified as Guinea worm-free by the WHO and only single cases are recorded in Chad and South Sudan although possible reservoirs of infection in dogs may be a problem. Dracunculiosis may be totally eradicated in the world in the near future. However, recent findings of naturally infected dogs in Chad have spurred concerns that this goal might be more difficult to be reached than anticipated.

3.3.4.11 *Enterobius vermicularis*

The pinworm (also known as threadworm or seadworm) is a member of the third suborder Oxyurina of the order Spirurida (Table 3.10). The family Oxyuridae contains medium-sized nematodes (females up to 13 mm long, males smaller), parasites in the digestive tract of invertebrates, and mostly herbivorous vertebrates, with a direct, one-host life cycle, in which eggs with the infective larva develop in the external environment. Oxyurids are characterized by a sharply pointed posterior end of the females (Figure 3.55a), which has given the name to the taxon (Greek: *oxys* = pointed, *ura* = tail). In males, the body end is truncated (Figure 3.55b).

Enterobius vermicularis is a common intestinal parasite of humans, called pinworm in the United States and threadworm in the United Kingdom. It has a worldwide distribution with approximately 200 million people infected, most of them being children.

Development *E. vermicularis* has a monoxenous life cycle. Fertilized females – males die soon after mating – live in the lower part of the small intestine (ileum) and in cecum, appendix, and ascending colon of the large intestine, where they are attached to the mucosa. At the end of their life, lasting 3–6 weeks, the gravid females migrate to the rectum and emerge from the anus where they lay their eggs (11 000–16 000 per female). The worm then dies. The eggs have a sticky surface and tend to adhere to the perianal folds. They contain

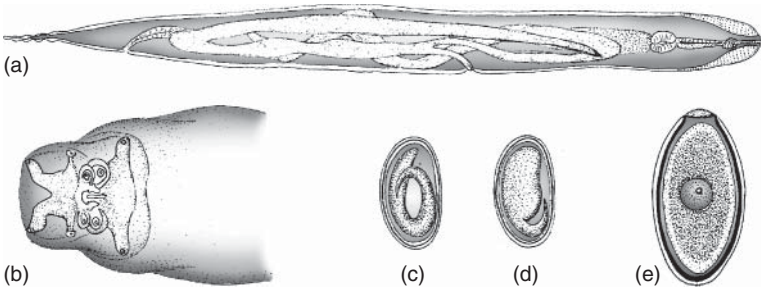


Figure 3.55 *Enterobius vermicularis*. (a) Female, didelphic. (b) Posterior part of a male worm. (c) Newly released egg. (d) Egg with infectious L3 stage. (e) Egg of *Oxyuris equi*.

a first-stage larva (Figure 3.55c), which, in contact with oxygen, molts twice to become an L3 with the shape of a tadpole (Figure 3.55d) within 4–6 h. Eggs are easily recognized as they are slightly flattened on one side. They have to be ingested by another or by the same host, where the larvae hatch in the duodenum and, molting twice, migrate as adults through the small intestine to the lower intestine and colon.

Morphology Females are 8–13 mm long, males only 1–4 mm. The males have a truncated bursa copulatrix (Figure 3.55b) and a tiny spiculum of 70 µm. The eggs measure 50–60 × 20–30 µm and are flattened on one side (Figure 3.55c,d).

Epidemiology Autoinfection is a typical trait of *E. vermicularis,* occurring mainly in children. The migration of female worms to the anus and the deposition of eggs cause intensive itching, mainly at night. Scratching and the resulting anus-finger-mouth contact lead to continual reinfection. In addition, the highly infective eggs may contaminate all household objects and infect whole families. Often, infections are observed in cramped conditions and where hygiene conditions are poor – also in institutional care. A second mode of infection is possible by hatching of third-stage larvae on the anal mucosa and migration backward to the intestine. This retroinfection potentially leads to a heavy parasite load. *Enterobius* species of simians do not infect humans.

Pathology, Treatment Symptoms are caused by the nocturnal egg-laying of the females, which result in fierce itching. The night-long scratching, especially in children, with resulting sleep deprivation, may cause pallor, impairment of concentration, irritability, and decreased school performance. Worms can also invade the appendix, vagina, or fallopian tubes and cause inflammation. Diagnosis is carried out by the "Scotch tape test" (the sticky side of a cellophane tape pressed onto the anus at night and surveyed microscopically for eggs). The small, whitish worms are clearly visible in the stool. Infection can persist in a family in which household members who are symptom-free are not treated. They remain a source of infection in what is often thought to be treatment-refractory cases. Treatment with appropriate drugs is possible but has to be applied to the whole family or household. Strict hygiene measures are necessary: careful washing of the hands and scrubbing of the fingernails and daily change and laundry of underwear and bedclothes.

Oxyuris equi of the horse has large operculated eggs (Figure 3.55e). They are deposited within a quickly solidifying, viscous fluid on the anus and cause itching and irritations that affect the horse's health. ***Passalurus ambiguus***, the Rabbit pinworm, is extremely common worldwide, normally not pathogenic, but difficult to prevent even when animals are well kept, because of autoinfections through cecotrophy (obligatory consumption of cecal pellets). Rarely, cecal impaction, stasis, pain, and bloating may occur. ***Syphacia muris*** is a common pinworm of laboratory mice and rats. It is often difficult to eradicate even in highly controlled facilities.

3.3.4.12 **Filariae**

Nematodes of the superfamily Filarioidea (order Spirurida, suborder Spirurina) are commonly called filariae (Table 3.13). The worms are long and filiform (Latin: *filum* = thread, filament), and inhabit tissues or body cavities, but never the intestine. They do not lay eggs but give birth to larvae, which are called microfilariae. These are transmitted by bloodsucking arthropods, mostly by insects (rarely by mites). While the family Filariidae contains parasites of veterinary significance, the Onchocercidae include all important human filarial parasites. Human filarial infections count among the most important tropical diseases. They account for significant morbidity, with widely varying clinical pictures, depending on exposure and individual immune reactivity. The infections are difficult to control as no safe and rapidly acting drugs against the long-lived adult worms (life spans of >10 years are not an exception) exist, while the microfilariae can be temporarily eliminated.

Immunobiology Living directly within the tissues of their host, filarial nematodes are in intimate contact with the host's immune system and modulate it strongly.

Table 3.13 Overview of the human pathogenic filarilal nematodes.

Species	Site of adults	Microfilariae	Geographical distribution	Vector
Wuchereria bancrofti	Lymphatic system	Sheathed in blood	Tropics of Asia, Africa, China, Pacific, South America (few countries)	*Culex, Aedes, Anopheles*
Brugia malayi	Lymphatic system	Sheathed in blood	Indian–Malayan region, East Asia	*Mansonia, Anopheles, Aedes*
Brugia timori	Lymphatic system	Sheathed in blood	Timor, Southeast Indonesia	*Anopheles barbirostris*
Onchocerca volvulus	Subcutaneous, also deeper connective tissue	Unsheathed in connective tissue of the skin	Tropical Africa, Central and South America	*Simulium* spp.
Loa loa	Subcutaneous connective tissue	Sheathed in blood	Rainforest of Arica	*Chrysops* spp.
Mansonella perstans	Visceral cavities	Unsheathed in blood	Angola to Mozambique, east of South America	*Culicoides* spp.
M. ozzardi	Visceral cavities	Unsheathed in blood	The Caribbean, Mexico to North Argentina, Bolivia	*Culicoides* spp., *Simulium* spp.
M. streptocerca	Subcutaneous connective tissue	Unsheathed in connective tissue	Africa: Ghana, Democratic Republic Congo	*Culicoides* spp.

As in other helminth infections, immune responses of individuals infected with filariae are typically skewed toward the Th2 type, as seen by high levels of IgE antibodies and a strongly increased proportion of eosinophil granulocytes. This type of immune response entails less-aggressive inflammation than Th1 responses do, increasing the parasites' chances of survival and sparing at the same time the host from severe immunopathology. These changes are believed to be induced by worm products that have been optimized during evolution. Analyses of excreted products have allowed characterization of filarial immunomodulators. A secreted 62-kDa glycoprotein of the rodent filarial *Acanthocheilonema viteae* was shown to contain carbohydrates that present phosphorylcholine – a ubiquitious phospholipid – in a particular manner, inhibiting proliferation of a large variety of immune cells. *A. viteae*-Cystatin, a secreted protease inhibitor, induces macrophages after an initial classical activation to adopt a regulatory phenotype. The macrophages then instruct T cells to produce the immunomodulatory cytokine IL-10. These filarial products are currently used as lead components for the development of anti-inflammatory drugs.

Box 3.1

Typically, filarial nematodes contain intracellular, Gram-negative symbiotic bacteria of the genus *Wolbachia* (class α-Proteobacteria, order Rickettsiales) that usually infect arthropods. The endosymbionts are vertically transmitted from one generation to the next through infection of the oocytes, but not through sperm. Therefore, in order to increase the chances of transmission, *Wolbachia* of arthropods have developed various spectacular strategies to augment the proportion of females within the arthropod population, among others the feminization of their host. The *Wolbachia* of filarial nematodes have probably been picked up from arthropod hosts during evolution and live in cells of the worms' hypodermal chords. They are members of two particular groups – C and D – and have evolved into true symbionts, without which the worms cannot live. They do not occur in other nematodes. However, a few filarial species have secondarily lost their symbionts, probably because essential bacterial genes have been transferred to the host's nucleus. Antibiotics (doxycycline) given to the mammalian host eliminate the bacteria, prevent the development of microfilaria, and finally kill the adult nematodes. This approach has been successfully applied in human filarial infections, where no safe macrofilaricide exists, and is currently being further developed.

3.3.4.13 *Wuchereria bancrofti* and *Brugia malayi*

Wuchereria bancrofti (Figure 3.56) and *Brugia malayi* cause lymphatic filariosis of humans. *W. bancrofti* occurs in tropical areas of Asia, Africa, and the Americas, as well as in the Pacific. *Brugia malayi* is present in South and Southeast Asia. Both species are transmitted by mosquitoes. About 120 million people in more than 80 countries of the world are infected. Approximately one-third of them suffer

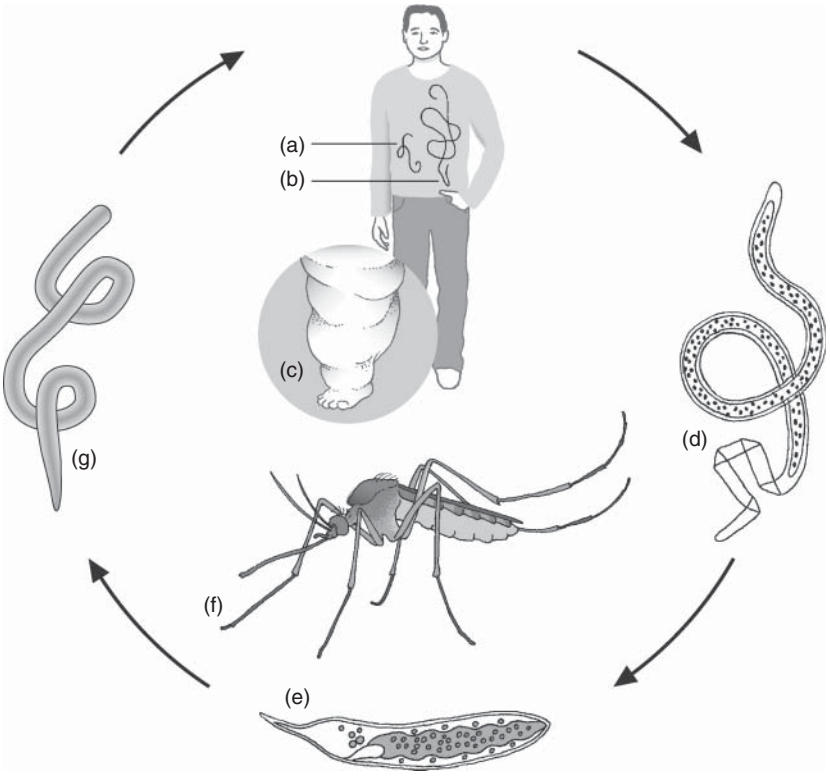


Figure 3.56 Life cycle and morphology of *Wuchereria bancrofti*. (a) adult male, (b) adult female, (c) elephantiasis, (d) microfilariae, (e) L2 larva, (f) female mosquito, (g) L3 larva.

from the worst consequence of the infection, elephantiasis (Figure 3.57), which is manifested by extremely swollen limbs or other body parts, rendering a normal life very difficult.

Development The adult worms mainly live in the large lymphatic vessels of the extremities. Their life span is 5–15 years. The microfilariae shed by the female invade the blood stream. Their presence in the blood is called microfilaremia. These first-stage larvae occur with regular periodicity in the peripheral blood. Those of *W. bancrofti* are typically present between 22:00 and 02:00 h ("nocturnal activity"). In Pacific islands, a strain of *W. bancrofti* is diurnally subperiodic with microfilariae appearing in the peripheral blood circulation at any time, but maximally during daytime. The microfilariae of *B. malayi* are diurnally periodic, detectable during the day. When not in the peripheral circulation, the microfilariae remain in the lung capillaries with high oxygen tension. These facts indicate that for diagnosis, blood must be taken at the appropriate time. The periodicity of the microfiliarae coincides with the activities of the transmitting mosquitoes. These are species of the genera *Culex, Aedes, Anopheles, Mansonia,* and *(* only for *W. bancrofti) Coquillettidia.* (see section 4.4.8.4).

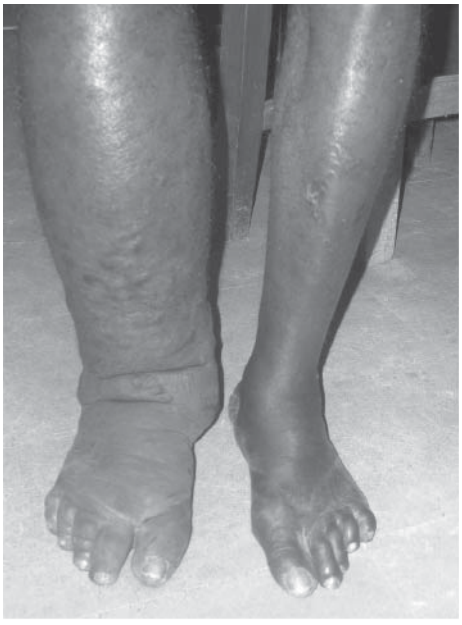


Figure 3.57 *Wuchereria bancrofti*, lymphoedma (elephantiasis) of leg. (Image: Courtesy of Achim Hörauf.)

In the mosquito the sheath of the micofilaria is shed. The L2, the so-called sausage stage, develops in the thoracic muscles of the mosquito. It is short, plump, 120–250 um long and moults to the infective L3. Development to the L3 needs 7 – 10 days. L3 are passed via the probscis into the next human invade a lymph vessel, become adults after two molts, and subsequently mate. The prepatent period until the first emergence of microfilariae in the blood takes approximately 9 months in *W. bancrofti* and 3 months in *B. malayi*.

Morphology The adults of *W. bancrofti and B. malayi* are very similar. Females are 65–100 mm long and 0.2–0.3 mm thick, males are smaller, measuring 40 × 0.1 mm. The microfilariae (Figure 3.58a) of the two species can be differentiated in blood smears. They are sheathed and measure 290 µm (*W. bancrofti*) and 222 µm (*B. malayi*).

Genome The genome of *B. malayi* was chosen as representative for the filarial nematodes, as this worm can be maintained in some strains of, which eases gaining access to material. The draft genome published in 2007 revealed a genome size of about 90 Mb, a repeat content of approximately 15%, and between 14 500 and 17 800 predicted protein-coding genes, organized in 2n = 10 chromosomes. Approximately 15% of the genes are present in polycistrons of two to five genes, and trans-splicing occurs. The data show clear adaptations at the parasitic lifestyle. For example, the cuticle of *B. malayi* is supposedly much less

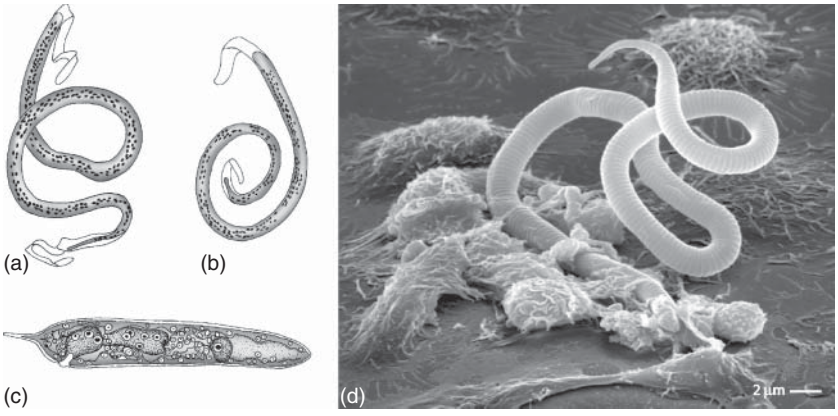


Figure 3.58 Stages of Onchocercidae. (a) Sheathed microfilaria of *Wuchereria bancrofti*. (b) Sheathed microfilaria of *Loa loa*. (c) L2 stage larvae of *W. bancrofti*. (d) A mouse macrophages attack a microfilaria of the rodent filaria *Acanthocheilobema viteae*. EM image: Department of Molecular Parasitology, Humboldt Universität.

complex as compared to *C. elegans* (82 vs 180 collagen genes), and the capacity for the de *novo synthesis* of purines and riboflavin is lost, as suggested by loss of key genes. These compounds have to be acquired from either the bacterial *Wolbachia* symbiont (see Box 3.1) or the host. In turn, a family of secreted proteins involved in immune modulation is expanded. Among others, *B. malayi* produces cytokine-like proteins and expresses antioxidant enzymes deployed at the cuticle surface to protect against oxyradical attack by imune effector cells.

Epidemiology *W. bancrofti* is responsible for 90% of the 120 million cases of lymphatic filariosis worldwide. Of these, 40% are found in India, and one-third in Africa. One of the contributing factors in the expansion of the disease is the occurrence of mosquito species, which breed in small water bodies and polluted water that are especially frequent in slums.

Pathology While in endemic areas about one-third of the exposed population appears to be resistant, in others, a high but mainly asymptomatic microfilaremia develops. This may be followed, in some people, by a phase in which inflammation induced by infection causes obstruction of the lymph vessels. This leads to lymphedema mainly of the limbs, which is accompanied by fever, headache, and painful lymph nodes. The most conspicuous sign of this chronic disease is elephantiasis, a gross swelling of legs and arms (Figure 3.57). In women, the breast can also become affected and in men the scrotum. This disfiguring condition incapacitates the patients severely and isolates them socially. Although the majority of infected people in endemic regions are asymptomatic, virtually all of them may have subclinical lymphatic damage and as many as 40% have kidney damage, with proteinuria and hematuria.

Control Mass treatment has been undertaken in India from 2003 onwards with diethylcarbamazine citrate plus albendazole, targeting microfilariae and adults, respectively. The aim was elimination of filariosis by 2015. It is stressed, however, that additional vector control has to be implemented together with better education for the population in endemic regions.

3.3.4.14 *Onchocerca volvulus*

This species is the causative agent of river blindness in humans and occurs in Africa, especially in the Volta basin, less frequently eastward as far as Sudan and Tanzania, and south of it as far as Angola (Figure 3.59). Small endemic areas are present in Yemen and in the Americas (Mexico, Guatemala, Venezuela, and Ecuador). In the New World, however, onchocerciosis is relatively unimportant, as blindness is rare or can be relatively easily prevented. In Africa, however, before the introduction of a major control program in 1975, vast areas of cultivated land and thousands of settlements along rivers were abandoned because of the high percentage of working people affected by blindness.

Development Intermediate hosts of *Onchocerca volvulus* are dipteran Nematocera of the family Simuliidae, the black flies (Figure 3.59c). With their short, broad mouthparts, they take up blood, lymph, and fluid from tissues. This enables them to ingest the microfilariae from subcutaneous tissue. The microfilariae develop into short second-stage larvae of the so-called "sausage" stage (Figure 3.59d) in the thoracic musculature, then molt to the infective third-stage larva (Figure 3.59e) and migrate to the proboscis of the Simuliidae, from where they are transmitted to humans. Within the human host, they molt to become forth-stage larvae, which migrate actively in tissue and lymph vessels. They then settle in the subcutaneous tissue and are surrounded by connective tissue, forming raised nodules of pea or hazelnut size under the skin (Figure 3.60a), called onchocercomas, where they become sexually mature after about 1 year. Very often, several worms lie in one nodule and several nodules can form aggregates, which is believed to facilitate insemination. The life of a female worm lasts 9–11 years. During this time, it produces between 700 and 1500 microfilariae per day. These leave the nodules and are found in the skin, where they are supposed to be actively migrating; they have a life span of about 6 months. In heavy infections, more than 20 million microfilariae can be present in one person.

Morphology As in all Filarioidea, the mouth region is symmetrical (Figure 3.59f). The females are 20–70 cm long and have a genital opening near the proximal end (Figure 3.59g). Males measure 3–12 cm in length. Their distal end is coiled, with the two spicules of unequal length (Figure 3.59h). The females lie usually beneath the skin and induce the formation of a nodule consisting of connective tissue, in which they are firmly embedded, the anterior end with the genital opening sticking out. By contrast, the males are mobile and migrate between the nodules to inseminate the female worms. The unsheathed microfilariae measure 300×8 µm (Figure 3.59b). They do not live in the blood vessels, but move through

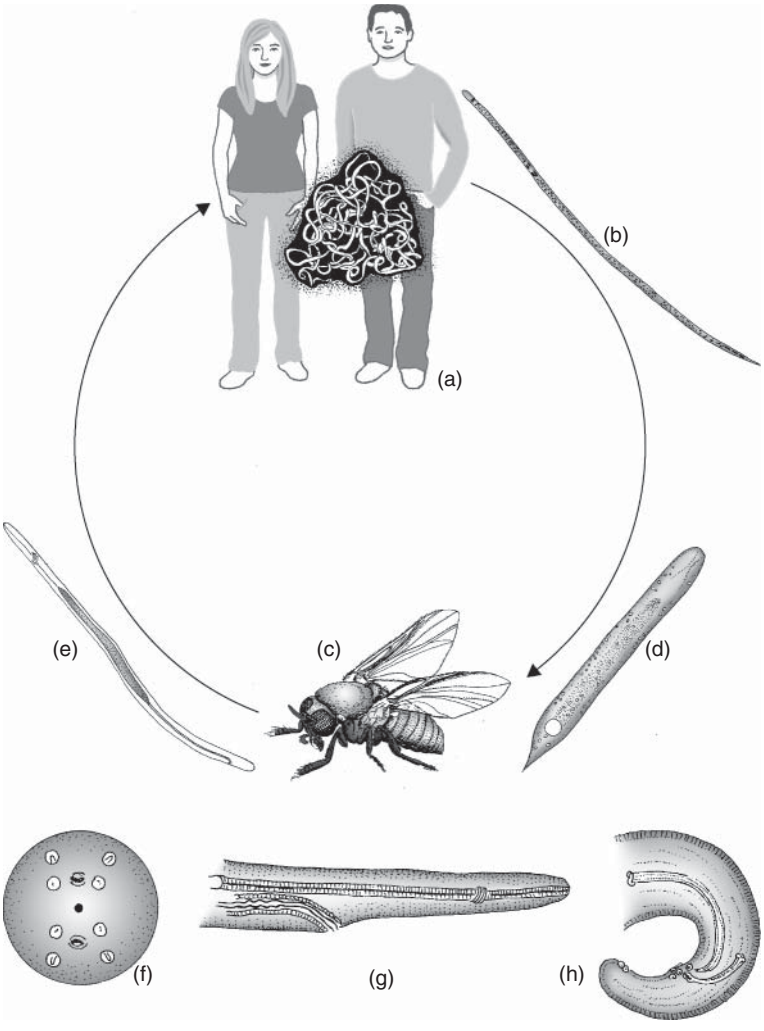
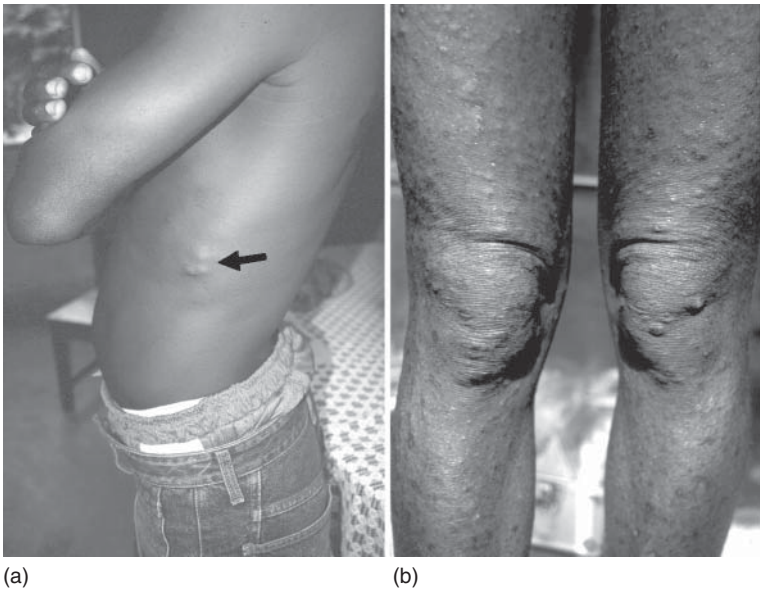


Figure 3.59 Life cycle and morphology of *Onchocerca volvulus*. (a) Adult males and females in connective tissue of humans. (b) Microfilaria in skin. (c) Blackfly (*Simulium*). (d) L2, sausage stage. (e) Infective L3. (f) Mouth region of adult with papillae and mouth opening. (g) Anterior end of female with vagina. (h) Posterior end of male with the two unequal spicules.

the subcutaneous connective tissue. As in all tissue-dwelling microfilariae, a periodicity of microfilaremia does not exist.

Epidemiology Simuliidae, the intermediate hosts of *O. volvulus*, are bound to fast-flowing, oxygen-rich waters by their breeding biology (see section 4.4.8.6), which is reflected by the name of the resulting disease "river blindness." Black flies are diurnally active and are persistent bloodsuckers, which are not easily discouraged. They are capable of flying over distances in the range of a few



(a) (b)

Figure 3.60 *Onchocerca volvulus*. (a) Skin nodules, Onchocercoma. (b) Sowda, a clinical picture of onchodermatitis. (Images: Courtesy of: Dietrich Büttner.)

hundred meters up to several kilometers, and can be carried by the wind over hundreds of miles, thus may disperse widely.

One particular strain of *O. volvulus* in the West-African savanna is transmitted by several *Simulium* species belonging to the *Simulium damnosum* complex. This savanna strain is of stronger pathogenicity than that found in West African and East African forest areas. In East Africa, *O. volvulus* is transmitted by black flies of the *Simulium neavei* complex. Its members occur in rivers harboring particular freshwater crabs, to which the larvae and pupae of *S. naevei* attach themselves. In Latin America, onchocerciosis (named Roble's disease) is transmitted by *Simulium ochraceum* and, less importantly, by the *Simulium metallicum* complex. Here, *O. volvulus* mainly occurs in coffee plantations at altitudes between 460 and 1400 m.

Pathology The pathogenic stages of *O. volvulus* are microfilariae within the skin that are target of immune reactions, while the onchocercomas themselves do not provoke symptoms. The clinical picture of the disease is determined by immune reactions of the patients and can, therefore, be very different. The majority of patients have the generalized type of onchocerciosis, while relatively few patients suffer from localized onchocerciosis.

In the generalized type, the severity of disease is correlated with the microfilarial density. Their number increases with the number of nodules, or females within them. Patients react mainly to dead and decaying microfilariae, which are surrounded by inflammatory cells, while living parasites are obviously not efficiently

killed. Soon after the appearance of the painless onchocercomas **onchodermatitis** arises, which begins with intense itching. This is followed by papule-like inflammation and - on the long run - loss of elasticity of the skin. Typical symptoms are leopard skin (pigmentation disorders), elephant skin (inflammatory swellings) or "hanging groin," indicating lobes of skin hanging down. Microfilariae invade among others the cornea and other parts of the eye, die there, and cause at first a temporary opaqueness of the cornea (snowflake keratits), which, in chronic infection, is followed by a more general white opacity, together with inflammation of the iris and retina and atrophy of the optic nerve. These processes are irreversible and lead to blindness. In the West African savanna, blindness is common; in rainforest areas and East African regions, it is relatively rare.

Localized onchocerciasis (Figure 3.60b) called sowda (arabic: black, for hyperpigmented skin parts) arises when patients develop strong inflammatory reactions against living microfilariae released by female worms. This extremely strong dermatitis can be very localized in the vicinty of a nodule, or afflict larger skin areas. Before the advent of ivermectin as a treatment against microfilariae, this condition was sometimes a cause of suicide.

In Africa, the onchocercomas occur mainly in the lower parts of the body, because the black flies prefer to bite legs and lower body regions. In Latin America, the insects usually bite on the upper part of the body because of the denser clothes usually worn in the cool mountain areas. Here, blindness caused by infection is relatively rare because of the surgical removal of nodules (nodulectomy), undertaken by trained laypersons.

Control A huge international control program in the Volta Basin (West Africa) was initiated by the WHO and other organizations in 1974. It started with insecticide spraying of rivers to diminish the numbers of the aquatic blackfly larvae, and from 1988 onward treatment of the population with the anthelmintic Ivermectin was initiated. At its peak, the program covered 30 million people in 11 countries. The program was successful and ended in 2002. In 1995, another African program began, covering a further 19 countries, but based solely on anthelmintic treatment with Ivermectin, a drug that kills microfilariae and temporarily inhibits embryogenesis in female worms. Another very exciting treatment is through the use of conventional antibiotics, which, in fact, kill the adult worms by destroying the bacterial symbiont *Wolbachia*, which have been discovered to be essential for *O. volvulus* survival.

Interestingly, exposure to the cattle filaria ***Onchocerca ochengi*** can also protect to a certain degree against *O. volvulus*. The larvae of this nematode do not fully develop in humans, but induce partial cross-immunity against *O. volvulus*.

Other species of the genus *Onchocerca* occur in wild and farm animals outside Africa; for example, ***Onchocerca cervicalis*** and ***Onchocerca reticulata*** are found worldwide in horses and, the less pathogenic, ***Onchocerca gutturosa, Onchocerca lienalis*** (worldwide), and ***Onchocerca gibsoni*** (North America, India, Australia) are found in cattle.

3.3.4.15 *Loa loa* and *Dirofilaria immitis*

The so-called African eyeworm *Loa loa*, also a member of the Onchocercidae, lives in subcutaneous tissues. The microfilariae migrate into the bloodstream and are taken up by two species of deer flies (Tabanidae) of the genus *Chrysops, Chrysops silacea* and *Chrysops dimidiata,* also known as mango flies or mangrove flies (see section 4.4.8.9). Development and molts take place in the insect's fat body and finally in its mouthparts, from where the infective L3 is transferred to the human skin by bite. The worms become sexually mature after 1 year or more. The females measure 57 mm, the males 34 mm. They migrate 2–3 cm/day and are sometimes seen as meandering prominences of the skin. Occasionally, they cross into the subconjunctival tissues of the eye, where they can be easily observed. Vision is not affected, but the movements of the worms are painful. The migrating worms can cause local edemas ("Calabar swelling") that reach the size of a hen's egg and persist for some days. These fiercely itching swellings are probably allergic reactions against released metabolic products. Loiosis is a disease occurring in high-canopied rain forests of West Africa and Central Africa.

Dirofilaria immitis, the heartworm, is a spirurid of the nematode superfamily Filarioidea. It lives in the pulmonary arteries and sometimes in the right ventricle of the heart of its definitive host, the dog (cat). The adult females (230–310 mm long) produce L1 larvae (microfilariae), which are ingested by mosquitoes (*Aedes, Culex, Anopheles, Mansonia, see section 4.4.8.4*). In the insect molts to the infective L3 takes place. The L3 are transferred to the next definitive host where they first reside in the subcutis, molt twice and as young worms migrate into the pulmonary arteries. It takes 5–6 months to develop a manifest heartworm infection and production of microfilariae.

Heartworm disease or cardiopulmonary dirofilariasis of dogs (and cats) occurs in North-America and mediterranean countries of Europe. The illness can include cough, exhaustion upon exercise, fainting, coughing up blood and severe weigh loss. Humans may also be infected.

3.3.4.16 Rodent Models of Filariosis

As most human pathogenic filariae are strictly host-specific, experimental filariosis research often has to resort to rodent models. The study of rodent filariids in their natural hosts is likely to yield important clues on the pathogen–host relationship. *Acanthocheilonema viteae* adult worms live in the subcutaneous tissues of gerbils, while the microfilariae circulate in the blood. Transmission is by ticks of the genus *Ornithodoros*. Golden hamsters and Mastomys natalensis can also be infected. *Litomosoides sigmodontis* is a natural parasite of cotton rats, where it dwells the pleural cavity, while microfilariae circulate in the blood. Transmission is by the small mite *Ornithonyssus bacoti*. This filaria can infect laboratory mouse strains and has therefore become an important model for research. *Brugia malayi* can also infect certain mouse strains, although the development of the parasite is restricted.

Further Reading

Anderson, R.C. (2000) *Nematode Parasites of Vertebrates. Their Development and Transmission*, CABI Publishing, Wallingford and New York.

Bachmann-Waldmann, C., Jentsch, S., Tobler, H., and Mueller, F. (2004) Chromatin diminuation leads to rapid evolutionary changes in the organization of the germline genomes of the parasitic nematodes *A. suum* and *A. univalens*. *Mol. Biochem. Parasitol.*, **134**, 53–64.

Blaxter, M. and Koutsovoulos, G. (2014) The evolution of parasitism in nematoda. *Parasitology*, **25**, 1–14.

Crump, A., Morel, C.M., and Omura, S. (2012) The onchocerciasis chronicle: from the beginning to the end? *Trends Parasitol.*, **28**, 280–288.

Dold, C., Holland, C.V. (2011) Ascaris and ascariasis. *Micobes. Infect.* **13**, 622–637.

Foth, B.J., Tsai, I.J., Reid, A.J. *et al.* (2014) Whipworm genome and dual-species transcriptome analysis provide molecular insights into an intimate host-parasite interaction. *Nat. Genet.*, **46**, 693–700.

Ghedin, E., Wang, S., Spiro, D. *et al.* (2007) Draft genome of the filarial nematode parasite *Brugia malayi*. *Science*, **317**, 1756–1760.

Gottstein, B., Pozio, E., Noeckler, K. *et al.* (2009) Epidemiology, diagnosis, treatment, and control of trichinellosis. *Clin. Microbiol. Rev.* **22**, 127–145.

Hayes, K.S., Bancroft, A.J., Goldrick, M. *et al.* (2010) Exploitation of the intestinal microflora by the parasitic nematode *Trichuris muris*. *Science*, **328**, 1391–1394.

Hotez, P.J., Diemert, D., and Bacon, K.M. (2013) The human hookworm vaccine. *Vaccine*, **31** (Suppl. 2), B227–B232.

Jex, A.R., Liu, S., Li, B., Young, N.D. *et al.* (2011) *Ascaris suum* draft genome. *Nature*, **479**, 529–533.

Jex, A.R., Nejsum, P., Schwarz, E.M. *et al.* (2014) Genome and transcriptome of the porcine whipworm *Trichuris suis*. *Nat. Genet.*, **46**, 701–706.

Laing, R., Kikuchi, T., Martinelli, A., Tsai, I.J. *et al.* (2013) The genome and transcriptome of *Haemonchus contortus*, a key model parasite for drug and vaccine discovery. *Genome Biol.*, **14**, R88.

Lee, D. (ed) (2002) *The Biology of Nematodes*, Taylor & Francis, London, pp. 1–648.

Leles, D., Gardner, S.L., Reinhard, K., Iñiguez, A., and Araujo, A. (2012) Are *Ascaris lumbricoides* and *Ascaris suum* a single species? *Parasites Vectors*, **5**, 42.

McSorley, H.J., Hewitson, J.P., and Maizels, R.M. (2013) Immunomodulation by helminth parasites: defining mechanisms and mediators. *Int. J. Parasitol.*, **43**, 301–310.

Mitreva, M., Jasmer, D.P., Zarlenga, D.S. *et al.* (2011) The draft genome of the parasitic nematode *Trichinella spiralis*. *Nat. Genet.*, **43**, 228–235.

O'Regan, N.L., Steinfelder, S., Venugopal, G., Rao, G.B., Lucius, R., Srikantam, A., and Hartmann, S. (2014) *Brugia malayi* microfilariae induce a regulatory monocyte/macrophage phenotype that suppresses innate and adaptive immune responses. *PLoS Negl. Trop. Dis.*, **8** (10), e3206.

Pineda, M.A., Lumb, F., Harnett, M.M., and Harnett, W. (2014) ES-62, a therapeutic anti-inflamatory agent evolved by the filarial nmatode *Acanthocheilonema viteae*. *Mol. Biochem. Parasitol.*, **194**, 1–8.

Rebollo, M., Bockarie, M.J. (2013) Towards the elimination of lympahatic filariasis by 2020.: treatment update and impact assessment for the endgame. *Expert. Rev. Anti Infect. Ther.* **11**, 723–731.

Smythe, A.B., Sanderson, M.J., and Nadler, S.A. (2006) Nematode small subunit phylogeny correlates with alignment parameters. *Syst. Biol.*, **55** (6), 972–992.

Summers, R.W., Elliott, D.E., Urban, J.F. Jr., *et al.* (2005) *Trichuris suis* therapy in Crohn's disease. *Gut*, **54**, 87–90.

Taylor, M.J., Hoerauf, A., Townson, S. *et al.* (2014) Anti-Wolbachia drug discovery and development: safe macrofilaricides for onchocerciasis and lymphatic filariasis. *Parasitology*, **141**, 119–127.

Telford, M.J., Bourlat, S.J., Ecocnomou, A. *et al.* (2008) The evolution of the Ecdysozoa. *Philos. Trans. R. Soc. London, Ser. B*, **363**, 1529–1537.

Test Questions

1. Where do nematodes live?
2. What are the characteristic morphological features of nematodes?
3. Which is the typical form of nematode eggs?
4. Which developmental stages occur in the life cycle of parasitic nematodes?
5. Which ectoparasitic nematodes do you know?
6. What or who is the intermediate host of *Trichinella*?
7. Which stage of *Trichinella* is pathogenic?
8. Which kind of reproduction occurs in *Strongyloides stercoralis*?
9. How are humans infected by *Angiostrongylus cantonensis*?
10. What is the significance of *Haemonchus contortus*?
11. What or who is the intermediate host of *Ascaris lumbricoides*?
12. What is the significance of *Toxocara canis* for humans?
13. How are pubs infected by *Toxocara canis*?
14. What is anisakiosis?
15. What is the vernacular name of the genus Anisakis?
16. How and when does a person note an infection with the Guinea worm?
17. Is dracunculiosis contagious for other persons?
18. What does *Enterobius vermicularis* do in its host?
19. Which is the intermediate host of *Enterobius vermicularis*?
20. In which organs does *Wuchereria bancrofti* live?
21. What are microfilariae?
22. What is river blindness and by which nematode is it caused?
23. Where does river blindness occur?
24. Why can antibiotics kill *Onchocerca volvulus*?

4
Arthropods

Brigitte Loos-Frank and Richard P. Lane

The Biology of Parasites, First Edition. Richard Lucius, Brigitte Loos-Frank, Richard P. Lane, Robert Poulin, Craig W. Roberts, and Richard K. Grencis.

4.1 Introduction

Arthropoda, which include arachnids, crustaceans, myriapods, and hexapods, is a phylum invariably described with superlatives. More than 85% of the 1.8 million described animal species are arthropods. They have radiated into every ecosystem from the ocean floor to high altitudes, and its members are extraordinarily diverse in their structure and life histories. The unifying feature of the phylum is a

segmented body and segmented limbs covered in a resistant cuticle, which is often hardened to form an exoskeleton. Flexible cuticle between sections of the limbs and body segments form joints and allows movement by muscles attached internally to the cuticle. The principal component of the cuticle is the polysaccharide chitin, the second most abundant polymer on earth after cellulose. The exoskeleton consists of a thin, impermeable, nonchitinous layer (epicuticle) and a thick, elastic, permeable, laminated inner layer (endocuticle) composed mainly of protein and chitin. The endocuticle can be hardened by either the incorporation of calcite, as in many marine crustaceans, or sclerotization of proteins in the cuticle of insects and arachnids by covalent cross-linking. The exoskeleton also extends into the fore and hindgut and lines the trachea, the tubes used in respiration in insects and myriapods.

The hard exoskeleton, which confers many advantages, such as resistance to external environmental stresses, mechanical advantage, and permitting extraordinary morphological plasticity, also has limitations:

- It restrains the size of individuals; the largest are marine crabs weighing 6.4 kg, but most arthropods are "small," the largest insects weighing 100 g ranging down to mites and parasitic wasps less than 0.25 mm in length; despite their complex structures and behavior, they can weigh less than the nucleus of a large cell.
- It limits growth necessitating a process of molting (ecdysis); the inner layers of the cuticle are digested by a series of enzymes to separate the "old skin" from the newly formed cuticle. After molting, the arthropod swallows air or water to inflate its flexible cuticle until it chemically hardens. The molting process is controlled by a complex interplay of specific hormones (similar to nematodes).
- The skeleton needs to be perforated with sensory organs (sensilla) to monitor the outside world. The most important in a parasitological context are sensory organs to locate hosts or mates via pheromones.

Respiration in arthropods has been solved in different ways. Insects and myriapods have small tubes, tracheae, through which oxygen diffuses to all parts of the body; this can be very efficient and able to operate with very small differences in the partial pressure of gases between the tissue end of the trachea and the outside atmosphere. Tracheae deliver oxygen to insect muscle, some of the most active tissue in the animal kingdom. Aquatic arthropods either use gills or diffusion across the cuticle. Arachnids (ticks, mites, and spiders) have book lungs, which are enclosed gills.

The phylogeny of arthropods has been a matter of controversy for over a century. The most recent views, incorporating both comparative and developmental morphology (of contemporary and fossil organisms) and molecular data (single "target" gene and genomic sequences) conclude that the arthropods are monophyletic but that relationships within and between the four main groups – Eucheliceratа (arachnids, scorpions, pseudoscorpions, etc.), hexapods ("insects"), crustaceans (a paraphyletic assemblage) and myriapods – are still open to debate (Figure 4.1).

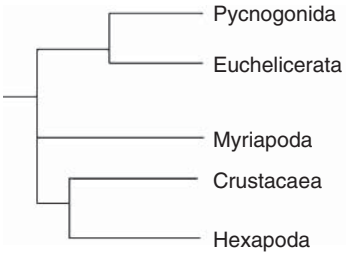


Figure 4.1 Phylogeny of arthropods. (After Giribet, G. and Edgecombe, G.D. (2013) in *Arthropod Biology and Evolution: Molecules, Development, Morphology* (eds A. Minelli, G. Boxshall, and G. Fusco), Springer, pp. 17–40.)

Arthropods are a key group in parasitology, as direct parasites of vertebrates and invertebrates, intermediate hosts, vectors of parasites and as hosts themselves of an extraordinary range of parasites. As "direct" parasites of vertebrates, they are mostly ectoparasites (e.g., lice, fleas, ticks, and mites) and flying bloodsuckers, but a few have become true internal parasites, such as the larvae of some Diptera (flies), a relationship known as myiasis.

4.1.1 Vector Concepts

Perhaps the most important and well-researched role of arthropods in parasitology is their role as vectors. The term vector is used when arthropods transmit parasites from one vertebrate host to another, most often during the process of bloodfeeding. Parasites have evolved many mechanisms to exploit the vector's need to feed on blood as a means of transmission from one host to another and often affect the behavior of the vector species to enhance the probability of transmission (see Section 1.73).

Arthropods feed on blood as a primary source of nutrition in all life stages in some groups (e.g., ticks, mites, triatomine bugs, and lice), while only the adult females feed on blood as a prerequisite for egg development in other groups such as Diptera (mosquitoes, midges, blackflies) or both sexes in tsetse flies.

Intermediate hosts of eukaryote parasites (the subject of this book) and vectors of bacteria, and viruses are found in the arachnids/acarines (ticks and mites) and the insects (lice, fleas, triatomine bugs, and Diptera: mosquitoes, midges, etc.). There is very little evidence yet that parasitic copepod crustaceans (fish lice) transmit infections between fish.

There are increasing levels of intimacy in the relationship between parasites and bloodfeeding arthropods. In general, pathogens can be transmitted mechanically between successive hosts, without any obligatory development of the parasite. In these cases, the arthropod has a pure role as a vector. Transmission in these cases is mainly fortuitous, by contamination, and is usually only an alternative to other methods of transmission. Omnivorous insects inhabiting human dwellings, such as house flies (*Musca*) or cockroaches, which feed on feces or human food, have the potential to transmit enteric pathogens mechanically. There are many laboratory reports of bloodsucking insects transmitting parasites on their mouthparts, mechanical transmission, but it is probably rare in nature. The main constraint is

the viability of parasites on the external surface or mouthparts of the putative vector. However, the transmission of myxomatosis, a viral disease, between rabbits is solely by mechanical means via mosquitoes, blackflies, or fleas. Here, the etiological agent is viable for up to 90 days, which is sufficient to cause large epizootics of myxomatosis.

Most bloodfeeding arthropods are intermediate hosts, also known as "biological vectors" in that the parasite undergoes either development or multiplication in the vector before reaching an infective stage capable of invading a new host. Various mechanisms of transmission of parasites by vectors of increasing levels of ecological and evolutionary sophistication are possible:

- **Transmission by eating the vector**. This is the simplest mode of biological transmission when the host eats the vector. Only one host can be infected for the life of each vector. Eggs of the dog tapeworm *Dipylidium caninum* are taken up by flea larvae and transmission takes place when adult fleas are eaten by canids. *Borrelia* spirochaetes, which cause louse-borne relapsing fever, are transmitted when infected lice are crushed between the fingers of their human host and subsequently infect mucous membranes.
- **Transmission during or after bloodfeeding**. Cyclically transmitted parasites are acquired by their intermediate hosts when they feed on an infected host's blood, but there are several routes by which the parasites get out of the vector and into a new host:
    - **Proliferation in the gut and transmission in vector feces**. The parasites develop in the gut or gut cells only. *Rickettsia prowazekii* develop in the gut cells of clothing lice and the cells rupture after 8–10 days, releasing infective bacteria into the louse feces. The feces can remain infective for up to 3 months and invade the human host by being scratched into wounds (e.g., louse bite) or through mucous membranes. The most important example in this category is that of the protozoan *Trypanosoma cruzi* in triatomine bugs. The metacyclic protozoans developed from an earlier infected bite are excreted during the bug's diuresis (elimination of excess fluid) at bloodfeeding or in the bug's feces, which are subsequently rubbed into the eyes or ingested by the new human host.
    - **Proliferation in the vector's gut and transmission by bite**. After ingestion with a bloodmeal, the parasites remain in the gut before migrating forward into the foregut or mouthparts for transmission when the vector next feeds. *Leishmania* parasites undergo a complex development in various parts of the sandfly vector gut (depending on parasite species) before forward migration and attachment to the chitin-lined area of the foregut. Here, they are embedded in a carbohydrate matrix before being "regurgitated" into the new host when the sandfly next feeds. Metacyclic stages of the cattle parasite *Trypanosoma congolense* and the pig parasite *Trypanosoma simiae* migrate from the midgut to the proboscis of their tsetse fly vector.
    - **Penetration of the vector's gut and transmission by bite**: many parasites do not stay in the intermediate host's gut to become involved

in aggressive digestive enzymes and potential entanglement in the peritrophic membrane (an envelope formed around a bloodmeal in insects) but rapidly penetrate the gut wall. The parasite then becomes intimately involved in the vector's immune system. Once development has taken place, the parasite has to get out of the vector. Filarial parasites, such as *Onchocerca* and *Wuchereria*, having entered their vectors as microfilariae molt two or three times to reach the infective stage (L3) before migrating to the thick, fleshy labium of the mouthparts. When the fly feeds, the highly mobile worms break out through the soft intersegmental cuticle and on to the host's skin, which they rapidly penetrate. There is no multiplication of the filariae in the vector. Many other parasites replicate in the hemocoel of the vector and migrate to the salivary glands. This is an effective strategy because saliva is produced early in the vector's bite and the parasite is inoculated directly into the new host's tissues. Many widely known parasites use this mode of transmission: *Plasmodium*, and all the arboviruses (e.g., yellow fever and dengue). In some cases, there is interference by parasites with the production of saliva to maximize chances of transmission.

Vertical transmission occurs when pathogens, usually viruses or important commensals, such as *Wolbachia*, are transmitted from one generation of a vector to the next via their eggs.

The ability of a vector to transmit a particular parasite is dependent on a number of components: physiological and immunological factors within the vector and collectively known as vector competence, host-seeking behavior (host specificity), and aspects of the population biology of the vector (see Box 4.1).

Physiological factors include the timing and cascade of digestive enzymes within the gut of the vector following a bloodmeal and potential binding sites on gut and salivary gland cells. Arthropod immune mechanisms are of the innate immunity type, indicating that they are genetically fixed, recognize defined structures, act very quickly, and have no memory responses. They include phagocytosis; antimicrobial peptides, such as cecropins, defensins, and attacins; and proteolytic cascades resulting in the clotting of hemolymph and melanin encapsulation.

Box 4.1 Vectorial Capacity

By far the most important method of comparing the relative importance of different vector species is to use a quantitative measure of transmission. The simplest estimates are made by combining the biting rate (bites per unit time) with the infection rate (proportion of vectors infected with a parasite) to give the *infective biting rate*, usually expressed per month or per year. A more precise measure is the vectorial capacity. It is based on MacDonald's model of malaria transmission. Mathematically, vectorial capacity represents the number of secondary cases arising per day from one infective case in a susceptible

population of hosts. It can be represented by the equation:

$$C = \frac{ma^2p^n}{-\log p},$$

where m = the number of individual vectors per individual, a = number of bloodmeals per vector individual on humans, p = the daily survival rate of the vector, and n = the extrinsic incubation period. Practically, obtaining field data on each parameter is extremely difficult, vectorial capacity is mainly used to explore the relative importance of different aspects of the biology of vector and parasite on the epidemiology of disease. The vectorial capacity is most sensitive to the longevity of the vector, particularly the proportion of the vector population that lives long enough to transmit a parasite, and the biting rate on the specific host (anthropophily, in the case of biting humans). Note, C is the daily *rate* of the appearance of secondary cases while the familiar epidemiological parameter R_0 is the total *number* of secondary cases arising from an infected, primary case. The Vectorial capacity model is used to explore the potential effect of disease interventions (e.g., insecticide residual spraying to reduce longevity) or climate change (e.g., Ceccato, P., Vancutsem, C., Klaver, R., Rowland, J., and Connor, J.C. (2012) A vectorial capacity product to monitor changing malaria transmission potential in epidemic regions of Africa. *J. Trop. Med.*, **2012**. doi: 10.1155/2012/595948).

4.1.2 Impact of Bloodfeeding

Even when arthropods are not carrying parasites or pathogens to a host, the effect of their bloodfeeding can have a very deleterious effect. This can range from extreme annoyance by biting or even feeding on secretions (e.g., head flies on cattle) leading to weight loss in domestic animals, anemia in humans and animals, depression in people, and economic losses to tourism. The massive quantity of alien proteins and other bioactive agents injected when large numbers of bloodsucking arthropods feed leads initially to delayed hypersensitivity and later to more profound systemic responses. However, bloodfeeding by arthropods can also lead to anemia and delayed development. For example, severe consequences of flea bites are observed in young birds from heavily infested nests (see Section 1.3).

Further Reading

Minelli, A., Boxshall, G., and Fusco, G. (eds) (2013) *Arthropod Biology and Evolution: Molecules, Development, Morphology*, Springer, 532 pp.

Tree of Life Web Project http://tolweb.org/ (accessed 28 April 2016).

Wiegmann, B.M., Trautwein, M.D., Winkler, I.S. *et al.* (2011) Episodic radiations in the fly tree of life. *Proc. Natl. Acad. Sci. U.S.A.*, **108**, 5690–5695.

Test Questions

1. What are the main advantages and disadvantages of an exoskeleton?
2. What is the structure of the exoskeleton and what is its main component?
3. What are the four main groups in Arthropoda?
4. What does vectorial capacity measure?
5. Give two examples of parasites or pathogens transmitted in the feces of a vector.

4.2
Acari – Mites and Ticks

- Free-living or parasitic arachnids, mostly minute to small.
- Body consists of two parts: a complex of the mouthparts (gnathosoma) and an unsegmented idiosoma containing the gut and reproductive organs.
- One pair of spiracles (except in Notostigmata).
- Six-legged larva, several eight-legged nymph stages
- Parasitic forms usually as ectoparasites.

The Acari (mites and ticks) are the largest group of the Arachnida and doubtlessly have the greatest biological diversity. Approximately 45 000 species have been described, but there might be up to 20 times this number. They are truly ubiquitous, having colonized nearly every terrestrial, marine, and freshwater habitat from arctic and alpine snowfields to the oceanic abyss at 5000 m and in soil to a depth of 10 m. As soil-inhabiting organisms, they form 7% of the dry mass of all invertebrates living there. Most mites are very or extremely small, for example, the follicle mites that inhabit hair follicles of humans, are smaller than 0.1 mm. The largest are fully bloodfeeding ticks up to 3 cm. Mites are predators, saprophages, plant pests, parasites, and commensals of plants and animals. Dispersion is often by phoresy (attachment to a carrier host). The diversity of biotopes of the Acari and their way of living is expressed by an enormous richness of forms, life cycles, and species. One of the groups of the Acari, the entirely parasitic ticks, are important as vectors of diseases to vertebrates.

Together with the Aranae (spiders), the Acari can be easily distinguished from other arachnids by the absence of somatic segmentation. In spiders, the head and thorax are fused to form the cephalothorax, which carries the mouthparts and legs. To this, the abdomen is connected by a narrow pedicel. In the Acari, there is no such division. Rather, they have a compact body without segmentation and without a separate head.

The phylogeny and higher classification of the Acari (ticks and mites) is problematic for several reasons: they are highly evolved and have lost many external features, including their primary segmentation; there are few homologous morphological characters across the whole group; there is often a lack of concordance between morphological and molecular approaches and there are

Table 4.1 Summary classification of the Acari showing important parasitic groups.

Order	Suborder	Supercohort (superfamily)	No. of stigmata (spiracles) and (position)	Way of life	Genera (examples only)
Anactinotrichida = Parasitiformes	Mesostigmata = Gamasida	Monogynaspida (Dermanyssoidea)	2 (between coxa 3/4)	Free living and parasitic	*Varroa, Dermanyssus, Ornithonyssus*
	Metastigmata	(Ixodoidea)	2 (behind coxa 4 and above coxae 3/4)	Obligatory parasites	*Ixodes, Rhipicephalus Argas, Ornithodoros*
Actinotrichida = Acariformes	Cryptostigmata = Oribatida		None	Free living	*Sceloribates*
	Prostigmata = Actinidia = Trombidiformes	Promatides (Cheyletoidea) (Trombidioidea)	2, on gnathosoma or on proximal idiosoma	Free living and parasitic	*Demodex, Neotrombicula*
	Astigmata = Acaridida = Sarcoptiformes	Sarcoptoidea	None	Mostly parasitic	*Sarcoptes*

few examples of an "integrated approach" to higher-level classification using comparative morphology and molecular analyses. At present, the consensus is for a single, monophyletic origin of the acarines, but there the consensus ends. Major authorities even disagree on the number of orders in the acarines and their names. Taxonomy at the family, generic, and species level is also in a state of flux and identification requires very careful preparation and microscopic examination. Many species and even families are still being described.

Table 4.1 shows a summary classification of the Acari; there are two distinct subgroups, most probably each of them monophyletic, the Anactinotrichida and Actinotrichida. Their names signify the absence or presence of the birefringent actinopilin in the cuticle, especially in some setae (hairs), visible in polarized light. Both groups have alternative but not very apt names, since the Parasitiformes (=Anactinotrichida) do not contain many parasitic groups, and the Acariformes (Actinotrichida) are by no means the only ones to have "mite-like" shapes. Two Acari groups of entirely tropical and free-living mites will not be dealt with here, the Notostigmata (or Opilioacarida) and Tetrastigmata (or Holotyrida), both belong to the Anactinotrichida.

The taxonomic rank of the groups in the first three columns of Table 4.1 varies between superorder, order, suborder, supercohort, and cohort by different authors. Not noted within the Actinotrichida are the Endeostigmata, which have no parasitic members

4.2.1 Morphology

Most Acari are very small, usually less than 1 mm, some much larger. The body comprises a complex of mouthparts, called the gnathosoma (Greek: *gnáthos* = jaw, *sóma* = body) and unsegmented idiosoma (Greek: *ídios* = belonging to one's self) (Figure 4.2a). The gnathosoma is delimited from the idiosoma by a circumcapitular suture. There are no antennae.

The **gnathosoma** (Figure 4.2a) consists of (i) the hypostome (Greek: *hypo* = beneath, *stóma* = mouth), formed from the fused coxae of the pedipalps, which delimits the gnathosoma ventrally, and dorsally forms a furrow for food and saliva. (ii) The paired chelicerae are situated above the hypostome enclosed in a long protrusion of the exoskeleton, the cheliceral sheath, and from which they can be protruded or retracted (Figure 4.2b). The basic chelicerae are forceps-like with a movable part (*digitus mobilis*) working against a fixed part (*digitus fixus*), but they may be variously modified into stylets, chisels, swords, or combs. (iii) Between the two chelicerae lie the labrum or upper lip; and, finally, (iv) the distal segments of the pedipalps serve as tactile receptors. The gnathosoma is not a "head" in that it does not have a central ganglion or "brain."

The **idiosoma** has lost all evidence of segmentation. Consequently, the various dorsal and ventral plates of sclerotized cuticle found on different developmental stages do not correspond to the tergites and sternites of other arthropods. The body and extremities have an enormous range of bristles, the setae (Latin: *saeta* = bristle, animal hair), which act as mechano- or chemoreceptors. The number, shape, and distribution of setae are important for systematics and identification. The four pairs of legs (denoted I, II, III, and IV) are attached to the idiosoma, two of which are directed anteriorly and two posteriorly. The first pair often has an important tactile function, and is usually longer than the others. As in all arachnids, the legs have one segment, the genu (Latin: = knee), more than in insects. Thus, there are, from proximal to distal, coxa, trochanter, genu, femur, tibia, and tarsus. On the tip of the tarsus is a pretarsus securing the acarine's attachment to the substrate and which varies significantly between taxonomic groups and habitats.

The genital opening is ventral, but its position varies between the sexes and taxonomic groups. The stigmata are respiratory pores (spiracles) found on the idiosoma and their position or absence is a very important feature in the classification of acarines (Table 4.1). The stigmata of the Mesostigmata and Metastigmata are surrounded by a visible sclerotization of the cuticle, called the peritreme. In the Mesostigmata, it projects forward as a narrow band, and in the ticks, it is disk-shaped.

Sense organs are firstly eyes of rhabdomeres and retinula, which are particularly conspicuous in ticks. Secondly, there is the multitude of bristles already mentioned.

Internally, the idiosoma is dominated by the gut. A fused synganglion acts as the central nervous system.

4.2.2 Development

After the egg, there are up to six developmental stages with a molt between each, as follows:

- A hexapod (six-legged) reduced *prelarva*, within the egg shell.
- A hexapod larva.
- Three octopod (eight legged) *nymphs* without externally visible genital organs (protonymph, deutonymph, tritonymph).
- Fully developed octopod *adults*.

Some of the immature stages may be missing in some groups.

4.2.3 Anactinotrichida (= Parasitiformes)

4.2.3.1 Mesostigmata

- Mostly free-living; parasitic species on terrestrial vertebrates, some residing in the respiratory tract.
- Spiracles between coxae III and IV, missing in the larva.
- Peritreme narrow, usually projected forward.
- Developmental stages: six-legged larva protonymph, deutonymph, adult.

Members of the Mesostigmata live as predators or saprophytes in soil and litter, as pests in stored products and commensals and parasites in/on invertebrates and vertebrates. Many of them are nidicolous (inhabit nests), from where they developed into parasitism and bloodsucking. This transition was accompanied by a transformation of the chelicerae into piercing tools, which can penetrate skin or other tissues. The superfamily Dermanyssoidea contains all families parasitizing vertebrates. Most of their ectoparasitic members are hematophagous.

Morphology The chelicerae can be protruded or retracted. In parasitic groups, they have the form of stylets with a reduced *digitus mobilis* (Figure 4.2). The pretarsi consist of a membranous lobe, which, when the foot is put down, is expanded between the two paired claws, adhering to the substrate, and is folded again, when the foot is retracted. Sometimes, a conspicuous bristle, the tritosternum, is present at the anterior ventral margin of the idiosoma, serving as a sense organ (Figure 4.2a). The location of the stigma between coxae III and IV explains the name of the group. The peritreme surrounds the stigma and extends forward as a long furrow.

Development Four developmental stages are present: A six-legged larva and eight-legged protonymph, deutonymph, and adult. A tritonymph is lacking. The Dermanyssoidea tend to shorten the life cycle by retaining the egg or even the larva within the female, so that the protonymph is the first free stage.

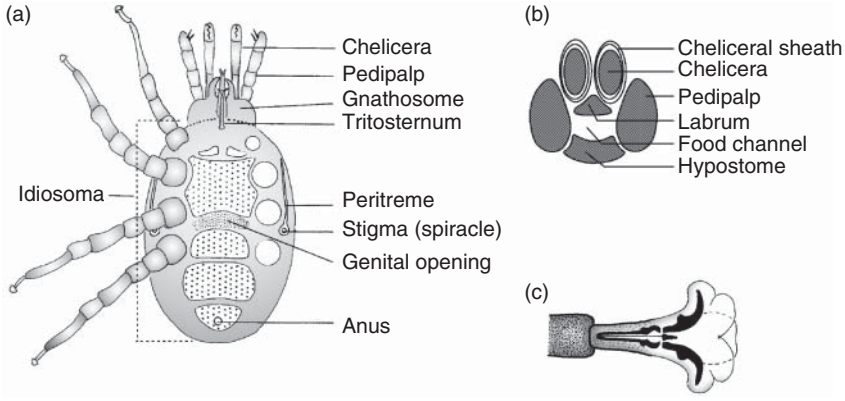


Figure 4.2 Acari: Mesostigmata. (a) Female (ventral aspect). (b) Gnathosoma in cross section. (c) Pretarsus.

4.2.3.2 *Dermanyssus gallinae*

The red poultry mite can cause serious damage in not only intensive poultry management, but also ornamental and pet birds. If the proper hosts are not available, the mite will attack humans causing a dermatosis. As the mite is nocturnal, it can be overlooked for some time, until the hosts show agitation and diminished production from blood loss. During daytime, the mites hide in cracks and crevices and are easily overlooked. The larva does not feed, but nymphs and adults feed on blood. ***Ornithonyssus bacoti*** (=*Bdellonyssus*), the tropical rat mite, is a parasite of captive and, in America, of many wild rodents that can live on humans. Morphologically, it is very similar to *Dermanyssus gallinae* (identification requires a specialist). It is an intermediate host of the filaria *Litomosoides carinii*, serving as a model for human filariae. *O. bacoti* is also a reservoir and vector of Korean hemorrhagic fever of humans. ***Pneumonyssus simicola*** is a lung parasite of the rhesus monkey occurring frequently but asymptomatically except in long-nosed monkeys, where it is highly pathogenic and can be fatal. ***Sternostoma tracheacu-lum*** is a parasite of the respiratory tract of canaries and Gouldian finches. In heavy infections, audible dyspnea, open-mouthed breathing, and mortality can occur.

4.2.3.3 *Varroa destructor*

This infamous mite is a devastating pest of honey bees causing "varroosis." It has a major economic impact on the beekeeping industry and can be a contributing factor to colony collapse disorder. *Varroa destructor* and its sister species, *Varroa jacobsoni,* are more or less harmless to the Asian honey bee *Apis cerana,* but not when *V. destructor* moves on to the Western honey bee *A. mellifera*. The initial transference was in the Philippines, from where it was introduced with bees to Japan and Russia, then to Europe in the 1960s and 1970s, to South America in the late 1970s, and to the United States in 1987. The devastation of bee hives leading to a reduction in pollination, and the additional expense of control is a considerable financial cost to the beekeeping industry.

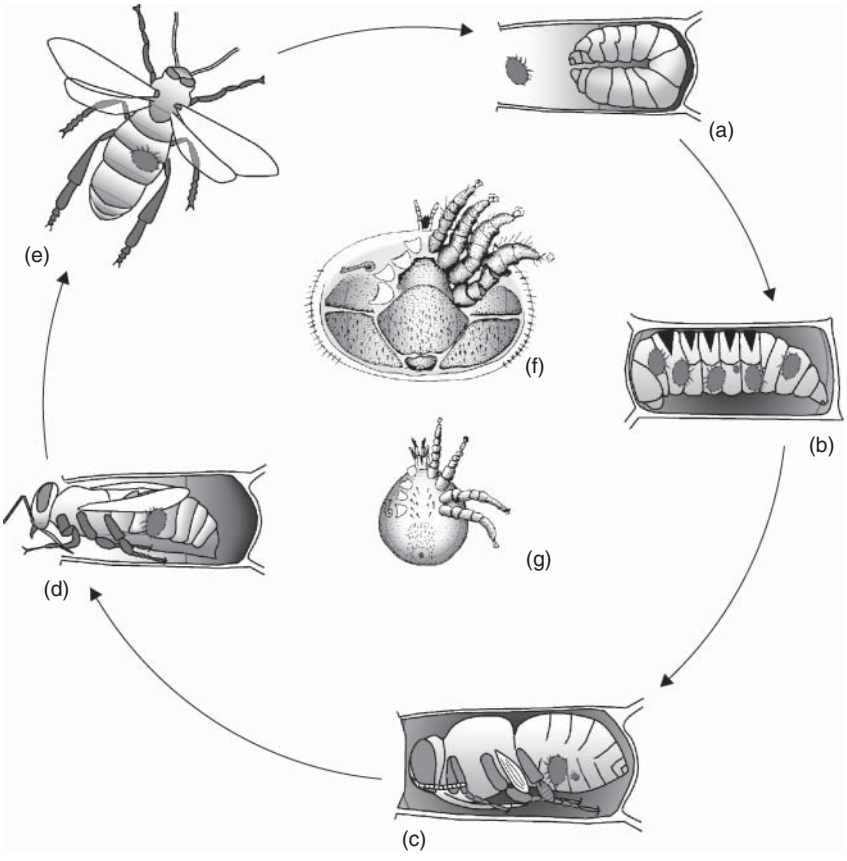


Figure 4.3 Life cycle of *Varroa destructor*. (a) Female mite in brood cell of worker bee. (b) From eggs laid on a bee larva one male and up to six female mites develop. (c) Copulation within brood cell. (d) Fertilized female mite enters the outside world when adult bee hatches. (e) Mites suck hemolymph from flying bee. (f) Female of *Varroa*. (g) Male of *Varroa*.

Varroa mites have an unusual appearance. The females (Figure 4.3f) are broader than long (1.1 × 1.6 mm), weakly concave, dark brown, and covered with strong, spine-like setae. The legs, which serve to hold the mite on the flying bee, are unusually robust. The peritreme is directed outward. The heteromorphic males (Figure 4.3g) are rounded in top view, measure only 0.8 mm in diameter, and are weakly sclerotized. Their chelicerae are modified for the transmission of sperm packets during copulation.

The female varroa mite enters the brood cell containing larvae of drone or worker bees shortly before the cells are capped. The female feeds on hemolymph from the developing bee before ovipositing (Figure 4.3a). The first egg to be laid is haploid (unfertilized) and develops into a male mite, and subsequent eggs are diploid and develop into females (Figure 4.3b). The male mates with its "sisters"

and dies without having fed. Larval mites develop within the egg before emerging as an eight-legged protonymph, then deutonymph, and finally an adult mite. Proto- and deutonymphs suck hemolymph from the bee larvae (Figure 4.3c) and can reduce the proteins in bee hemolymph by up to 50% and significantly affect the bees' life span and health. They can also transmit viral and bacterial infections. Pathology is highly correlated with mite abundance per cell. The entire process from egg to adult mite takes 6–7 days for both sexes.

The *Varroa* females attach to adult bees between the abdominal segments, up to six per bee, and are carried to other parts of the hive and other hives, especially by drones. Because drones have a longer development time (24 days) than worker bees (21 days), more adult mites can develop in the brood cell of drones. One possible control measure therefore is to cut away and destroy the capped drone brood cells. In the autumn, drone-rearing ceases and only worker larvae are available and losses in the hive consequently increase. A hive perishes without control measures, usually in the winter as soon as about 30% of the worker bees are infested. Increasing resistance makes chemical control of varroatosis (another name for varroosis) more difficult. Nonchemical control methods are costly in terms of labor and cannot eradicate the parasite.

4.2.3.4 **Metastigmata (= Ixodida or Ixodoidea, Ticks)**

- Members of the Anactinotrichida.
- All stages of the life cycle are obligatory hematophagous ectoparasites of terrestrial vertebrates.
- Stigmata behind coxae IV, not present in larvae.
- Peritreme round or oval, surrounding stigma.
- Hypostome with strong backward facing teeth.
- Haller's organ dorsally on tarsus I.
- Stages: six-legged larva, nymph(s), adult.
- Vectors of many diseases.

The Ixodida, or ticks, are the only group of Acari consisting exclusively of obligatory hematophagous parasites. They are the largest Acari and fully fed females of tropical species can be as large as 30 mm. Approximately 900 species are known from a wide range of hosts. Some ticks are very colorful while most are brown or buff. Ticks cause toxicoses and dermatoses from their physical presence as well as transmitting viruses, bacteria, rickettsiae, protozoa, and nematodes, which cause disease in their hosts.

Research on ticks has developed independently from the rest of the mites resulting in a different nomenclature for homologous structures. Thus, the gnathosome is called the capitulum, and its broadened base is the basis capituli, while the dorsal shield in the ticks is called the scutum.

The ticks consist of three families with many biological and morphological differences between them: the Ixodidae (hard ticks) with 14 genera, Argasidae (soft

ticks) with five genera, and finally the Nuttalliellidae (no vernacular name) with one species (*Nuttalliella namaqua*) in Africa.

Morphology Ticks show the typical features of the Anactinotrichida in that the coxae are independently movable, the pretarsus bears two claws and between them is a "pulvillus," a soft, cushion-like pad serving as an adhesive structure. Ticks have a number of unique features: The **hypostome** (Figure 4.4c,f,g,i) has a dorsal groove, and ventrally is covered with strong backwardly directed and symmetrically arranged teeth. Therefore, it does not matter if the extraction of a tick from the skin is done clockwise or counterclockwise. The **chelicerae** have

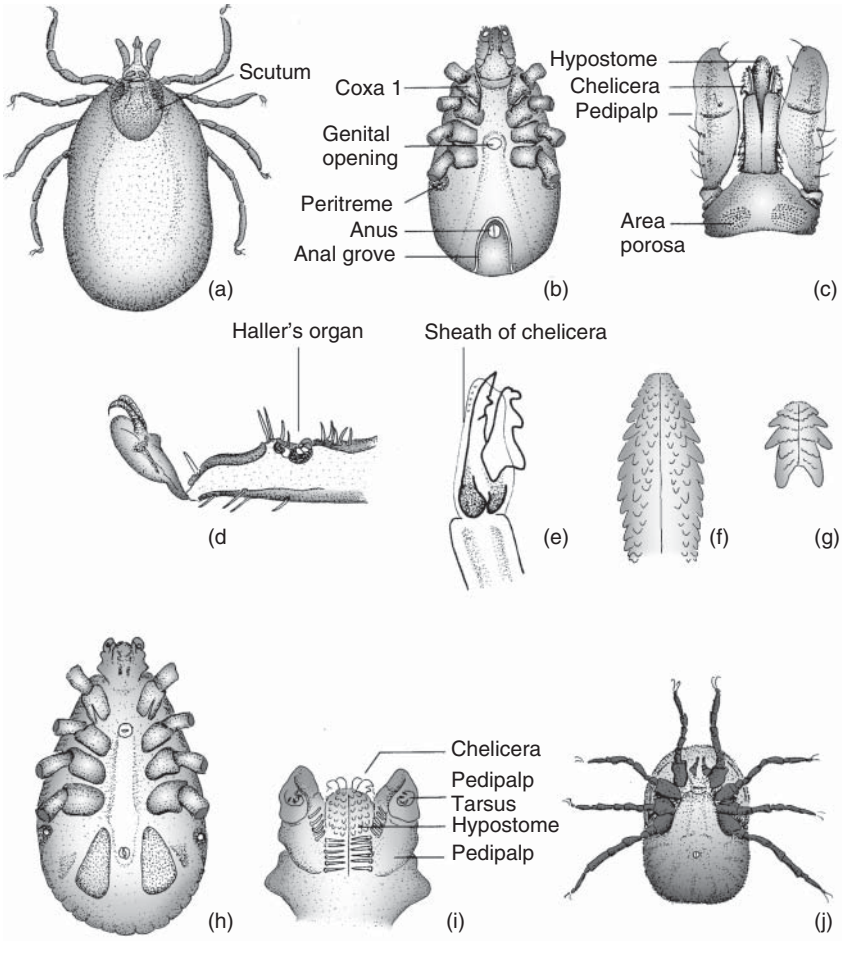


Figure 4.4 Metastigmata, ticks. (a) *Ixodes ricinus*, female, dorsal view. (b) *I. ricinus* female, ventral view. (c) Capitulum with mouthparts of *I. persulcatus*, dorsal view. (d) Tarsus of a tick with Haller's organ. (e) Tick chelicera. (f) Hypostome of female *I. ricinus*. (g) Hypostome of male *I. ricinus*. (h) Male of *Rhipicephalus sanguineus*, ventral view. (i) Capitulum of *R. sanguineus*, ventral view. (j) Female *Ornithodoros moubata*, ventral view.

only two segments and outwardly directed teeth to cut open the skin of the host. Ticks are poolfeeders, taking up blood, lymph, and cell fluid (telmophagy). Leg I bears an important sensory organ, **Haller's organ**, on the tarsus (Figure 4.4d). It consists of a proximal pit and a distal capsule, both with sensillae, to detect host odors, humidity, and temperature. A unique structure in all arthropods, **Géné's organ**, is situated dorsally beneath the proximal margin of the female's scutum. During oviposition from a fold separating the capitulum and scutum, two horn-like, thin-walled sacs filled with secretions are protruded (Gene's organ). While the capitulum is bent downward, the egg leaves the ventral genital opening and is clasped by these horns. They secrete waxes during pulsating movements covering the egg with a waterproof layer to prevent desiccation. Subsequently, each egg contacts the "porose area" (Figure 4.4c), which secretes antioxidants helping to prevent degradation of the secretions of the gene's organ. The scutum is the only sclerotized plate dorsally. If eyes are present, they are situated on the margins of the scutum. The stigmata (absent in the larva) are surrounded by a round peritreme (= stigmal plate; Figure 4.4b). Their position behind the fourth pair of legs gives the Metastigmata their name.

The two major families of ticks – the Ixodidae (hard ticks) and Argasidae (soft ticks) – can be distinguished relatively easily. The diagnostic features of the Argasidae are that the capitulum is not visible from above; the scutum is absent and the body is covered in a leathery and often papillate surface; the peritreme is inconspicuous and anterior to coxae I (Table 4.2). By contrast, in the Ixodidae,

Table 4.2 Important characteristics of ticks.

	Ixodidae	Argasidae
Way of life	Not nidicolous	Nidicolous[a)]
Sex differences	Females larger than males	Equal size
Capitulum	Visible from above	Not visible from above except in larva
Shields	One dorsal shield (Scutum) In females covering only front part of the idiosoma, in males the whole back Males with several ventral shields	Absent
Surface of idiosoma	Smooth	Leathery, tubercular
Stigmata	Behind coxae IV	Between coxae III and IV
Peritreme	Round, oval or elliptical	Round, small, inconspicuous
Hypostome	Stout, with strong teeth	Slender, week teeth
Tarsi of pedipalps	Retracted into tibia	Normal, visible
Pulvilli of pretarsi	Present	Absent in nymphs and adult
Coxal glands	Lacking	Between coxa I and II, absent in larva
Area porosa	Present	Lacking
Position of eyes	Lateral on scutum	Usually above and between coxa II and III

a) Living in or near nests or borrows (of hosts).

the capitulum projects forward and can be seen from above; an obvious scutum is present, which can cover the entire dorsum in males or the anterior dorsum in females; the peritremes are large and posterior to coxae IV.

The argasids are mainly found on birds but some important species attack mammals, including humans, and transmit significant diseases. They usually seek out dark recesses in which to hide during the day in and around the "nests" of their hosts (nidicolous). By contrast, Ixodids are mainly found on mammals and range freely on vegetation when seeking new hosts.

4.2.3.5 Development

The larvae are hexapod, as in all mites. There is one eight-legged nymphal stage in the Ixodidae, but up to eight nymphal stages in the Argasidae. All of these stages have to suck blood. Fertilization is achieved by the transference of spermatophores from the male with the aid of the chelicerae into the genital opening of the female.

4.2.3.6 Tick Bites and Saliva

The chelicerae of Ixodidae move horizontally and lacerate the epidermis of the host's skin as a prelude to the insertion of the hypostome. Its backwardly pointing teeth secure a very effective anchorage in the skin and then blood and lymph are sucked from the ensuing lesion. The pedipalps are not inserted into the lesion but lie laterally on the surface of the host's skin. In hard ticks, 5–30 min after the initial bite, the salivary glands begin to secrete a matrix, the cement, which can continue for up to 3 days. The cement hardens around the hypostome and chelicerae, reinforcing the attachment and preventing the tick falling off. Soft ticks, which only feed for a short time, have finer mouthparts and do not produce cement.

The two pairs of alveolar salivary glands consist of several types of cells with additional functions to the generation of cement. As in all hematophagous arthropods, the coagulation of the host's blood has to be prevented. This is done firstly by vasodilatators causing the relaxation of the muscles of blood vessels; secondly by inhibiting thrombocyte aggregation; and thirdly by inhibiting the protease thrombin, which usually activates the thrombocytes and the subsequent generation of fibrin. The bioactive secretions necessary for these events have been extracted and characterized in ticks. Furthermore, hard ticks being in contact with the host for a long time need to reduce inflammation, as this would compromise the tick's feeding and survival. Therefore, the adhesion and trans-endothelial migration of neutrophils together with histamine and serotonin, both important inflammation factors, are inhibited. Substances for the inhibition of the complement cascade, which could lead to a lysis in the tick's intestine, have also been isolated. Salivary gland products generate host immunity against ticks, causing a reduction of the number of blood-sucking females, a decrease in the intake of blood, and consequent reduction in fertility and increase in mortality. In order to prevent such effects, ticks are able to downregulate T cell proliferation, the formation of the cytokines interleukin and interferon-γ, as well as the production of the macrophage cytokine interleukin-1, tumor necrosis factor, and antibody responses.

Tick saliva can be a target for anti-tick vaccines. A commercially available recombinant vaccine against the cattle tick *Boophilus microplus,* which transmits *Babesia bovis,* was released in Australia in 1994. Given that antigens are common to several tick species, there is the potential for more generic anti-tick vaccines in future. A serpin elastase inhibitor, "Iris," from *Ixodes ricinus* saliva which inhibits hemostasis and the immune response in mammals, is an anti-tick vaccine candidate.

4.2.3.7 **Ixodidae – Hard Ticks**

The hard ticks, with more than 700 species in 14 genera, are distributed worldwide, absent only in the Arctic and Antarctica. Their characteristic strong and smooth cuticle is reduced to a small dorsal shield in females (Figure 4.5). Many species, especially tropical species, are highly conspicuous, caused by interference colors of the cuticle and underlying guanine deposits. Most hard ticks live in open habitats such as grassland, heaths, or open woodland and in this respect are "outdoor ticks."

The ixodids are long-term feeders, all developmental stages adhering firmly to the host, taking several hours to penetrate the skin and several days to fully feed. Females of some of the larger *Amblyomma* species can increase from just less than 1 cm to over 2.5 cm in length and from about 0.04 g to over 4.0 g in weight after a bloodmeal.

The life cycles of ticks are complex and involve regular alternation between a bloodfeeding and free-living stage with a change of host. The on-host existence, which is in many species less than 10% of a tick's life, is adapted to blood-feeding, whereas the off-host phase focuses on survival during development and finding a new host. Ticks that need a new host for each developmental stage, larva, nymph, and adult are termed three-host ticks and include the genus *Ixodes,* as well as most species of *Amblyomma, Haemaphysalis, and Dermacentor*. In two-host ticks, the larva and nymph use the same host and the adult another (e.g., species of

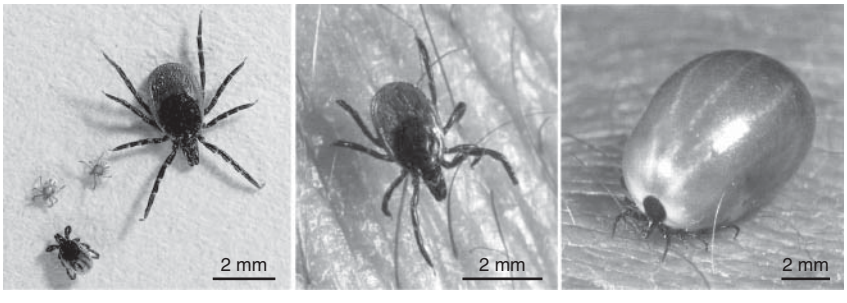


Figure 4.5 *Ixodes ricinus. (*a) Young female, nymph, and two six-legged larvae. (b) Young female questing for place of penetration. (c) Replete female. (Images: Courtesy of Heiko Bellmann.)

Rhipicephalus and *Hyalomma*). One-host ticks feed on one-host individual as larva, nymph, and adult (e.g., *Boophilus* and some species of *Dermacentor*).

Except in the genus *Ixodes,* copulation takes place on the host, with males often copulating with several females. Only mated females become fully engorged after which they drop off the host and in the vegetation enter a quiescent phase while the bloodmeal is digested and the eggs mature. Away from the host, the hatching of eggs and molting demands very exacting micro-climatic conditions for survival. Host-finding by field-inhabiting ticks is very risky as, unlike most insects, ticks only move short distances and have to lie in wait for a new host (questing). Passing hosts are detected and physical contact is required for transfer. Mortality is very high in questing. Each female can lay between 2000 and 20 000 eggs.

Host specificity varies from generalists to highly coevolved relationships, although this can be modified with the introduction of domesticated animals into the normal habitat of the tick–host association.

Ixodes *I. ricinus*, the European sheep tick or castor bean tick (Figures 4.4a,b, 4.5, and 4.6), is the most common tick in temperate parts of Europe and occurs in low densities in North Africa.

Engorged females are 11 mm long. Favored biotopes are margins of woodland areas with shrubs or dense vegetation of herbs. The relative humidity of the microenvironment has to be 80%. It has a three-host life cycle and has been found to feed on over 300 vertebrate species. Stages seeking a host ascend grass and twigs and assume a "questing" position with the first pair of legs extended so that the Haller's organ can detect the signals of a passing host. The different stages climb plants according to their host's size, larvae are usually found on rodents

(a) (b)

Figure 4.6 *Ixodes ricinus*. (a) Capitulum, frontal view, below: hypostome, above: chelicerae, right and left: pedipalps, on capitulum: the two areae porosae. (b) Hypostome, ventral view, tip of a chelicera underneath left-hand side. (EM Images: Courtesy of Eye of Science.)

and mustelids, nymphs on birds (very often blackbirds), squirrels, and hedgehog, adults on all big mammals. All stages occur on humans. The life cycle usually takes 2–3 years.

I. ricinus is the principal vector of tick-borne encephalitis, the most important arthropod-borne viral disease in Europe. *Ixodes persulcatus* transmits the virus in Eastern Europe and Asia. *I. ricinus* is also the primary vector of Lyme disease in Europe and members of the same-species complex, especially *Ixodes scapularis*, the black-legged tick or deer tick, and *I. pacificus*, the western black-legged tick, transmit the pathogen in North America and have been the topic of much research. As in *I. ricinus*, the immature stages infest rodents, especially the white-footed mouse (*Peromyscus leucopus*) and the adults larger mammals, especially white-tailed deer (*Odocoileus virginianus*) which are expanding their distribution and Lyme disease with them. The *Borrelia* spirochaetes can be acquired early in the tick's life and maintained trans-stadially from larvae to nymphs to adults.

Dermacentor *Dermacentor* species are conspicuous by the bright blue, enamel-like patterns on the scutum, especially the large male scutum and by "festoons," more or less rectangular bulges on the posterior body margin. Most species are three-host ticks (Figure 4.7). In Eurasia, *Dermacentor marginatus*, the ornate sheep tick, occurs in warm, dry habitats where the adult infests large herbivorous mammals, especially sheep. *Dermacentor reticulatus*, the ornate cow tick, is difficult to distinguish from *D. marginatus* and has the same distribution, although found in more wooded habitats.

The most important *Dermacentor* species are found in North America, especially the Dog Tick *Dermacentor variabilis* in the east, which has a large silver spot behind the "head," and the Rocky Mountain dog tick *Dermacentor andersoni* in the west with a white scutum. Both transmit the Rocky Mountain spotted fever (RMSF) parasites (*Rickettsia rickettsii*) and Tularemia (*Francisella tularensis*).

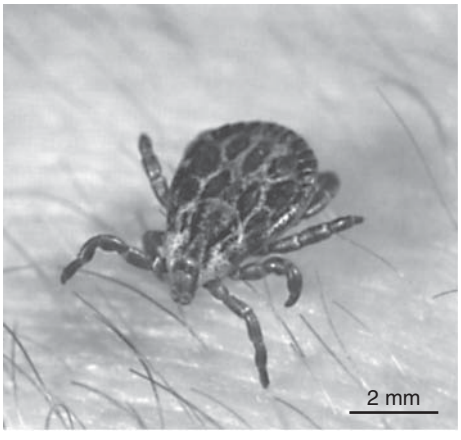


Figure 4.7 Female of *Dermacentor* sp. (Image: Courtesy of Heiko Bellmann.)

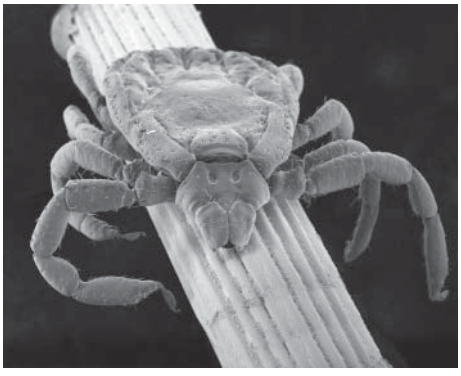


Figure 4.8 Female *Rhipicephalus sanguineus*. (EM Image: Courtesy of Eye of Science.)

Rhipicephalus *Rhipicephalus* species are found in Africa and Europe and most are three-host ticks, that is, the larva, nymph, and adults leave the host and molt or lay eggs in the surrounding environment. The brown ear tick, *Rhipicephalus appendiculatus*, of east and southern Africa is a very important parasite of domestic livestock. Although a three-host tick, considerable numbers of adults and immature stages infest the same host, usually in the ears but overflow populations occur elsewhere. It transmits East Coast Fever (*Theileria parva*) among cattle and antelopes, the virus of Nairobi sheep disease in East Africa and is an important vector of African tick typhus (*Rickettsia* spp), in the grasslands of the South African veldt, where it bites humans frequently.

The brown dog tick *Rhipicephalus sanguineus* (Figures 4.4h,i and 4.8) has expanded its range from Africa to a worldwide distribution by human transport of its dog host. It is unusual in that, as an endophilic species, it is the only ixodid (hard tick), which can complete its whole life cycle indoors and can become a real pest of domestic dogs and humans.

Although a three-host tick, all stages can develop on a dog in a "domestic setting." Up to four generations can be completed per year. In contrast to *Ixodes* species, which find their hosts by a questing behavior, *R. sanguineus* is a "hunter tick," seeking out its host more actively.

Amblyomma These are large and beautifully ornate ticks. They have particularly long mouthparts, which cause deep lesions that can become secondarily infected. Many species are economically important in the tropics, such as the African bont tick *Amblyomma variegatum,* which transmits heartwater fever (*Cowdria* = *Ehrlichia ruminantium*) between cattle. In Southern Africa, *Amblyomma hebraeum* larvae and nymphs will swarm toward and avidly feed on humans in grasslands where they transmit tick typhus (*Rickettsia conori*). The center of species diversity is the Americas, where half of all species occur. Two species, the more northerly *Amblyomma americanum* (lone star tick, North and South America) and the more southerly distributed *Amblyomma cajennense* (Cayenne tick, South and Central America) attack humans in all stages and transmit Rocky Mountain spotted fever (RMSF).

4.2.3.8 Argasidae (Soft Ticks)

There are about 200 species in five genera, the two most important being *Argas* and *Ornithodoros.* With a few exceptions, soft ticks occur in the tropics and subtropics. They are nidicolous parasites in that they live in or nearby the nests of their hosts. Because they live so close to their host, they can feed regularly and, with the exception of the larvae, relatively rapidly. Consequently, the hypostome is a weak structure and the legs are relatively long and slender, not needing to cling on to the host like hard ticks. They feed within minutes, unlike hard ticks, which feed for days, taking 5–10 times their body weight. This is made possible by the exudation of superfluous fluid during and after meal, via the coxal glands, in a similar manner to the diuresis of bloodfeeding insects. Mating and oviposition follow a bloodmeal away from the host. Fewer eggs are produced than in hard ticks, probably because they are laid in the proximity of the host.

Argas This genus occurs mostly in arid regions, where they feed on bats and birds, which nest or roost in colonies. The ticks hide in crevices and cavities in rocks or under bark coming out to feed on their hosts. Larvae remain attached to the host for several days. *Argas persicus* attacks wild birds and poultry and has been transported around the world. It is an important vector of *Borrelia anserina* and can kill birds by exsanguination. The European pigeon tick (*Argas reflexus)* occurs in the Middle East and Europe, and will attack other birds and mammals when their pigeon hosts are not available.

Ornithodoros There are more than 110 species of *Ornithodoros*, most relatively large, which parasitize reptiles, birds, and mammals. *Ornithodoros savignyi*, the eyed or sand tampan, is widely distributed from Africa to Sri Lanka. It is an outdoor tick with eyes, unlike most argasids, inhabiting loose sand in arid regions where animals rest, such as under bushes or trees. The notorious "*Ornithodoros moubata*" (Figure 4.4j) is a species complex of probably four species. *Ornithodoros moubata moubata* is anthropophilic, inhabiting the soil, cracks, and crevices of adobe huts in Africa. In a similar manner to triatomine bugs, the ticks are easily transported between houses in people's possessions. Larvae molt without a bloodmeal to nymphs. By contrast, *O. moubata porcinus* inhabits the burrows of wart-hogs, porcupines, and antbears. Both species have wild and domestic populations, and it is the domestic ones that transmit *Rickettsia duttoni* (tick borne relapsing fever) to humans and African swine fever to pigs. In the New World, species of the genus *Ornithodoros* (*Ornithodoros hermsi*, *Ornithodoros parkeri*, and *Ornithodoros turicata*) are vectors of three homonymous *Borrelia* species, causing tick-borne relapsing fever (TBRF).

Otobius There are only two species in this genus: *O. megnini*, the spinose ear tick, originated in the New World from where it has spread to India and Africa, becoming a serious pest of horses and cattle. It is a one-host tick. The larvae enter the ear of domestic mammals, rabbits, birds, primates, and humans, where they feed and molt into spinose nymphs. The nymphs will feed for several months before

detaching and molting into nonfeeding adults. The females oviposit without feeding (autogeny).

4.2.3.9 Tick-Borne Diseases

An important consequence of tick bites can be tick paralysis, caused by neurotoxins in the saliva of many tick species, both hard and soft ticks. Tick paralysis mainly affects motor pathways by blocking neuromuscular junctions and can be fatal. The most severe disease is caused by *Ixodes holocyclus* in Australia, leading to cardiogenic pulmonary edema in humans and animals. In North America, *D. andersoni* and *D. variabilis* are the most common causes of paralysis.

Ticks transmit a range of pathogens (viruses, bacteria, etc.), protozoa, and some are intermediate hosts of nematodes. The impact of tick-borne pathogens has increased significantly over the past few decades due to growing awareness of the diseases, improved diagnosis, increased contact through recreational activities, and changing distributions as a consequence of climate change. The acquisition of tick-borne diseases is increasing more in North America than Europe.

More than 100 arboviruses have been isolated from ticks, several are of public health and veterinary importance.

Tick borne encephalitis (TBE) is a viral infection caused by tick-borne encephalitis virus (Flaviviridae) related to the yellow fever virus. The disease is found in north Eurasia. Vectors are *I. ricinus* in the western areas and *I. persulcatus* in Russia and the east, and *I. scapularis* in America. The disease can affect the brain, meninges, or both (meningoencephalitis). Mortality is 1–2% and serious handicaps can remain as a consequence of infection. Viruses are maintained in enzootic foci by transovarial transmission.

Louping ill of sheep is caused by another *Flavivirus,* and as yet there is no cure for the disease but vaccination is available for people.

Crimean-Congo hemorrhagic fever (CCHF) is caused by a *Nairovirus* (Bunyaviridae) in steppe, savanna, and semideserts in Asia, Africa, and Europe up to a northern latitude of 50°. It is transmitted by *Hyalomma* spp. between small mammals and *Boophilus* between cattle. Transmission to humans is by aggressively host-seeking *Hyalomma* spp. and epidemics are more common when tick populations are high following mild winters.

Ticks transmit several groups of bacteria, the most important being the Gram-negative, intracellular rickettsiae and spirochaetes of the genus *Borrelia*.

RMSF, caused by *R. rickettsi,* occurs in North America. It is transmitted by the dog tick *D. variabilis*, the wood tick *D. andersoni,* and the brown dog tick *R. sanguineus*. The disease has increased during the last decade, from less than two cases per million persons in 2000 to more than six cases per million in 2010. It can be severe or even fatal unless treated with antibiotics. Two other tick-borne *Rickettsia* species occur in North America, *Rickettsia parkeri*, transmitted by the Gold Coast tick *Amblyomma maculatum*, and *Rickettsia* species 364D transmitted by the Pacific coast tick *Dermacentor occidentalis.*

Boutonneuse fever (South African tick typhus, etc.) occurs around the Mediterranean basin, in Africa, and S.W. Asia, and is caused by *R. conori*. It is transmitted by *A. hebraeum* and *R. sanguineus* and in southern France at least is strongly associated with rabbits, cases falling dramatically when rabbit populations crash.

Siberian tick typhus caused by *R. siberica* occurs from the eastern seaboard of Russia to Armenia in the west. It is transmitted by *Dermacentor* and *Hyalomma* species and the Rickettsiae are trans-standial and transovarially transmitted within the tick population. Similar rickettsial infections are *Rickettsia slovaca* causing tick-borne lymphadenopathy (TIBOLA), transmitted by Dermacentor and *Haemaphysalis* species and *Rickettsia helvetica*, transmitted by *I. ricinus*.

Lyme disease or **Lyme Borreliosis** is an extremely aggravating, systemic infection caused by *Borrelia* spirochaetes. A total of 12 of the 36 species cause Lyme borreliosis; *Borrelia burgdorferi sensu stricto* (=in the strict sense) in North America, and the genospecies *Borrelia burgdorferi afzelii* and *Borrelia burgdorferi garinii* in Europe. *I. ricinus* in the main vector in Europe while in North America the vectors are the black-legged tick *I. scapularis* and the western black-legged tick *I. pacificus*. In highly endemic areas up to 40% of ticks are infected, adults more so than nymphs, and nymphs more than larvae, indicating that transovarial transmission does not often take place. Reservoirs are rodents and birds, including the white-footed mouse (*Peromyscus*) in North America, where almost all mice are infected in enzootic areas. The increase in the number of cases in North America and Europe has been connected to the rise in the number of deer, which although they are not reservoirs of *Borrelia*, they significantly increase tick populations and bring the ticks closer to humans. In the United States, it was the sixth most common Nationally Notifiable Disease in 2011, concentrated mainly in the Northeast and upper Midwest. In Europe, 60 000 – 100 000 people are infected with this disease every year. After a tick bite, 3 – 6% of people will develop an infection, usually starting with a skin rash called erythema migrans or bull's-eye rash; a characteristic red ring 5 – 7 cm in diameter with a smaller red patch centrally. Fever, headache, fatigue, and even depression can follow leading to joint, heart, and central nervous system symptoms. As a chronic disease, it will last for decades. Quick removal of the tick and appropriate antibiotics will limit the disease. No licensed vaccine is available.

Tick Borne Relapsing Fever (TBRF), like Lyme disease, is caused by *Borrelia* species, but the vectors are soft ticks of the genus *Ornithodoros,* not hard ticks. *Borrelia duttoni*, transmitted by the African *O. moubata*, is responsible for the relapsing fever found in central, eastern, and southern Africa. In the United States, relapsing fever is caused mainly by *B. hermsii* and additionally by *Borrelia parkeri* and *Borrelia turicatae*. Vectors are the homonymous *Ornithodoros* species *O. hermsi*, *O. parkeri*, and *O. turicata*. A Lyme disease-like infection in the south-central and southeast US is probably caused by *Borrelia lonestari*, transmitted by *A. americanum*.

Anaplasmosis (human granulocytic ehrlichiosis) is a rickettsial infection of neutrophils caused by *Anaplasma phagocytophilum* and transmitted by *I. ricinus.* The role of rodents, ungulates, and birds as reservoirs for the human strain is not clear. No fatal cases have been recorded in Europe, but the disease may still be largely unrecognized. In the United States, the disease increased from 1.4 cases per million in 2000 to 6.1 cases per million in 2010. Also belonging to the Anaplasmataceae are three *Ehrlichia* species, which occur in south, central, and eastern US: *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, and a species provisionally called *Ehrlichia muris*-like, all transmitted by the lone star tick *A. americanum*. Reports on the disease increased steadily since it became reportable in 2008. A new pathogen was detected in the Norway rat and *Ixodes ovatus* in Japan, which is still termed "Candidatus *Neoehrlichia mikurensis*" (a provisional status applied for incompletely described prokaryotes, not yet formally recognized by the International Code of Nomenclature of Bacteria). The disease was since found in humans, dogs, and small mammals in many parts of Europe and the Far East.

Q-fever is caused by *Coxiella burneti* and occurs worldwide in many domestic mammals and humans transmitted by ixodid ticks. It can be transmitted by *D. reticulatus*, but more often by inhalation and by contact with substances of infected animals.

Tularemia is caused by *F. tularensis.* It is a disease of rabbits, hares, rodents, and humans in North America. It is transmitted not only by *D. variabilis, D. andersoni*, and *A. americanum,* but also by various insects.

Ticks transmit two important protozoal diseases, both of which affect livestock:

Babesiosis, occurring in a broad range of climate zones and causing losses in cattle and other livestock, is transmitted mainly by *Rhipicephalus*. American babesiosis is caused by *Babesia microti* in parts of the Northeast and upper Midwest, and is transmitted by *Ixodes scapularis,* usually by the nymphs. This protozoan infects rodents and humans (see Section 2.6.4.1).

Theileriosis or East Coast Fever is caused by the piroplasm *Theileria parva* and transmitted to Africa by *R. appendiculatus*. The parasite is extremely pathogenic to cattle and has had a profound effect on the agricultural development of Africa. It has wild mammal reservoirs (see Section 2.6.4.2).

4.2.4
Actinotrichida (= Acariformes)

The second major group of the Acari has actinopilin, a birefringent material, in their cuticle, only visible in polarized light. One large group of mainly soil-dwelling mites, the Oribatida, are intermediate hosts of the cestode family Anoplocephalidae (see Section 3.1.2.8).

4.2.4.1 Prostigmata = Actinedida = Trombidiformes

- Very heterogenic, nonmonophyletic group.
- Mostly free-living.
- Some are ectoparasites, rarely endoparasites of vertebrates and invertebrates.
- Setae often plumed.
- Developmental stages with six-legged larva, deuto- and tritonymph, adult. Life cycle often with abbreviations.

***Demodex* – Face Mites** The family Demodecidae involves minute mites living on and in the skin of mammals, including humans. Some have been reported on internal skin, for example, tongue, esophagus, and nasal passages. It is possible that every mammal species possesses its own species of *Demodex*, if not several on different parts of the body. The tiny mites look completely different to other mites, being elongated and slender with extremely short legs and a finely annulated, worm-like body.

Two *Demodex* species occur on humans, mainly on the face. *Demodex folliculorum* inhabits the hair follicles, while *Demodex brevis* lives in sebaceous glands. The females of *D. folliculorum* measure only 430 × 52 µm (Figure 4.9a,b) and *D. brevis* 250 × 50 µm (Figure 4.9c). Mites feed by pushing their needle-like cheliceral digits through the epithelium and sucking out the contents of the underlying cells. The two mites are common, inhabiting 60–80% of people, usually without any clinical signs, although they have been associated with folliculitis and blepharitis (inflammation of hair follicles and eyelids, respectively). Similarly, infestations in other mammals are asymptomatic, although *Demodex canis* can occasionally cause skin conditions and alopecia (loss of hair) in dogs (Figure 4.9d).

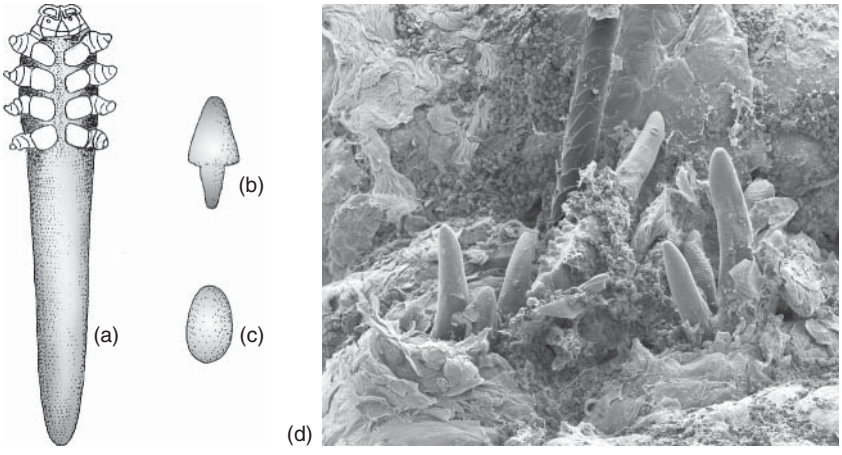


Figure 4.9 Acari, Prostigmata. (a) Female *Demodex folliculorum*, ventral view. (b) Egg of *D. folliculorum*. (c) Egg of *D. brevis*. (d) *D. canis* sticking head first within a hair follicle from the skin of a dog. (Image: Courtesy of Eye of Science.)

4.2.4.2 **Trombiculidae – Harvest Mites, Chiggers**

The larvae of trombiculid mites are known as harvest mites, chiggers, or scrub itch mites and cause intensely itchy reactions and dermatitis in their hosts (trombidiosis). In Europe, *Neotrombicula autumnalis,* which parasitizes a range of birds and mammals, will attack humans at harvest time in the early autumn – hence its colloquial and scientific name. In the Americas, *Trombicula alfreddugesi,* the American chigger, attacks humans. Trombiculids have an unusual life cycle in that the six-legged larvae are parasitic on terrestrial vertebrates, but the eight-legged deutonymph and adult are free-living predators in the soil and known as velvet mites on account of their bright red and velvet appearance. The stages protonymph and tritonymph are immobile resting stages (Figure 4.10). After hatching, the larvae climb up grasses and await a passing host and migrate to the skin around the ears in rodents and eyes in birds. On humans, they congregate where clothing is tight. During initial feeding, a feeding tube (stylostome) is formed from salivary secretions, which anchors the larva to the skin and enables it to feed continuously for up to several days, depending on species. The larvae feed on liquefied tissue

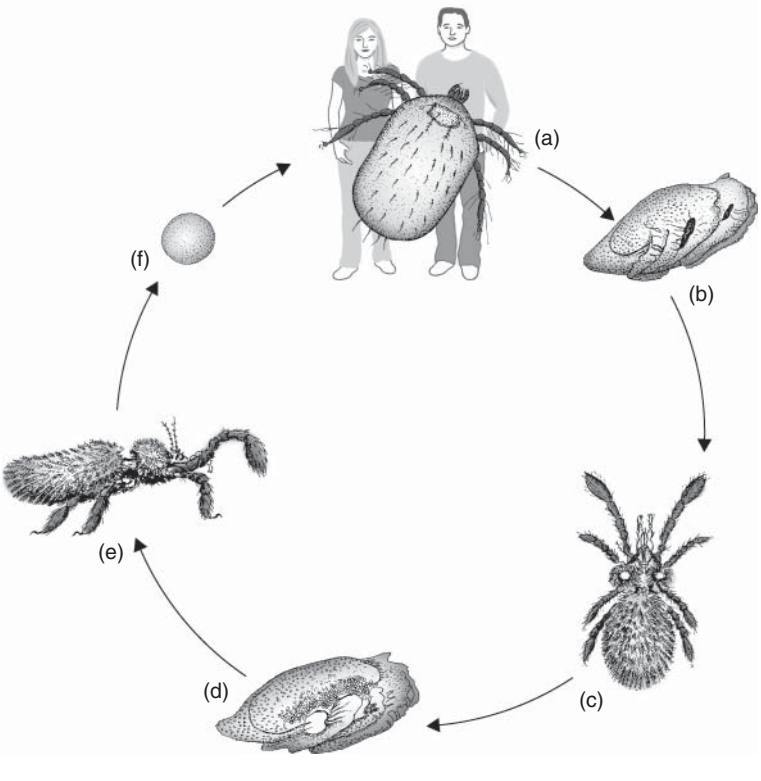


Figure 4.10 Life cycle of *Neotrombicula autumnalis*. (a) Replete larva (chigger) on humans. (b) Hypobiotic protonymph. (c) Free-living deutonymph. (d) Hypobiotic tritonymph. (e) Adult mite. (f) Egg. (According to Jones, B.M. (1951) Parasitology 41, 229–248.)

digested by their saliva. They do not feed primarily on blood. In the tropics, development takes about 40 days, but in temperate regions there is only one generation annually.

From East and Southeast Asia down to Indonesia and northern Queensland and some Melanesian islands, species of *Leptotrombidium* – mainly *Leptotrombidium deliense* – transmit scrub typhus or tsustugamushi disease caused by the rickettsiae *Orienta (=Rickettsia) tsutsugamushi*. The chigger acquires the infection from rodents living in the margins between forests and grasslands or scrub and once infected passes it to the next generation by transovarial transmission. Thus, the mites are the main reservoir of the infection in an area.

4.2.4.3 Astigmata = Acaridida = Sarcoptiformes

- Mostly free-living.
- Stigmata missing.
- Often with sexual dimorphism.
- Two groups: Acaridia free-living or associated with arthropods, and Psoroptidia, ectoparasitic on birds and mammals.

The Astigmata constitute a fairly uniform group of mites without obvious shields and without stigmata or tracheae. The deutonymph is often a phoretic stage, termed the hypopus. One group, the Psoroptidia (with the exception of the notorious free-living house dust mite, *Dermataphagoides*) are almost exclusively harmless ectoparasites living in the plumage and fur of birds and mammals. Occasionally, they aggravate their hosts such as *Psoroptes ovis*, the sheep scab, causative agent of the psoroptic mange of sheep, *Chorioptes bovis* of cattle (both of them highly contagious) and *Otodectes cynotis*, the ear mite of dogs and cats.

***Sarcoptes* – Scabies Mites** The family Sarcoptidae involves mites that live in the skin of birds and mammals and spend their entire life in burrows in the skin. They are globose with finely striated cuticle and cutting chelicerae (Figure 4.11). They cause scabies or sarcoptic mange in humans and other mammals and "scaly leg" in birds. The itch mite *Sarcoptes scabiei* occurs on a wide variety of hosts causing scabies when populations are large. Different strains of *S. scabiei* occur in various domestic and wild animals (e.g., pig, dog, cattle, and fox). Some of the morphologically very similar strains are highly host-specific, others can also infect humans. The infection can result in severe disease, with formation of bark-like skin alterations and total loss of hair.

About 1 h after copulation, the minute female (300–400 µm) starts to burrow into the upper epidermis at a rate of 0.5–5 mm per day with their chelicerae and legs. The burrows, about 1 cm long and parallel to the surface, are air-filled. One to two eggs are produced per night, which block the tube so that the hatching larvae have to dig to the outside. Larva, protonymph, tritonymph, and young adults move on the skin for a very short time before making shallow burrows, in which they stay until the next molt. The entire cycle takes 10–14 days. The life span of the mites is 30–60 days, during which a female will lay 40–50 eggs.

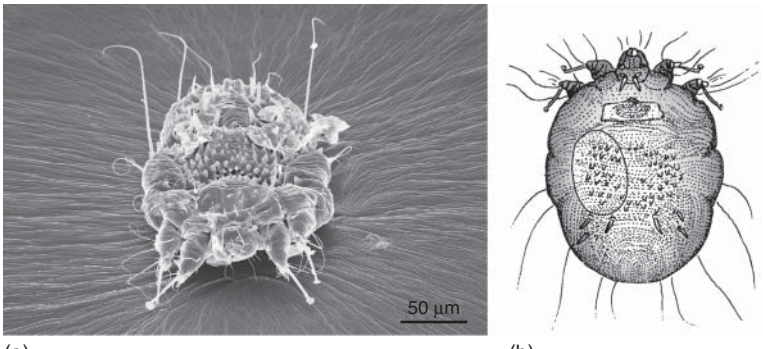


Figure 4.11 (a) *Sarcoptes scabiei* var. *suis*, frontal view from above. Some bristles on the back lost. (EM Image: Department of Molecular Parasitology, Humboldt Universität.) (b) Female *Sarcoptes scabiei* with egg, dorsal view.

Predeliction sites in humans are the inner face of fingers and wrist, as well as armpit, abdomen, and penis; in children, they are the palms of the hand and soles of the feet. The symptoms are severe itching with pruritic, erythematous, papular, and vesicular lesions. Secondary bacterial infections are common. Typically, itching starts 4 weeks after establishment of the mites. Their population dies off after about 100 days, leaving behind potential lifelong immunity. If occasionally a new infection occurs, the preceding sensitization causes heavy itching after only 1–3 days. In this case, hypersensitivity due to cellular immunity leads to such a strong production of lymph that the burrows are flooded and the mite population drowns. There is a theory that pandemics occur globally every 20 years, when the number of young susceptibles is sufficiently high.

Scabies is contagious only by prolonged and close physical is not transmitted by a transient touch or by the considerable amount of fomites produced (cases of crusted scabies excluded). Epidemiologically, the most important routes of transmission are intrafamilial ones, sexual ones among adults, and in long-term care facilities. As for diagnosis, the only reliable method is the proof of live mites, by either skin scrapings or a needle probe. Therapy is possible with certain acaricides.

Further Reading

Barker, S.C. and Murrell, A. (2004) Systematics and evolution of ticks with a list of valid genus and species names. *Parasitology*, **129**, 15–36.

Currier, R.W., Walton, S.F., and Currie, B.J. (2012) Scabies in animals and humans: history, evolutionary perspectives, and modern clinical management. *Ann. N.Y. Acad. Sci.*, **1230**, E50–E60.

Dabert, M., Witalinski, W., Kazmierski, A., *et al*. (2010) Molecular phylogeny of acariform mites (Acari, Arachnida): strong conflict between phylogenetic signal and long-branch attraction artifacts. *Mol. Phylogenet. Evol.*, **56**, 222–241.

Dantas-Torres, F. (2010) Biology and ecology of the brown dog tick, *Rhipicephalus sanguineus*. *Parasites Vectors*, **3**, 26.

Dobson, A.D.M. and Randolph, S.E. (2011) Modelling the effects of recent changes in climate, host density and acaricide treatments on population dynamics of *Ixodes ricinus* in the UK. *J. Appl. Ecol.*, **48**, 1029–1037.

Fischer, K., Holt, D., Currie, B., and Kemp, D. (2012) Scabies: important clinical consequences explained by new molecular studies. *Adv. Parasitol.*, **79**, 339–373.

Guglielmone, A.A., Robbins, R.G., Apanaskevich, D.A., *et al.* (2010) The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world: a list of valid species names. *Zootaxa*, **2528**, 1–28.

Jones, B.M. (1951) The growth of the harvest mite, *Trombicula autumnalis SHAW – Parasitology* **41**, 299–248.

Roy, L., Dowling, A.P.G., Chauve, A.M., and Buronfosse, T. (2009) Delimiting species boundaries within *Dermanyssus* Dugès, 1834 (Acari:Dermanyssidae) using a total evidence approach. *Mol. Phylogenet. Evol.*, **50**, 446–470.

Tree of Life Web Project http://tolweb.org/ (accessed 28 April 2016).

Test Questions

1. Which well-known organisms (pests) are contained within the Acari?
2. To which higher-ranking group of arthropods do the Acari belong?
3. How are they distinguished morphologically from this group?
4. How are they distiguished biologically from this group?
5. Which developmental stages (instars) occur in the life cycle of Acari?
6. Which part of the host body is usually inhabited by Acari?
7. What is the causative agent of a devastating disease of bees?
8. Which Acari inhabit the face of humans?
9. What is a hypopus?
10. What is mange?
11. Is there something like mange in humans, and which is the causative agent?
12. Where and how does this agent live?
13. What is the morphological difference between ticks and other Acari?
14. What is the biological difference between ticks and other Acari?
15. Name two (or even three) important tick families.
16. Name some tick-borne diseases occurring in the United States.
17. By which ticks are they transmitted? (The genus is enough.)
18. What is the medical importance of this tick?
19. Which parasites of economic importance are transmitted by ticks in which parts of the world?
20. What is the habitat of the second of the two tick families?

4.3 Crustacea

- Subphylum of the Arthropoda, water-bound with the exception of Isopoda.
- Majority marine and free-living.
- Biramous appendages on head, thorax, and abdomen.
- Two pairs of antennae, the first one uniramous.
- Nauplius as first larva common to all crustaceans.

The Crustacea (>67 000 species) are primarily marine (crabs, lobsters, barnacles, shrimps), but some groups occur in freshwater and the Isopoda on land. The group contains parasites of marine and freshwater invertebrates and vertebrates (fish and amphibians). They are intermediate hosts of some important parasites such as the lung fluke *Paragonimus westermani* in freshwater crabs and crayfish as well as the tapeworm *Diphyllobothrium* and the well-known Guinea worm, *Dracunculus medinensis* in freshwater copepods such as *Cyclops*.

Crustaceans are characterized by two pairs of antennae, a pair each of mandibles and maxillae, and at least five pairs of appendages. The more advanced forms have three body parts: head, thorax, and abdomen. The head and thorax are often fused to a cephalothorax and covered by a large carapace. The hard exoskeleton protects the animal and has to be molted as in all arthropods. The many segmental appendages vary hugely in number and form between groups. There are several larval stages, which also vary greatly in number and form between the different crustacean groups. The only larva common to all groups is the first, the nauplius, which has one unpaired eye and three pairs of swimming legs.

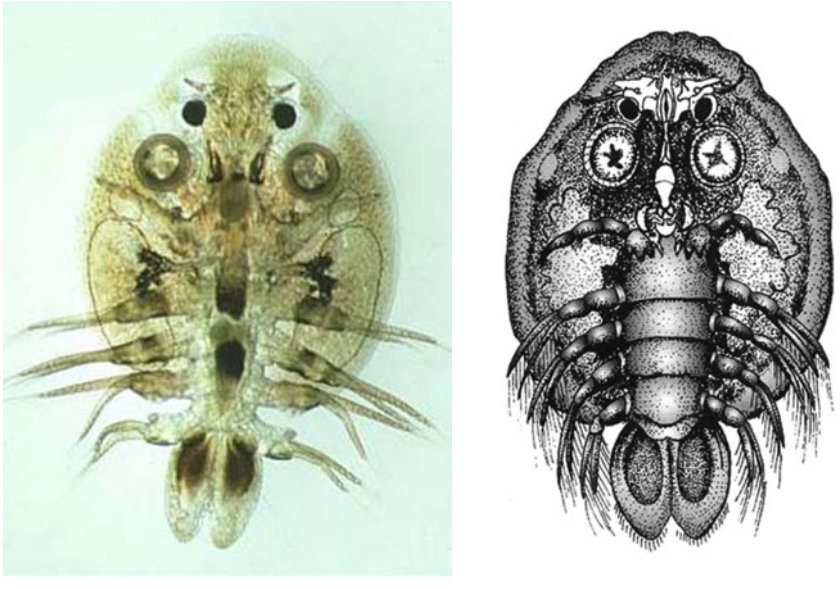
Adaptation to a parasitic lifestyle varies from ectoparasites, which very much resemble free-living forms, to endoparasites with strongly reduced or completely lacking appendages in crustaceans. Here, we present two examples, the ectoparasite *Argulus* (fish louse) and the truely extraordinary *Sacculina*, which is only recognizable as a crustacean in its nauplius larval stage.

4.3.1
Argulus foliaceus

Parasitic copepods of fish (fish lice) have become well known recently with the increase in fish farming, especially of trout and salmon. The genus *Argulus* is found throughout the world and belongs to a small group of freshwater ectoparasites, the Branchiura, containing about 150 species. The common fish louse or carp louse *Argulus foliaceus* (Figure 4.12) is native to temperate regions of Europe but is also common in aquarium and pond fish worldwide and has become a real pest. It is found mainly on carp, especially on the fins and around the fin bases, where it feeds on mucus and sloughed-off scales or pierces the skin and feeds on blood. *Argulus japonicus* was introduced into Europe and *Argulus appendiculosus* occurs in North America. *Lepeophtheirus salmonis* is a major pest in salmon farming.

Fish lice suck blood on the gills and the base of the fins. Adult females leave the host every few days to lay up to 1000 eggs on submerged solid surfaces in two to four rows. After 6 days, the first larva attaches to a host and molts to a larva sometimes called the metanauplius. It grows by several molts, but without metamorphosis, that is, without changes in its morphology. It takes 34–80 days from hatching to adult.

A. foliaceus is dorsoventrally flattened and measures $3–7 \times 2.5–5$ mm, with females larger than males. The cephalothorax is covered by a large disk-shaped carapace. There are two pairs of antennae and a pair of eyes. The first maxillae are transformed into large, round, and movable suckers. Between the eyes and the



(a) (b)

Figure 4.12 *Argulus foliaceus*. ((a) dorsal view. Image: Coutesy of Chris Williams. (b) Ventral view.)

"suckers," a labrum (upper lip) and labium (lower lip) form a mouth tube, from which a stylet can be everted. Presumably, the stylet is used to deliver a toxin that helps break down the host's epithelial cells or induces hemorrhaging. The thorax bears four swimming legs. The posterior body, the urosome, consists of rounded lobes that are covered with small spines on its margin. *Argulus* is hardly ever a problem in nature, but has been reported to wipe out trout populations and cause problems in carp farming. Symptoms include abnormal swimming, rubbing, and deteriorating physical condition. Because of the tissue damage caused by the parasite, secondary bacterial and fungal infections frequently occur.

4.3.2 *Sacculina carcini*

Sacculina species are barnacles (Cirripedia) of the group Rhizocephala, with adult females mainly consisting of a network of thread-like extensions penetrating the body of decapod crustaceans. It is only the nauplius, the first instar larva that gives clues to the identity of this parasite as a crustacean. *Sacculina carcini* is a parasitic castrator of the shore crab *Carcinus maenas,* where up to 50% of crabs can be infected.

The free-living nauplius larva molts into a second-stage cypris larva, which attaches itself to a host's antenna and searches for a joint in the exoskeleton. It then sheds its thorax and legs (Figure 4.13), while its inner structures are

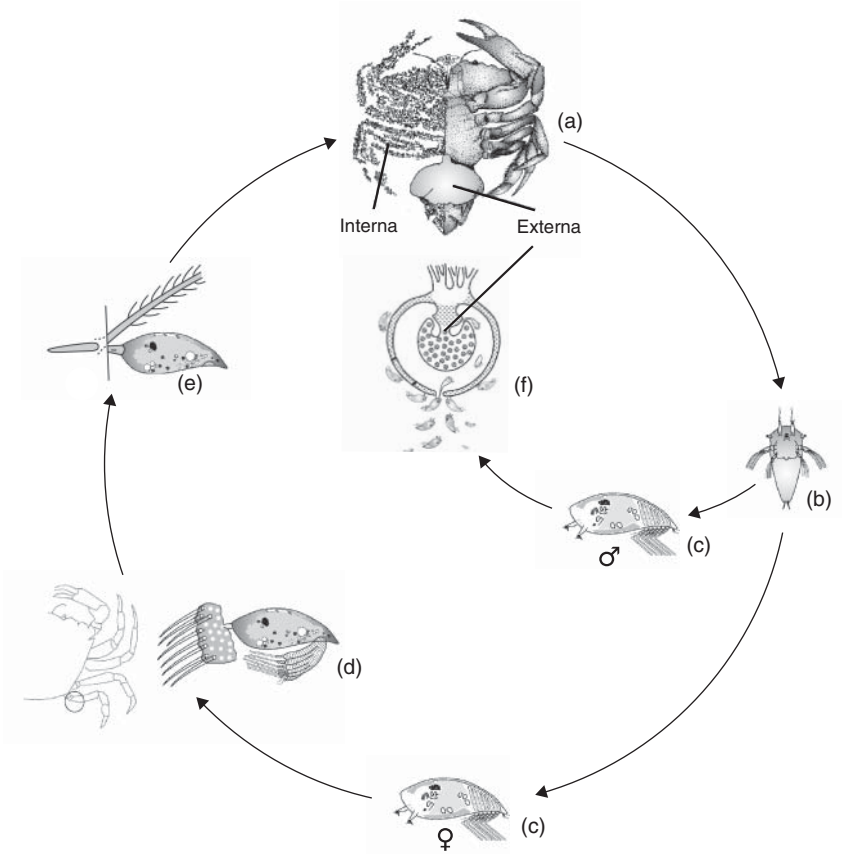


Figure 4.13 Life cycle of *Sacculina carcini*. (a) Infected crab with interna and externa. (b) Nauplius larva. (c) Cypris larva. (d) Cypris larva with developing kentrogon. (e) Kentron penetrates basis of a crab's bristle and cells ("vermigon") are injected. (f) Young externa fertilized by male cypris larvae.

disaggregated and a new stage develops, the kentrogon, which contains a hollow spine, the kentron. This is bored into the host's cuticle and cells from the kentrogon are injected into the host. These cells grow to form a "vermigon" and then root-like structure called the "interna," wrapping around the internal organs before spreading into the abdomen of the crab. Here it breaks through the cuticle and appears as conspicuous yellowish-white sac-like bulk, the externa under the tail flap. Microscopic male cypris stages burrow into the "externa" and fertilize the female. As the externa resides at a position where the female crab carries its own eggs, it is not recognized as a foreign body but as its own egg mass and therefore triggers brood care by the parasitized crab. Male crabs with *S. carcini* are feminized by the parasite's influence on crab hormones, so that they behave like females carrying eggs. When the *Sacculina* nauplii are ready to be released,

the crab exhibits the same behavior as it would when releasing its own eggs of vibrating up and down and paddling the water (Figure 4.13).

Further Reading

Glenner, H., Høeg, J.T., O'Brien, J.J., and Sherman, D.T. (2000) Invasive vermigon stage in the parasitic barnacles *Loxothylacus texanus* and *l. panopaei (*Sacculinidae*):* closing of the rhizocephalan life-cycle. *Mar. Biol.*, **136**, 249–257.

Høeg, J.T., Maruzzo, D., Okano, K., *et al.* (2012) Metamorphosis in balanomorphan, peduculated and parasitic barnacles: a video-based analysis. *Integr. Comp. Biol.*, **52**, 337–347.

Johnson, S.C., Treasurer, A.W., Bravo, S., *et al.* (2004) A review of the impact of parasitic copepods on marine aquaculture. *Zool. Stud.*, **43** (2), 229–243.

Torrissen, O., Jones, S., Asche, F., *et al.* (2013) Salmon lice-impact on wild salmonids and on salmon aquaculture. *J. Fish Dis.*, **36**, 171–194.

Walker, P.D. (2008) Argulus*, the Ecology of a Fish Pest*, Radboud Repository, Nijmegen.

Test Questions

1. What is *Argulus foliaceus* and what are its hosts?
2. What does *A. foliaceus* feed on?
3. What is the evidence for *Sacculina carcini* being a member of the crustacea?
4. What is the host of *S. carcini*?
5. What is visible of *S. carcini* on the crab?

4.4
Insecta

- Body (usually visibly) divided into head, thorax, and abdomen.
- Head with one pair of antennae, mandibles, and maxillae.
- Three pairs of uniramous legs on thorax.
- Often two pairs of wings (on meso- and metathorax).
- Development as either incomplete or complete metamorphosis.

The insects are members of the superclass hexapoda, that is, arthropods with three pairs of uniramous legs. The Hexapods also include the primarily wingless classes of Collembola (springtails), Protura, Diplura, and Archaeognatha. The huge class of insects (>1.8 million species) possess wings on the thorax that can be lost secondarily. There are several aspects of insect biology that have made them so successful, some of these are typical of all arthropods, such as the presence of an exoskeleton, but other features, especially the development of flight are unique to insects.

Metamorphosis is a dominant feature of the insects and is of two types: incomplete (hemimetabolous) and complete (holometabolous). In the former, the egg hatches into an immature stage, usually referred to as a nymph. The nymph superficially resembles the adult, although it is not sexually mature and may have wing buds but not functional wings. Most importantly, the nymphs are usually found in the same place as the adults and feed on the same type of food. In the case of

bloodsucking insects (e.g., lice and triatomine bugs), this indicates that all stages are potentially involved in the transmission of pathogens and parasites. In insects with a holometabolous metamorphosis, the egg hatches into an immature stage, usually called a larva, which grows and eventually molts into a nonfeeding, transitional stage, the pupa, which then transforms into the adult. The larva lives in a completely different habitat to the adult and consequently has a fundamentally different diet.

Systematics The phylogeny and hence classification of the Hexapoda, the six-legged animals, is still being actively researched and there is no universally accepted approach. The first hexapods were the wingless Apterygota (Collembola, Protura, Diplura, Archaeognatha) with no relevance for parasitology. The main groupings, the Pterygota, have wings and include species that feed on vertebrate blood or parasitize vertebrates. Some contain groups which are parasitic on other arthropods, such as in the Diptera (two-winged flies) or Hymenoptera (bees, ants, and wasps). Most of these, especially the numerous parasitic wasps, are termed parasitoids rather than "parasites," because they kill their host. They are not dealt with in this book. The first Pterygota are the Palaeoptera, which lack the ability to fold the wings back over the abdomen (Ephemeroptera (mayflies) and Odonata (dragonflies or damselflies). They are followed by the Neoptera (Table 4.3),

Table 4.3 Selected members of the Neoptera.

	Other scientific names and vernacular names
Exopterygota[a)]	Hemipteroid Assemblage, thrips, lice
Psocoptera	Psocodea, lice
Amblycera	Chewing lice, part of the former, Mallophaga
Anoplura	Biting lice, Phthiraptera
Rhynchophthirina	Elephant lice, not dealt with here
Ischnocera	Chewing lice, part of the former, Mallophaga'
Hemiptera	Bugs
Heteroptera	True bugs
Reduviidae	Kissing bugs
Cimicidae	Bedbugs + bat bugs
Polyctenidae	Bugs of bats, not dealt with here
Endopterygota[b)]	Holometabola
Siphonaptera	Aphaniptera, fleas
Coleoptera	Beetles
Strepsiptera	Twisted-wing parasites (not dealt with here)
Hymenoptera	Ants, bees, wasps (no vernacular name)
Diptera	True flies
Trichoptera	Caddis flies
Lepidoptera	Butterflies

Groups containing major parasites in bold letters.
a) Externally developing wings.
b) Internally developing wings.

literally the ones with new wings, actually having wings that can be flexed over the abdomen (with exception of butterflies and moths). The first major division of the Neoptera, the Exopterygota, contains insects with incomplete metamorphosis, among them the thrips (not listed in table), lice, and bugs. The Endopterygota or Holometabola have a complete metamorphosis with pupa in their development.

Morphology Juvenile stages, or nymphs, of hemimetabolous insects resemble adults except for wings and external genital organs. By contrast, holometabolous insects have larvae, which are very different from adults and occur in a great variety of forms. Some have well-developed head capsules (eucephalic), while others, like the familiar "maggot" of some Diptera have no obvious head (acephalic), which is often accompanied by a lack of legs (apodous). Larvae can be polypod with three pairs of thoracic legs and two to five pairs of abdominal prolegs (unsegmented stubs) such as that found in the beetles (Coleoptera) and Lepidoptera (butterflies and moths). Some dipterous larvae have fleshy abdominal stubs, the prolegs (e.g., Simuliidae). The remarkable transformative stage, the pupa, is of three types: obtect pupae have appendages (legs and wings) fused to the body, exarate pupae have appendages free from the body, and coarctate pupae are contained within the exoskeleton of the last larval instar, the puparium (found in the higher Diptera).

The adult head bears compound eyes, mouthparts, and a pair of antennae. The basic insect mouthpart plan is of the chewing type (Figure 4.14a) and consists of an upper unpaired labium, paired mandibles used for masticating, the hypopharynx arising from the base of the labium assisting in the movement of food, and paired maxillae with an inner appendage (lacinia) and outer galea. The floor of the

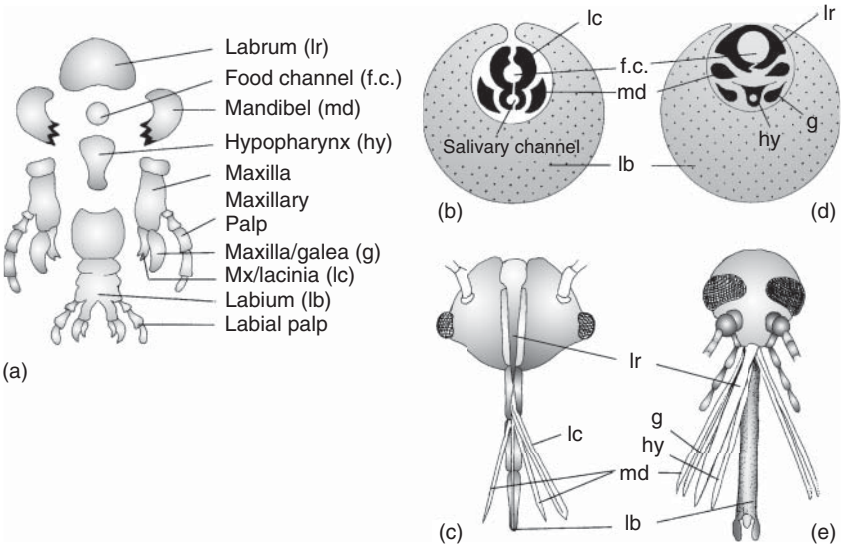


Figure 4.14 Mouthparts of insects. (a) General parts. (b) Bug, cross section. (c) Bug, front view. (d) Mosquito, cross section. (e) Mosquito, front view.

mouth is the labium. Also present are maxillary palps used for "tasting" the substrate. These mouthparts are highly modified in hematophagous insects to form a slender proboscis composed of different parts in various groups but playing a similar role: cutting into the host's skin. For example, in the bugs, the function of piercing and sucking is done by the lacinia, forming two cavities for blood and saliva (Figure 4.14b,c), whereas mosquitoes suck blood with the hollow labrum and inject saliva with the hypopharynx (Figure 4.14d), while the piercing is done with thin mandibles and the maxillary galea (Figure 4.14e). These thin mouthparts are enclosed and protected by a strong labium, which does not penetrate the host's skin.

The thorax is made up from three segments, pro-, meso-, and metathorax, with dorsal plates, the pro-, meso-, and metanotum. Each of the thorax segments carries a pair of legs, which consist of five segments from base to tip: coxa, trochanter, femur, tibia, and tarsus. The meso- and metathorax carry two pairs of wings, where they have not been lost as an adaptation to parasitism (e.g., fleas, lice, and keds). In Diptera, the second pair of wings is modified to form halteres, small knobbed gyroscope-like structures, which inform the insect about the body's position during flight. The abdomen contains the main part of the alimentary tract and genital organs.

Bloodfeeding and Its Consequences Insects feed on blood in two basic ways: poolfeeders, which use toothed, ridged, or spine-tipped mouthparts to tear or cut a wound in the host's skin (e.g., horse flies, midges, sand flies, and blackflies) and ingest the pool of blood, lymph, and cell fluid oozing into the wound. This is also called telmophagy. The alternative approach is used by insects with thin, slender mouthparts, which selectively pierce a capillary and take up blood directly (mosquitoes, tsetse flies). This is called solenophagy.

The gut (see Figure 4.15) is composed of an ectodermal, that is, chitinous, foregut, and hindgut and an endodermal midgut with epithelia, to which parasites adhere in different ways. The lower Diptera (=Nematocera, mosquito relatives) have a crop, which is a bag-like extension of the foregut. Blood ingested by the female goes to the midgut, while sugar-rich nectar is directed to the crop, which acts like a "fuel tank," decanting sugars into the gut as needed.

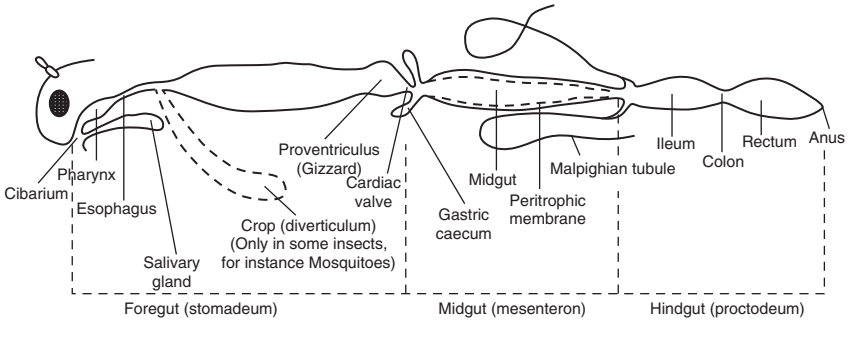


Figure 4.15 Alimentary canal of an insect.

The peritrophic membrane (Greek: *perí* = around, *trophé* = food), better called the peritrophic matrix, is a very thin chitinous layer secreted to protect the gut epithelium and for pelletizing undigested food. It occurs in two forms, one secreted around the bloodmeal by epithelial gut cells in response to a bloodmeal (type 1, e.g., in mosquitoes, blackflies, and sandflies) and the other secreted continuously by a collar of cells at the junction of the foregut and midgut (type 2, e.g., tsetse flies). The peritrophic membrane is a critical structure for many parasites to cross. A peritrophic membrane does not exist in fleas, lice, and most bugs.

Blood is a nutritionally deficient diet, especially in B vitamins, therefore those insects living on blood all their lives, for instance, bugs, lice, bat flies, and tsetse flies, need supplementary nutrients, which are supplied by bacterial symbionts. Symbionts inhabit certain parts of the gut or in specialized cells or masses called mycetomes and are transmitted transovarially to the offspring. Insects that feed as larvae on a different diet to blood, such as mosquitoes, sandflies, and blackflies, do not have symbiotic bacteria as they accumulate sufficient nutrients during their larval life and the adult males imbibe sugars.

Female insects can produce their first batch of eggs without feeding (anautogeny), but more often a bloodmeal is required (autogeny).

During bloodfeeding, hematophagous insects inject a multitude of substances such as anticoagulants, analgesics, vasodilators, and immunomodulators. These products not only have pharmacological effects, but they also induce immune reactions by the host. These responses are rarely protective to the host, because most insects feed for a very short time (mosquitoes, for instance) and certainly less than required for activation of the first immune response. However, those feeding continuously on the same host have mechanisms to avoid host immune responses.

4.4.1 Phthiraptera – Lice

- Obligate, stationary, and strictly host-specific ectoparasites.
- Secondarily flightless.
- Eggs ("nits") are glued to hairs or feathers.
- Three juvenile stages.

Lice are secondarily wingless, ectoparasitic parasites of birds and mammals. The group is not monophyletic and parasitism arose more than once within the order Psocoptera (booklice or barklice). Consequently, the chewing or biting lice (formerly: Mallophaga) are now split into two not closely related groups, the Amblycera and Ischnocera.

Despite their polyphyletic origins, the various groups of lice have many common features. They are hemimetabolous, wingless, and permanent ectoparasites of birds and mammals. Their eggs are oval and have an operculum (a lid) with micropyles (tiny holes) for respiration. During oviposition, the female secretes

an adhesive onto a hair or feather shaft, into which the egg is pressed and which covers the whole egg except for the operculum. There are three nymphal instars and like the adults, these are flattened dorsoventrally. The eyes are reduced or absent, and there is no ocellus. The three segments of the thorax are, in one way or the other, fused. The legs terminate in claws, which can be specialized to fit the host's hair or feathers. The abdomen has nine distinct segments. Symbionts, necessary in arthropods with an exclusively blood diet, are transferred trans-ovarially to the next generation via the eggs.

4.4.2 "Mallophaga" – Chewing Lice

The former Mallophaga or chewing lice consist of two distinct groups, the Amblycera and Ischnocera. All have mouthparts modified for chewing rather than piercing. Their head is as broad as the thorax or broader and the margins of the abdomen are slightly smooth. The eggs have hexagonal surface sculpturing.

Both groups feed on birds and mammals; 57% of the species parasitize mammals and 43% birds in the Amblycera and 13% on mammals and 87% on birds in the Ischnocera. The Ischnocera feed on the downy parts of feathers and soft fur and have marked preference for particular areas of the host. By contrast, the Amblycera roam more freely over the host, feeding on feathers, sebaceous exudates, and sometimes blood with mandibles modified for cutting. Chewing lice are highly species-specific for their hosts and often have specificity for a certain part of the host's body (Section 1.1.4, Figure 10). In order to avoid dislodging, chewing lice, particularly the Ischnocera, cling on to the hosts with their mandibles, which have striations on the inner margin. The striations correspond to the microsculpturing of host's hair or feather shafts. Many bird-inhabiting species are transported from host to host (phoresy) by clinging on to parasitic flies such as the Hippoboscidae (see 4.4.8.14) or less usually other insects (e.g., mosquitoes).

A few Amblycera and Ischnocera are vectors of nematodes (*Eulimdana* spp, Filarioidea) and, in the case of the dog louse *Trichodectes canis*, for the cestode *D. caninum*.

Heavy infestations of chewing lice can be deleterious to their hosts by causing irritation, leading to loss of sleep, restlessness, and eventually reduction in the production of egg or milk by domesticated animals. The main pest species are Amblycera: *Menopon gallinae* (poultry) G*liricola porcelli and Trimenopon hispidum (*both on guinea pig); Ischnocera: *T. canis* (dog), *Felicola subrostratus* (cats); and *Bovicola bovis* (cattle), and *Columbicola columbae* (pigeon).

4.4.3 Anoplura – Sucking Lice

The sucking lice only parasitize placental mammals, they are completely absent in birds. There are about 490 species in nine families, one of them, the

Echinophthiridae, even living on pinnipeds (seals), in their nostrils. As permanent, wingless parasites, physiologically adapted to the blood of their particular host, anoplurans have a strict host-specificity. The boundaries of the host genus are rarely transgressed. The three nymphal stages and the adults have to take a bloodmeal at least once, the adults often several times per day.

Adult Anoplura (Greek: *an* = without, *hóplon* = weapon, *ur* = tail) measure 0.5 × 8 mm. The head is narrower than the thorax, and is cone-shaped in front of the slender antennae (Figure 4.16h). A few species possess eyes. The mouthparts are modified for piercing and sucking and are coiled within the head when not

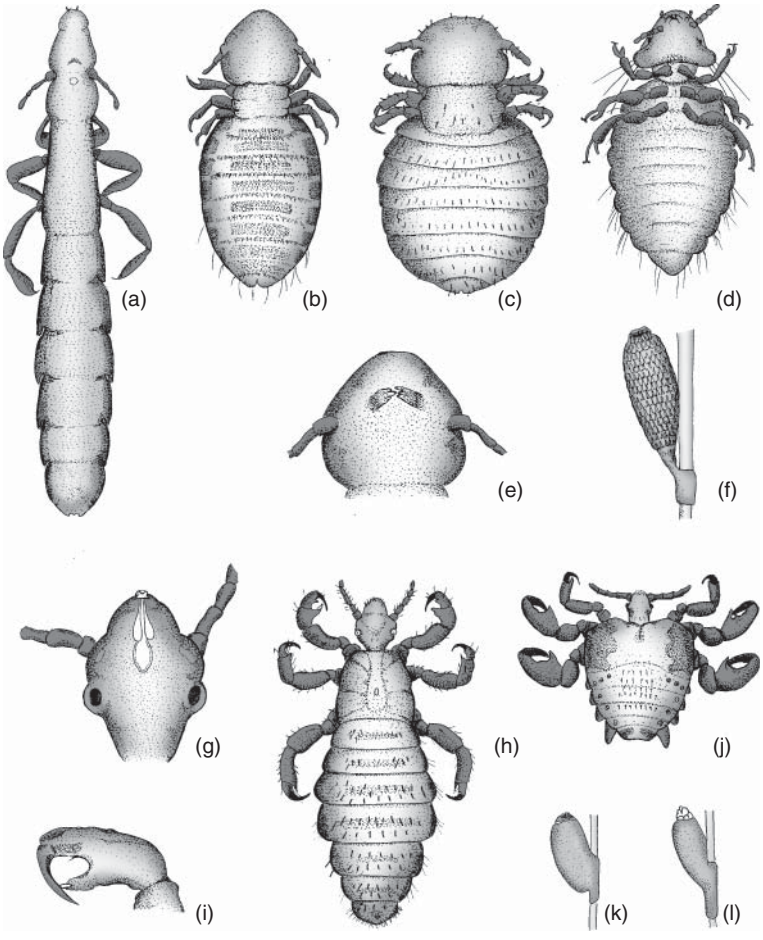


Figure 4.16 Phthiraptera. (a–c) Ischnocera: (a) *Columbicola columbae*. (b) *Bovicola bovis*. (c) *Trichodectes canis*. (d) Amblycera: *Menopon gallinae*. (e) Dorsal view of head of an ischnocera, with mandibles showing through. (f) Egg of *Trimenopon hispidus* of the guinea pig. (g–l) Anoplura. (g) Head of a louse with piercing–sucking mouthparts showing through. (h) *Pediculus humanus*. (i) Claspers of a louse leg, consisting of tibia and a thorn-like tarsus. (j) *Pthirus pubis*. (k) Nit of *P. h. capitis*. (l) Nit of *P. pubis*.

feeding (Figure 4.16g). It is difficult to homologize the three piercing stylets with the mouthparts of other bloodsucking insects. The stylets fit on top of one another, enclosing a food canal and salivary canal. At the start of bloodfeeding, the head conus armed with a ring of hooks is evaginated and pressed onto the skin. Then the three stylets are inserted into the skin, if necessary several times, until a capillary is found.

The three segments of the thorax are fused. The legs are furnished with strong claws, consisting of a spine-like extension of the tibia working against the tarsus to form a claw (Figure 4.16i). The abdomen is joined broadly to the thorax. The posterior end is cone-shaped in the male and bilobed in the female. The dorsal and the ventral sclerites are weakly sclerotized, to enable considerable distension for the bloodmeal. The lateral margings bulge distinctly (in contrast to the chewing lice). Males are slightly smaller than females. The tibial claw of leg I is larger than that of the other legs. Symbionts are housed in a mycetome ventral to the stomach. The white eggs (or nits) are, as those of the Amblycera and Ischnocera, glued singly onto a hair (Figure 4.16k). Their surface is smooth.

Humans are inhabited by three lice: the head louse and body louse, two subspecies (perhaps ecospecies) of *Pediculus humanus*, and the pubic or crab louse (*Pthirus pubis*). The body louse *Pediculus humanus humanus* and the head louse *Pediculus humanus capitis* are able to interbred experimentally, but do not do this in nature. Some consider them to be ectotypes of the same species.

4.4.3.1 *Pediculus humanus capitis*

The head louse (*P. h. capitis*) is on average slightly smaller than the body louse, but measurements overlap. The space formed in the claw by the tibia and tarsus in the claw is so remarkably well adapted to the diameter of a human scalp hair that the head louse cannot fall off. Only direct contact with the head of another person will allow adults (not nymphs!) to move from one host to another. A female head louse produces about 270 eggs during the 30–35 days of its life. The eggs are attached to the hair shaft predominantly on the neck or behind the ears, at a distance of about 1 cm from the skin in cooler climates and 15 cm or more in warm climates. The substance fixing the eggs to a hair consists of proteins similar to hair keratin. It is insoluble in water, so mere washing will not remove the fixed eggs, called "nits." The life cycle takes 17–22 days from egg to egg.

Head lice do not transmit infections, although they can do so experimentally. Damage is mainly caused by scratching of the itching bites and the effect of salivary proteins and other molecules on the host.

There are several myths surrounding infestation with head lice, especially in developed nations; it is not especially connected with unhygienic situations, with immigrants or long hair. Head lice are frequently transmitted in institutions such as nurseries and schools and infestations are highly contagious. There are an estimated 6–12 million children treated per year in the United States alone, carrying a significant financial load. The most effective control is combing with a fine "nit-comb," although topical insecticides are frequently used. Resistance to these insecticides is developing.

4.4.3.2 *Pediculus humanus humanus*

The body louse (*P. h. humanus*) evolved from the head louse 30 000 – 114 000 years ago when modern *Homo sapiens* spread to colder parts of the world, and where an increasing use of clothing developed. Because human body hair is sparse, the body louse cannot live directly on human skin, but instead resides in clothing, only moving on to bare skin to feed. The eggs are laid on clothing, usually along seams. In temperate climates, including the highlands of tropical countries, infestations frequently occur during cold seasons. Historically, infestations with body lice and the diseases caused by them occurred mainly during wars, in overcrowded hospitals, or prison camps, where hygiene standards were low and lice were easily transmitted from person to person. In developed countries, body lice remain a significant problem to homeless people, who cannot change and wash their clothes regularly. Body louse infestations cause stripe-shaped rashes with a postinflammatory brownish pigmentation, swelling, and bacterial inflammation, a condition known as "vagabond's skin."

4.4.3.3 *Pthirus pubis*

P. pubis, the pubic louse or crab louse, lives on hairs with a higher diameter than those inhabited by *Pediculus*, preferentially on pubic hairs and more rarely on hairs of the armpits, chest, or eyelashes (Figure 4.17). It is only found on the host like the head louse, and is transmitted through sexual contact. *P. pubis* is much broader than the head or body louse, 1.5 – 2.0 mm long and flattened, and looks indeed a bit like a crab (Figure 4.16). The thoracic segments are fused and the distal legs are longer and stronger than the proximal ones. Abdominal segments 5 – 8 have lateral cone-shaped appendages, the longest on the last segment. A total of 30 eggs (Figure 4.16) are laid during the 3- to 4-week life span of a female.

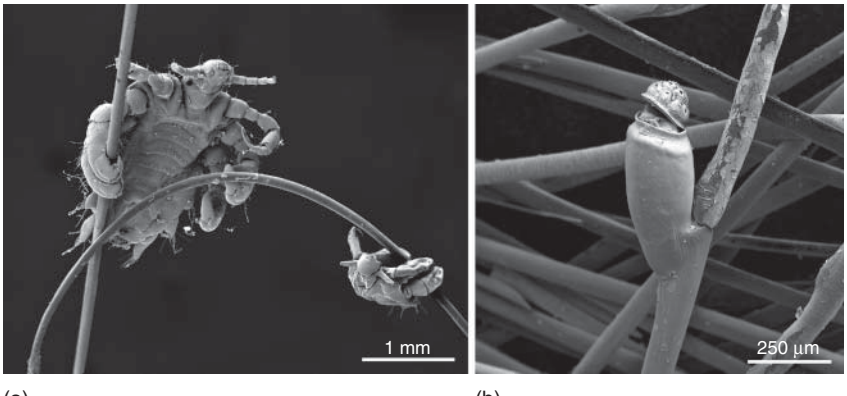


Figure 4.17 *Pthirus pubis* (crab louse). (a) EM Adult and nymph (b) Nit of *P. pubis*. (Images: Courtesy of Eye of Science.)

Other Anoplura: Our nearest relative, the chimpanzee, has its own *Pediculus* species: *Pediculus schaeffi*; but remarkably, we share *P. pubis* with the gorilla. Important lice of domestic animals are *Haematopinus suis* of pigs, *Haematopinus eurysternus* of cattle, *Polyplax serrata* of the white mouse, and *Linognathus setosus* of dogs. All these can cause multifactorial diseases, when they develop massive populations. The second group of the Anoplura, the Rhynchophthirina or elephant lice are not dealt with here.

4.4.3.4 Disease Transmission by Lice

Only the body louse, *P. h. humanus*, transmits diseases in nature:

- The most important disease is epidemic typhus or louse-borne typhus, caused by *Rickettsia prowazekii,* an α-proteobacterium. Humans are the only vertebrate hosts and are infected when louse bites start itching and louse feces containing bacteria are rubbed into the wound (the bacteria are not transmitted during bloodfeeding). *R. prowazekii* remains viable in dried louse feces for many days, and in dead lice for many weeks. The incubation period is 10–14 days. First signs are shivers, fever, aching head and limbs, and even confusion if the brain is infected. Later, a rash appears, blue-spotted at first, then red-spotted from petechial hemorrhages. Untreated, the disease is fatal in about 50% of cases. A subsequent infection takes a milder course due to acquired immunity. The high temperature of the patient during infection induces the lice to migrate to other people. In severely crowded situations, such as military hospitals or prison camps, contaminated blankets and clothing of the dead were transferred to healthy people, exacerbating an epidemic. The Russian campaign of Napoleon became a failure mainly due to mass mortality from epidemic typhus. In World War II, the infection caused high fatalities among soldiers and prisoners in German concentration camps. From 1940 onward, the control of lice with DDT was possible, and the devastating infection slowly vanished. The indicative names given to the disease such as camp fever, jail fever, hospital fever, famine fever, and putrid fever show the significance, the infection once had. At present, epidemic typhus, once diagnosed, can be controlled by antibiotics, leading to its control in most parts of the world.
- Trench fever is also transmitted by the body louse via its feces. It is caused by *Bartonella quintana* (former names: *Rochalimea quintana* or *Rickettsia quintana*) also an α-proteobacterium and occurs worldwide. The symptoms are sudden high fever, severe headache, pain in the back and legs, and a fleeting rash. It lasts only 5 days (quintan fever) and is not fatal. Recovery, although, takes at least a month, and relapses are common. It is often found in urban surroundings among homeless people and alcoholics. The infection can also cause bacillary angiomatosis in those infected with HIV.
- Louse-borne relapsing fever caused by *Borrelia recurrentis* is the third disease transmitted by body lice. The spirochetes reproduce in the hemolymph of the louse, and can be transmitted to humans only by crushing the louse (often by "cracking" between the fingers) and inadvertently rubbing the hemolymph into

a bite or mucous membranes. Louse-borne relapsing fever (different to TBRF caused by *B. duttoni*) occurs in epidemics among people in poor living conditions, famine, and war. It includes severe jaundice, severe change in mental status, severe bleeding, and the danger of heart attack. Mortality rates without treatment are 30–70%. The disease is currently present in Ethiopia and Sudan.

4.4.4 Heteroptera – True Bugs

- Hemimetabolous.
- Five nymphal stages.
- Mostly feeding on plants, some are bloodfeeders on vertebrates.
- Both sexes feed on blood.
- Reduviidae: vectors of *Trypanosoma cruzi* in Latin America.
- Cimicidae, includes the bedbug: no transmission of pathogens.

The Hemiptera, known as "true bugs," is a large order of hemimetabolous insects containing cicadas, leafhoppers, aphids, whiteflies, and others. All Hemiptera have very distinct piercing mouthparts to suck up tissues, mostly of plants, other insects, and, in a few groups, the blood of birds and mammals. One subgroup, the Heteroptera (Greek: *héteros* = another, different, *ptéron* = wing), is characterized by the forewings having the anterior half sclerotized, while the distal part is membranous like the hindwings, and a three-segmented proboscis. This group contains three families of ectoparasites, the Reduviidae (assassin bugs among others), Cimicidae (bedbugs), and Polyctenidae (parasites of bats, not dealt with here) (Table 4.3, Figure 4.18). The mouthparts, composed of two slender stylets (laciniae of the maxilla and the mandibles), ensheathed by the proboscis, are inserted during a bloodmeal.

4.4.5 Triatominae – Kissing Bugs

The Reduviidae (assassin bugs) contain species adapted to hunt and feed on other insects. However, one subfamily the Triatominae (kissing bugs) have become adapted to feed on the blood of terrestrial vertebrates from a nest-dwelling lifestyle, a common evolutionary route for ectoparasites. There are about 120 species of triatomines ranging from 5 to 45 mm long and predominantly occurring in the Americas. They form a relatively homogeneous group, similar in form, biology, and behavior. All species are predominantly bloodsuckers and over half have been shown naturally or experimentally to be susceptible to infection with *Trypanosoma cruzi,* which causes Chagas disease in humans (see Section 2.58). Because of their similar biology, all species in the Americas are potential vectors of disease, although only five species are of public health significance: *Triatoma*

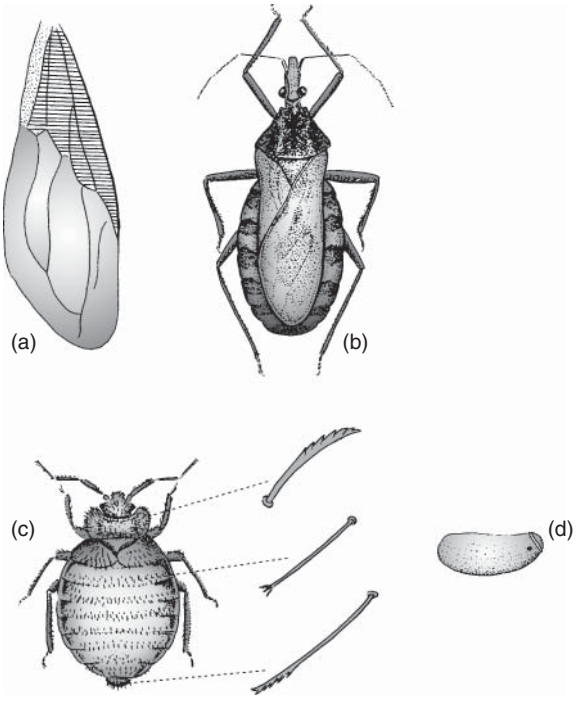


Figure 4.18 Heteroptera. (a) Anterior wing of a bug (corium hatched, clavus shaded). (b) *Triatoma infestans* (Reduviidae). (c) *Cimex lecticularius*, right-hand side: shape of bristles at different parts of the body. (d) Egg of a bedbug.

infestans, Triatoma dimidiata and *Triatoma brasiliensis, Rhodnius prolixus,* and *Panstrongylus megistus*. Of these, *T. infestans* is the most important.

Triatomines have an elongated head with prominent eyes and four segmented antennae. The thorax has a blunt triangular pronotum and small but obvious triangular scutellum (a feature of the Heteroptera). The wings are partly folded over one other and the margins of the abdomen (connexivum) extend from under the wings (Figure 4.18b) and are transversally striated in bright colors. The legs are slender and the tarsi bear weak claws. Males and females are very similar in appearance.

The female lays 100–600 eggs in small batches between bloodmeals. The eggs are oval and operculated, and the surface sculpturing differs between species. They are white at first becoming rose-red later. There are five nymphal stages, each of which has to feed on blood at least once. In the last nymphal stage, the wing buds are visible. It takes 3 to 4 months for the eggs to develop to adults in the smaller species such as *R. prolixus,* but most species take 5–8 months. The nymphs occupy the same habitat as the adults.

Triatomines are essentially sylvatic nest-inhabiting insects. They are found in burrows, caves, and the nests of rodents, armadillos, bats, birds, sloths, or opossums. A few species have moved from nests to live in houses in rural areas, especially those made of natural materials such as adobe or wood and roofs of palm

leaves. Here the bugs can find shelter easily in crevices and hide when not feeding. There can be as few as six bugs in an opossum nest up to several thousands in a house. Their diurnal activity matches that of their hosts. Hence, anthropophilic triatomines seek a human bloodmeal during the night, and retreat to their hiding places during the day. Locomotion is mainly by walking, rather than flying. Movement between houses is frequently by transfer of building materials such as palm thatching, clothing, bedding, or other activities.

Adults and fifth instar nymphs can ingest between 300 and 1000 mg of blood depending on size. Thus, in a house with a large bug population, where each bug feeds every 4–9 days, a person might receive 20–30 bites per night and lose several ml of blood per day. The bugs prefer to bite on the face, which is usually uncovered at night, especially the soft parts around the eyes or the lip; this behavior has given them their colloquial name of kissing bugs. Triatomines are relatively large insects that take about 30 min to fully engorge, yet unlike bloodsucking Diptera, they are unable to fly away quickly from a disturbed host. Therefore, in order to avoid painful bites, their mouthparts are adapted for quick insertion and release, and the saliva contains analgesics. Their bite is remarkably painless and is not noted during sleep.

Bugs acquire *T. cruzi* parasites during a bloodmeal from an infected animal or human host. The parasites grow and multiply in the gut and do not penetrate the hemocoel. They are transmitted when the bug either produces a droplet of liquid from its anus during feeding (rapid diuresis) or defecates. The species that defecate while feeding are the most efficient vectors of *T. cruzi*. The spread of Chagas disease over the past 100 years has been associated with changing land use, such as clearing land for subsistence agriculture and bringing sylvatic bugs and small mammal hosts of *T. cruzi* in close contact with humans.

Control of Chagas disease is mostly achieved by controlling the vector, particularly in rural areas, where the disease is more common. Indoor residual insecticides, such as pyrethroids, sprayed inside houses or incorporated into paint as part of well-structured control programs have been successful in many parts of Central and South America. However, there is the continual risk of reinvasion of dwellings by sylvatic vectors, either the original vector or another species.

4.4.6 Cimicidae – Bedbugs

The Cimicidae or bedbugs are a well-defined group of oval, flattened bugs that inhabit "nests" or roosting sites and feed on the blood of a disparate group of birds, bats, and humans. There are about 100 species, of which two feed on humans. Some species are pests of poultry, including *Cimex lectularius*, causing severe irritation, anemia, and weight loss from their abundant bites. Species associated with bats can transmit several trypanosome species.

4.4.6.1 *Cimex lectularius*

The common bedbug (Figure 4.18c) is a parasite of humans, birds, bats, and domestic animals. Originating in Southern Europe and the Middle East, it has spread around the world to be cosmopolitan today. It is such a familiar human parasite that it has featured in writings since ancient times. Experimentally, it can be infected with a wide range of blood-borne parasites and pathogens, including hepatitis B, HIV, and *T. cruzi,* but there is little evidence supporting its transmission of these infections in nature.

C. lectularius (5–7 mm) are brown, oval-shaped, and flat insects with large prominent eyes and with the proboscis tucked under the head as in all Heteroptera. The pronotum is very broad, the scutellum (mesonotum) triangular with the tip pointing backward, and the metanotum is covered by pads of the vestigal forewings. Hindwings are lacking. The females lay about 200 oblong and slightly curved eggs (Figure 4.18d). They are fixed onto a substratum with the convex side downward. Each of the five nymphal instars has to feed at least once. Fertilization in the Cimicidae occurs by an extraordinary process of traumatic insemination. The female's genital opening serves for oviposition only, but not for the dual function of receiving sperm and oviposition as in most other insects. The male possesses a heavily sclerotized "paramere," which is thrust through the female's body wall at a special point, where a small invagination in the distal margin of one of the abdominal tergites covers a cushion of hemocytes. The injected sperm migrates to a pair of receptacles next to the ovary and inseminates the egg cells.

Bedbugs feed at night and hide during the day in narrow cracks and crevices (thigmotaxis); in a dwelling, this will include locations behind wallpaper, in books, curtains, furniture, pictures, behind skirting boards, and in ventilation systems. Bugs are transported to new sites mostly via furniture and bedding. The bugs leave characteristic dots of dark feces around their hiding places and group together by the use of pheromones. Rooms contaminated with bugs have a very typical, intense, and nasty odor caused by secretions of the metasternal scent glands, situated between coxa II and III. The secretions are mainly hexanol and octanol and like many other groups of bugs are used both as a defense against predators and assembly cues for the bugs. Bedbugs locate their hosts by chemotactic and thermotactic cues, but only within a distance of a few centimeters, hence they hide close to where the host sleeps.

Bedbugs are capillary feeders using a very fine feeding tube consisting of paired mandibles and maxillae (Figure 4.14b,c) and hence their bites are painless. They preferentially feed on exposed skin, usually the face, neck, and arms of sleeping individuals. It takes between 5 and 10 min for a bedbug to completely engorge with blood. Their bites, often in lines, are not usually noted at the time, but develop slowly to itchy welts that can take weeks to vanish. Bloodmeals are taken every 5–10 days by nymphs and adults, but bedbugs can live for a year without feeding, coming out from their refugia when new hosts become available. Development is temperature-sensitive, bugs do not feed at temperatures less than 13 °C. Eggs

will only survive a maximum of 3 months and are unable to survive over winter in unheated buildings in temperate regions.

In the last two decades of the twentieth century, *C. lectularius* became scarcer than previously through the use of residual insecticides to control infestations. However, their numbers have grown significantly in developed countries in recent years to become a major pest in urban areas, especially in community housing and hotels.

Cimex hemipterus, the tropical bedbug, is restricted to regions between 30° north and south of the equator. It has the same hosts and biology as *C. lectularius,* although it is tolerant of higher temperatures. It is slightly larger and more slender than *C. lectularius*. The closely related *Cimex columbarius*, the pigeon bug, can attack humans when the bugs disperse from overpopulated pigeon nests or when the birds leave the nests in urban settings.

4.4.7 Siphonaptera – Fleas

- Obligatory ectoparasites of birds and mammals.
- Hematophagous in both sexes.
- Holometabolous, secondarily wingless.
- Three legless scavenging larval stages plus pupa.
- Vectors of plague.

Fleas are a well-defined order of the Endopterygota with remarkable adaptations to ectoparasitism on birds and mammals that have "nests." Of the approximately 2500 species of fleas, 93% infest mammals, and only 7% are parasitic on birds. Siphonaptera (Greek: *siphon* = tube, *a* = without, *pterón* = wing) occur throughout the world, wherever their hosts are present, from the tundra to mountain tops and from deserts to rainforests.

4.4.7.1 Biology and Development

Adult fleas rarely live permanently on the body of their host, but rather in the bedding of its nest or burrow only visiting the host to feed. The life cycle of fleas is closely related to the environment of the host and usually takes place within the dwelling place of the host or in the regularly patrolled home range of territorial species. As an adaptation to extreme environments, such as arctic hosts, development takes place entirely on the host.

Eggs are produced between bloodmeals and drop into the bedding of the nest or burrow of the host. The cat flea (*Ctenocephalides felis*), for instance, produces 25 eggs per day in its lifetime of 3–4 weeks, totaling 700–900 eggs during its lifetime. The life cycle of some fleas, for example, the rabbit flea *Spilopsyllus cuniculi,* is closely tied in with the reproductive cycle of the host, taking a cue from circulating hormones associated with pregnancy (Rothschild and Ford, 1964) to produce new flea adults when the young rabbits are born. The developing larvae feed on organic material in the nest using chewing mouthparts. Dried blood in the feces of

adult fleas or from the rapid expulsion of undigested blood by feeding adults is an important component of the larval diet. The gut of adult fleas can hold only 0.5 µl of blood, but a flea ingests 10–20 times as much, the remainder being expelled for the larvae. This link between adult feeding and larval development is an important reason why nonnesting mammals, such as apes, monkeys, elephants, kangaroos, or, with certain exceptions, free-living ungulates and felids, do not have fleas but have permanently resident lice. Domestic cats have only acquired abundant flea populations during the process of domestication.

The third instar larva weaves a silken cocoon and metamorphoses into a pupa. The cocoon is camouflaged by tiny particles of the surrounding habitat sticking to it. Pupation lasts from a few days up to 3 weeks. The adult flea can remain in the cocoon for a week to several months until vibration and CO_2 from a potential host stimulates its emergence. The first animal, or person, to enter a disused dwelling will often acquire a large number of fleas in a very short time.

The gigantic jumps of fleas are performed with the hind legs, a pad of resilin, an elastomeric protein, in the metathorax storing the energy.

Adult fleas take bloodmeals frequently (every 15–20 min in the well-studied *S. cuniculi*) and therefore bites are often seen close to one another on the host.

Adult fleas can be obligate parasites of one species, genus, or family or they can be nonspecific in their choice. Many are not so much host-specific as "nest-specific." Once adapted to a nest, they can feed on different hosts, although egg production is most successful when females feed on the main host. Under these circumstances, feeding on alternative hosts in the absence of the main host maximizes the chances of later finding an appropriate host.

4.4.7.2 **Morphology**

Fleas are small insects, 1–6 mm long, easily recognized by their winglessness, laterally compressed body for ease of movement on the host and large hind jumping legs, which they use to launch themselves at a passing host (Figure 4.19a). They are heavily sclerotized, and therefore are dark brown or black. The thoracic and abdominal tergites overlap each other like roof tiles and thus are streamlined (Figure 4.19b). In several genera, there are backwardly directed combs of spines called ctenidia on the head and the posterior margins of the thoracic segments (Figure 4.19b,d,e), which facilitate the adherence to the host fur or feathers. The body and legs are covered with bristles, also pointing backward. The head is keel-shaped and lacks compound eyes, though two small ocelli can be present. The club-shaped antennae are recessed in deep grooves in the side of the head and bear numerous sensillae that can detect hosts and monitor environmental conditions. Siphonapteran mouthparts are of the piercing–sucking type and are held against the ventral surface of the head when not feeding. As in the Diptera, to which the fleas are most closely related, the mouthparts lack mandibles (Figure 4.19g,h). The maxillary palps are easily mistaken for antennae.

The abdomen consists of eight visible segments (Figures 4.19b and 4.20), which are more or less rounded distally in the female but chamfered in the male. Males are usually smaller than females and have in their abdomen two long

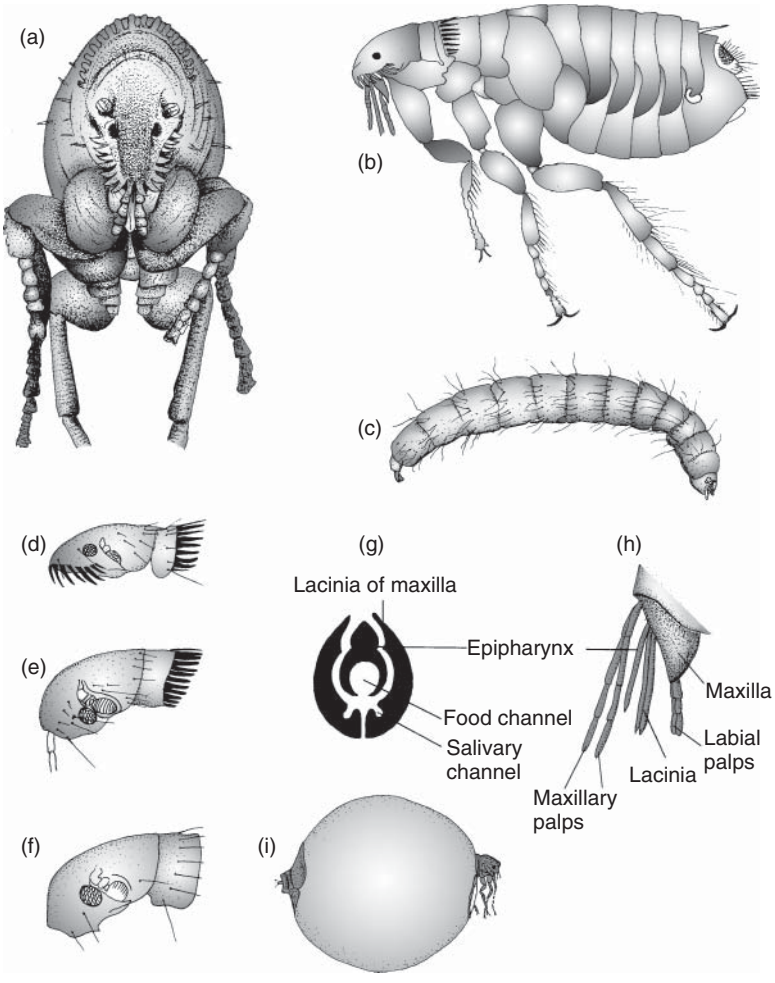


Figure 4.19 Siphonaptera. (a) *Ctenocephalides felis*, frontal view. (After a photo by P. Arnold.) (b) Female flea, lateral view. (c) Larval flea. (d) Head of *Ctenocephalides felis*. (e) Head of *Ceratophyllus gallinae*. (f) Head of *Pulex irritans*. (g) Scheme of a cross section through mouthparts. (h) Lateral view of mouthparts, strongly spread. (i) *Tunga penetrans*, gravid female.

chitinized filaments, which are inserted into the female via an aedeagus during copulation, after which sperm is stored within the comma-shaped spermathecae of the female.

The free-living and nonparasitic larvae are whitish and sparsely covered with hairs. They lack legs (apodous), but do have a well-defined head capsule (eucephalic, Figure 4.19c) bearing short, one-segmented antennae, chewing mouthparts, but lack eyes. They may thrive on excess undigested blood as a part of their diet, which is released by adult fleas. The 11th segment bears a pair of prolegs.

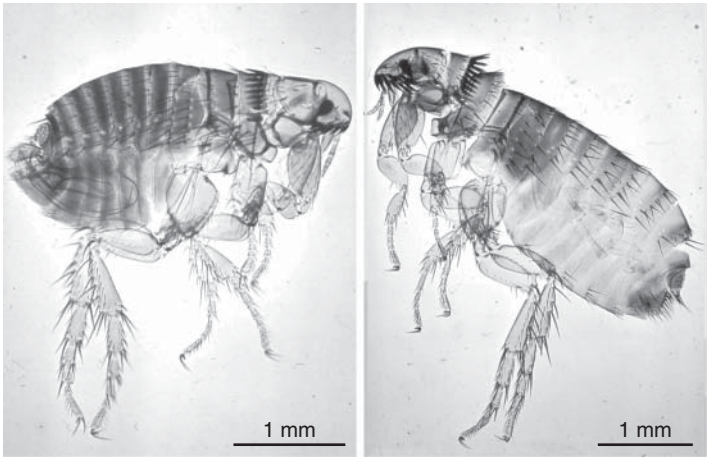


Figure 4.20 *Ctenocephalides felis* (cat flea). (a) Male, with copulatory apparatus in the abdomen. (b) Female. (Image: Archive of the Department of Parasitology, University of Hohenheim)

4.4.7.3 *Pulex irritans*

This cosmopolitan flea, usually misnamed the human flea, occurs on large and coarse coated mammals, including pigs, canids, mustelids, deer, tapirs, and peccaries as well as humans. It can transmit plague and erysipeloid (bacterial disease) in central Asia. It is an intermediate host of the cestode *Dipylidium caninum* in parts of Europe. It has become very scarce in many developed countries, where vacuum cleaning eliminates all the life stages, although it can become a pest in stables for the animals and people working there. *Pulex* species have no ctenidia (Figure 4.19f) on the head and only one row of bristles on each abdominal segment.

4.4.7.4 *Ctenocephalides*: Cat and Dog Fleas

The cat flea (*Ctenocephalides felis*, Figure 4.20) has become the real human flea in developed countries, far more than the dog flea *Ctenocephalides canis* or even *Pulex irritans*. The cat flea infests dog, cats, and other domestic and wild mammals, as well as cattle and sheep. In farms where calves are raised, and even in poultry farms outbreaks of *C. felis* can lead to anemias. It takes 27–40 days for the cat flea to complete one life cycle. Feeding on human blood appears to produce fewer eggs than feeding on cats. The dog flea is basically a parasite of dog, cat, and fox, but is much less common than *C. felis* in the domestic settings. Species of *Ctenocephalides* have a ctenidium on the pronotum with eight to nine teeth, and a ctenidium with seven to eight teeth on its head (genal ctenidium). *C. felis and C. canis* differ in the shape of the head (elongate in *C. felis*) and in the number of spine-bearing notches on the hind tibia (six in. *C. felis*; eight in. *C. canis*) (Figures 4.19d and 4.20).

4.4.7.5 *Tunga penetrans* – Jiggers

The sand flea, jigger or chigoe, is a good example of the evolutionary modification that a flea can undergo physically and behaviorally. A total of 10 species are known, of which two, *Tunga penetrans* and the more recently described *Tunga trimamillata*, infest humans by burrowing into the skin (tungiosis). Both originated in Central and South America. *T. penetrans* of neotropical Xenarthra (anteaters, tree sloths, and armadillos) and/or Caviidae (guinea pig, wild cavies, and the capybara) was subsequently spread to tropical Africa by humans. The larvae of the genus *Tunga* have only two (not three) instars and develop in sand and light soils in areas frequented by the hosts, rather than in their dwellings. The tiny adults (up to 1 mm) search for a host, usually attaching themselves to the feet of large mammals, including humans and pigs. Both sexes suck blood, but the male dies after mating. The female burrows head-first into the host's skin beginning in a crease or fold or even under the toenails. Only the tip of the female's abdomen is exposed bearing large spiracles for breathing, the anus, and the genital opening. It swells enormously, as hypertrophic giant cells of the epidermis of the second and third abdominal segments grow and enlarge to the size of a pea, a 1000 times her original size (Figure 4.19i). Several thousand eggs are produced. Irritation and inflammation cause scratching by the host. After the third week of penetration, the female dies and is eventually expelled from the skin. A high incidence of secondary bacterial infections is common.

4.4.7.6 Disease Transmission by Fleas

Flea bites are very characteristic with a central densely red area and paler corona. They induce a delayed hypersensitivity in many hosts, including humans. Irritating flea bites cause itching, scratching, secondary bacterial infections, disturbance, nerviness, emaciation, and in young or small animals iron deficiency from blood loss.

Fleas transmit protozoans, bacteria, rickettsiae, and viruses between mammals, but their status as vectors is not well established except for the infamous transmission of plague and, to a lesser extent, murine typhus and myxomatosis.

- Plague, or black death, as it was called in the Middle Ages, is caused by the bacterium *Yersinia pestis*, a Gram-negative obligate pathogen. Plague is a zoonosis occurring in many parts of the world, including North and South America, Africa, Central Asia, and Asia. It is transmitted by more than 100 flea species between about 20 rodent species and some lagomorphs, which vary in their susceptibility to the bacterium. Plague is usually a benign enzootic, but epizootics (epidemics in animals) can occur in the wild with very high mortality in some rodent species. If the rodents are peridomestic or urban, then the fleas will seek a bloodmeal from humans and establish an "urban" focus of infection. Subsequent human–human transmission can take place, as happened in the devastating plague epidemics in the past. The oriental rat flea *Xenopsylla cheopis* is particularly important in peridomestic transmission from *Rattus rattus*, but other species will transmit plague to humans in wild areas. The flea ingests *Y. pestis* in

a bloodmeal and when the infected bloodmeal cools to below 27 °C, a bacterial plasmid expresses a coagulase, which enhances solidification of the bloodmeal. This can occur when a flea leaves a dying host. The bacterium reproduces in the anterior gut (proventriculus), it does not enter the flea's hemocoel. The blockage caused by the multiplying bacteria and the coagulated blood prevents the flea from feeding properly. When the flea's temperature increases above 27 °C again, such as on a new host, the plasmid initiates expression of fibrinolysin, which causes the fibrin network of the blockage to break down and bacteria are regurgitated into a new feeding site when the flea next tries to feed. Bacteria can also be transmitted in the flea's feces or when the flea is eaten during grooming. *Y. pestis* produces products that affect both its vectors and hosts to maximize transmission and doubtlessly these vary between different ecological settings. In plague control programs, it is essential to attack the fleas with insecticides as well as rodent control, otherwise the problem can be exacerbated as infected fleas leave their dying rodent hosts.

- Murine typhus or endemic typhus, is a flea-borne rickettsial zoonosis caused by *Rickettsia typhi.* It occurs worldwide and is transmitted by the oriental rat flea *X. cheopis* and other cosmopolitan fleas from rats (*Rattus* sp.), and occasionally cats, to humans. The pathogens multiply in the gut epithelium and are transmitted when feces are inadvertently rubbed into a wound or mucous membranes of the host. There is evidence of transovarial transmission. In humans, after an incubation period of 5–18 days, high fever and violent headaches occur with mortality varying between 2% (New World) and 70% (Old World) depending on serotype. There is experimental evidence that *Ctenocephalides canis* can transmit the infection.
- Cat flea typhus, caused by *Rickettsia felis*, was discovered only in 1994. It is transmitted, to humans, too, by *C. felis*, the cat flea. The disease, occurring worldwide, manifests similar to louse-borne epidemic typhus (*R. prowazeki*), but is benign.
- Cat scratch disease, caused by *Bartonella henselae*, a Gram-negative bacterium related to *Rickettsia*, is the causative agent of a cat disease discovered 1990. The vector between cats is the cat flea *C. felis,* but there is increasing evidence of transmission by ticks, possibly *Ixodes ricinus*. Dogs can also be infected. The pathogen is transmitted to humans by scratched wounds of cats. Signs are papulous primary lesions, inclined to form abscesses, more rarely fever and general symptoms. After 2–4 months, spontaneous remission is seen. In HIV patients, it causes a severe "bacillary angiomatosis"
- Cat and dog fleas (*C. felis* and *C. canis*) are also intermediate hosts of the cestode *Dipylidium caninum* and probably, but rarely *Hymenolepis nana* (see Section 2.1.2.9). An infected flea is eaten during grooming. After maturation of the parasite in the definitive host, eggs are voided in the feces, which are then eaten by flea larvae living in the host's environment.

4.4.8
Diptera – Flies

- Only one pair of wings, the second pair replaced by "halteres."
- Mouthparts of adults sucking or piercing, never chewing.
- Apodous, eyeless larvae with chewing mouthparts.
- Pupa present.

The Diptera (Greek: *di* = two, *pterón* = wing) or true flies are a large order of holometabolous insects with some 150 000 species occurring in just about all habitats. By virtue of their bloodfeeding habits, the adults of certain groups are important vectors of parasites causing some of the most devastating diseases of humans and domesticated animals. The larvae of other groups are obligatory endoparasites in the tissues of land vertebrates, a condition known as myiasis. Diptera are characterized by having a single pair of wings, the hind pair having developed into small knob-like structures, the halteres, used as gyroscopes to aid their often sophisticated flight maneuvers. The order comprises two large groups: (1) the lower Diptera (=Nematocera) with several bloodsucking families containing the most important vectors of parasites and pathogens and (2) the Brachycera with the hover flies and horse flies ranging up to the familiar house flies, blowflies, and tsetse flies.

The larvae are legless (apodous) and have chewing mouthparts and invariably live in a completely different habitats to the adults, except in some of the most evolutionary recent groups, where the larvae are retained within the body of the female being "laid" (larvipary) as either first instar or mature larvae. The pupae are coated by the skin of the last larval stage.

The mouthparts of the adults are never chewing in function, but are either lapping/sponging or piercing-sucking in form. The mandibles are lost in the higher brachyceran flies. Parasitism in the larval stage is distributed throughout the Brachycera, some of which exploit vertebrates while many live on other insects (e.g., Tachinidae in Lepidoptera) and arthropods (Acroceridae in spiders). The higher classification of the Diptera is in a state of upheaval as phylogenetic studies attempt to ensure that the major groupings and families are monophyletic. Old divisions such as Nematocera (threadhorns), Brachycera in a narrow sense, and the Cyclorrhapha have been replaced with more precise terminology and definitions, but the old informal groupings are retained for convenience in many texts (Table 4.4). In many taxa, systematics are complicated by the existance of morhologically indistinguishable species complexes, which can nowadays be differentiated with molecular techniques (Box. 4.2).

4.4.8.1 Lower Diptera

This suborder contains slender, delicate insects with adults bearing filamentous, multisegmented antennae (contrary to the few antennal segments in the Brachycera). The antennae consist of two basal segments and a flagellum with 6–39 similar-shaped segments or flagellomeres. The larvae always have more

Table 4.4 Selected members of the Diptera (only groups containing parasites).

Lower Diptera (former Nematocera)
Culicomorpha
Culicoidea
Culicidae (mosquitoes)
Chironomoidea
Simuliidae (blackflies)
Ceratopogonidae (biting midgets)
Chironomidae (midges)
Psychodomorpha
Psychodoidea
Psychodidae
Phlebotominae (sandflies)
Tipuloidea (crane flies)
Brachycera
Tabanomorpha
Tabanidae (horse flies)
Muscomorpha (Cyclorrhapha)
Muscidae (house and stable flies)
Calliphoridae (blow flies, screwworms)
Oestridae (bot or warble flies)
Hippoboscoidea
Glossinidae (tsetse flies)
Hippoboscidae (louse flies or keds)
Nycteribiidae (bat flies)
Streblidae

than three instars and usually live in water or at least in moist habitats. The larvae have distinct head capsules (eucephalic) with chewing mouthparts. Adult lower Diptera feed on sugar-containing plant fluids, which is augmented by blood in females of several families for the production of eggs. In these cases, the term "host" refers to the species used as a blood source and the mouthparts are of the solenophagous type, that is, piercing-sucking (Figure 4.14c,e) or of the telmophagous type (poolfeeding).

The females of several families of the lower Diptera (Nematocera) suck blood and are the most important group of vectors in terms of the diversity of pathogens and parasites they transmit and the impact of those pathogens and parasites on their vertebrate hosts, including humans.

4.4.8.2 **Ceratopogonidae – Biting Midges, No-see-ums, Punkies**

- Smallest hematophagous insects.
- Females sucking blood of terrestrial vertebrates.
- Four larval instars.

- Apode larvae living in moist substrata, feeding on microorganisms.
- Vectors of several Arboviruses.
- Vectors of apicomplexan parasites and of filariids (genus *Mansonella* in humans and *Onchocerca* in other mammals).

The biting midges, also known as punkies, no-see-ums, or simply midges. They are vectors of viruses, protozoa, and filariids. About 5000 species occur worldwide, except in the Arctic and Antarctic. The females of the majority of species are predatory or ectoparasitic on other insects, but four genera are hematophagous on vertebrates (*Culicoides, Austroconops, Leptoconops*, and the subgenus *Lasiohelea* in the genus *Forcipomyia*). The genus *Culicoides* with more than 1000 species is the largest and most important genus parasitologically and occurs in all faunal regions of the world except the south of South America and New Zealand. At least 50 *Culicoides* species transmit pathogens and parasites to humans and other animals. The 150 species of *Leptoconops* occur in tropical or subtropical regions, despite not transmitting pathogens, can be major biting pests. Species of *Culicoides* and *Forcipomyia* (*Lasiohelea)* transmit several species of *Onchocerca* to cattle in Asia, Australia, and Europe.

Biology Adult *Culicoides* are crepuscular or nocturnal, whereas *Leptoconops* are day-biting. Midges are so small that they can penetrate mosquito nets. Males and females feed on nectar and the females only feed on blood for the production of eggs. However, some species can produce the first batch of eggs without a bloodmeal (autogeny), possibly an adaptation for survival in the absence of suitable hosts. Midges feed on birds as well as mammals and unlike mosquitoes and sandflies, they do not need to feed on exposed skin but will "burrow" into fur, hair, or feathers, making them difficult to brush off. Copulation takes place in swarms. The larvae develop in a wide range of semiaquatic habitats, including, decaying plant material, in the soil of salt marshes and mangrove swamps, shores of streams and ponds, muddy margins of pastures, and cattle feces (cowpats). They can achieve great abundances of 10 000 larvae per square meter. The larvae feed on small organisms or decaying vegetation. In temperate climates, the fourth instar can overwinter.

Midges can disperse over large distances when taken up into the upper air currents, which is important in the spread of arbovirus diseases.

Morphology Ceratopogonids are very small, compact biting flies only 1–3 mm long. They have large compound eyes, nearly contiguous above the antennal base. The rather long antennae are slender, have 10–13 flagellar segments and are plumous and name-giving (Greek: *kéras* = horn, *pógon* = beard, meaning bearded) in the males, similar to their sister group, the nonbiting midges (Chironomidae). They have well-developed mouthparts for bloodfeeding with cutting teeth on the tips of the elongated mandibles and the maxillae in a short proboscis. The mandibles work in a scissor-like manner cutting into the host, while the maxillae hold the mouthparts in place. They are poolfeeders, sucking blood and lymph up from the minute hole created by the mandibles.

The thorax is arched dorsally and extends slightly over the head. The wings, when at rest, are placed flat onto the abdomen and they often have contrasting dark and milky white spots in *Culicoides.* The wing patterns often differ between species, especially those transmitting pathogens.

The larvae have well-developed head capsules (eucephalic) and four instars. *Culicoides* larvae are snake-like, growing to 5–6 mm long. The last abdominal segment bears a gill-like structure, having an osmoregulatory function (respiration being cutaneous). The pupa is 2–4 mm long, the cephalothorax bears a pair of breathing trumpets, and the last segment bears a pair of horn-like processes.

4.4.8.3 Disease Transmission

Biting midges transmit numerous pathogens and parasites: 66 viruses, 15 species of protozoans, and 26 species of filarial nematodes. Their very small size and hence bloodmeals indicates that the chance of the acquisition of infectious agents is small. For example, a common viraemic peak in sheep infected with bluetongue virus (BTV) is $10^4 - 10^5$ infectious units per milliliter of blood; in a midge taking between 10^{-4} and 10^{-5} ml of blood, the chances of ingesting sufficient virus to infect a midge is small. The same argument applies to filariae although the enormous numbers in *Culicoides* populations ensure that transmission is maintained.

Their bites are irritating, painful, and can cause long-lasting lesions in some people. Some tropical members occur in such masses and bite so obstinately that the local tourism industry suffers.

Culicoides transmit several important viruses causing diseases of domesticated and wild mammals:

- One of the most important pathogens transmitted by *Culicoides* is Bluetongue, a worldwide disease of ruminants caused by the BTV an *Orbivirus* (Reoviridae) with 24 serotypes. The impact of this disease is significant with millions of dollars being lost to sheep and cattle farmers. BTV causes a catarrhal fever, mostly in sheep, more rarely, and usually asymptomatic, in cattle, goats, buffalo, deer, dromedaries, and antelopes. In red deer, it may be as acute as in sheep. Animals have high fever, excessive salivation, swelling of the face and tongue, and cyanosis of the tongue. High morbidity and mortality occur. The disease is transmitted in Africa by *C. imicola,* but its remarkable spread into Europe in the late 1990's via several routes was through the role of additional vectors such as *Culicoides obsoletus* and *Culicoides pulicaris*. The range extension is believed to be associated with climate change,
- African horse sickness virus (AHSV; Reoviridae) is related to bluetongue, and can kill horses with mortality as high as 90% during an epidemic.
- Epizootic Hemorrhagic Disease virus (EHD; *Orbivirus*, Reoviridae) is an often fatal disease of deer, mainly in the United States, and other Cervidae in Africa and Asia. It is closely related to BTV and cross-reacts on many blood tests.
- West Nile virus (WNV; Flaviviridae) is a zoonosis with bird reservoirs, but occasionally affects humans and horses with serious and fatal consequences. The usual vectors are mosquitoes and, in the United States, biting midges (*Culicoides arboricola, Culicoides biguttatus, Culicoides stellifer*) are involved. WNV

is another virus that has moved from its African origins in "West Nile District" in Uganda to Europe and Russia in the 1950s and 1960s and to the United States in 1999. In 2002, a major epidemic occurred in the United States with 284 deaths. The virus caused mild symptoms in most people, but in a neuroinvasive form severe disease developed with potential permanent neurological effects.

- Bovine ephemeral fever (BEF; *Ephemerovirus,* Rhabdoviridae) or 3-day fever is a debilitating disease of cattle in tropical and subtropical regions of Africa, the Middle East, and Australia. Vectors are *Culicoides oxystoma* and *Culicoides nipponensis*.
- Schmallenberg virus (SBV; *Simbu group* Bunyaviridae) has been recently isolated in Germany and subsequently in many European countries in sheep, cattle, and goats. It can cause fatal congenital defects by infection of fetuses in pregnancy.
- The only virus transmitted to humans by midges is Oropouche virus (OROV; *Orthobunyavirus*, Bunyaviridae) causing Oropouche fever. It occurs in the Amazonian region, the Caribbean, and Panama. It is a zoonosis with the sloth (*Bradypus tridactylus*) and marmosets (*Callithrix)* as reservoirs with occasional epidemics in humans. It causes a fever similar to dengue.The main vector is *Culicoides paraensis,* but mosquitoes are also involved.
- Several apicomplexan Haematozoea, causing "avian malaria" and trypanosomes, are transmitted by biting midges. Most notable is *Leucocytozoon caulleryi* of chickens, guinea fowl, and pheasants in Asia transmitted by *Culicoides arakawae.* Curiously, all other *Leucocytozoon* species are transmitted by *Simulium*. *Parahaemoproteus* (=*Hemoproteus*) *nettionis* is found in ducks, geese, and swans and transmitted by *Culicoides downesi* in North America*. Hepatocystis kochi* is a parasite of Old World monkeys, transmitted by *Culicoides adersi* in eastern Africa.
- Of the "filariae" transmitted by *Culicoides*, three infect humans: *Mansonella perstans* in Central and West Africa and the Caribean and Central America transmitted by *Culicoides milnei*, *Culicoides grahami* and so on; *Mansonella ozzardi* in Central America transmitted by the abundant *Culicoides furens;* and the less pathogenic *Mansonella streptocerca* in West Africa (see Section 3.3.2).

4.4.8.4 **Culicidae – Mosquitoes**

- Hematophagous, holometabolous insects.
- Only females suck blood, on vertebrates.
- Vectors of arboviruses, *Plasmodium*, and the filariae *Wuchereria* and *Brugia*.
- Four larval instars, aquatic, eucephalic, apode, feeding on microorganisms.
- Pupa actively swimming.

The mosquitoes are one of the most significant vectors of parasites and pathogens in the Diptera, as well as being an aggressive biter. Worldwide, there are more than 3500 species in 43 genera. Of the three subfamilies, the Toxorhynchitinae do not feed on blood; the others, the Anophelinae and Culicinae have

Box 4.2 Species Complexes

Species complexes are groups of morphologically indistinguishable species, which are reproductively isolated and differ biologically, often in their ecology (e.g., breeding sites), behavior (e.g., host preference), susceptibility to parasites, and vectorial capacity. Species complexes occur in many groups of organisms, but have been particularly well studied in bloodsucking insects. They were discovered first when *Anopheles* species thought to be vectors were present in areas without malaria – in fact they were biologically different species, which could only be distinguished from vector species by their chromosomes (banding on giant polytene chromosomes in *Anopheles* and later in *Simulium*), enzymes revealed by electrophoresis, cuticular hydrocarbons, and more recently by DNA sequences, including DNA barcodes.

Many important vectors are members of species complexes, for example, the major vector of *Plasmodium falciparum* in sub-Saharan Africa, *Anopheles gambiae,* is a member of a complex of at least seven species, including *Anopheles arabiensis, Anopheles bwambae, Anopheles merus, Anopheles melas,* and the nonvector, *Anopheles quadriannulatus*. Similar complexes exist in Europe (e.g., *Anopheles maculipennis* complex), Asia (e.g., *Anopheles dirus*, *Anopheles maculatus*, and *Anopheles culicifacies* complexes*),* Australia (*Anopheles punctulatus* complex), and South America (*Anopheles darlingi* complex).

The genus *Culex*, important vectors of several viruses and filariid nematodes, has one very well-studied complex, the *Culex pipiens complex,* which includes two "forms," *pipiens* and *molestus,* and the major filarial vector *Culex quinquefasciatus*. In the Pacific, the *Aedes scutellaris* complex with more than 35 forms has radiated dramatically between islands.

Research on species complexes has been at the forefront of evolutionary research, especially on gene flow, geographic gene frequency models, and modes of speciation. These theoretical studies are augmented by a huge body of field data.

hematophagous females. Because of their medical importance, a huge body of knowledge has been accumulated on their field biology, physiology, and more recently molecular biology, especially as *Anopheles gambiae* (*An. gambiae*) and *Aedes aegypti* (=*Stegomyia aegypti*) have become model organisms and their genomes sequenced. Many species are members of species complexes (Box 4.2).

Biology and Development Mosquitoes breed in all forms of standing water, each species having particular requirements. Breeding sites include ditches, ponds, lakes, among vegetation on the edges of flowing waters, puddles, animal footprints, paddy fields, or brackish pools. Some important vector species breed in natural or artificial containers such as empty tins, (e.g., *Ae. aegypti*) and recycled tire (*Aedes albopictus*). Some important virus vectors develop in the

axils of leaves, epiphytes, or tree holes (e.g., *Aedes africanus*, a vector of yellow fever). The important filarial vector, *Culex quinquefasciatus,* preferably breeds in heavily organically contaminated water such as latrines or cesspits.

Oviposition usually takes place after digestion of the bloodmeal, but some species do not require a bloodmeal (autogenous) for at least their first egg batch. Up to four or five cycles of bloodfeeding, oviposition can take place. Eggs can be laid directly on the surface of the water (e.g., *Anopheles* and *Culex*) or just above the waterline in the expectation of heavy rains (e.g., many *Aedes* sp.) and known as floodwater mosquitoes. *Mansonia* fixes the eggs to the underside of submerged water plants, where they obtain oxygen directly from the plant. *Culex* spp. produce a well-characterized pheromone secreted as a droplet on to the apex of each egg to attract other ovipositing females. Mosquito larvae and pupae are very active and avoid predators by rapid jerking movements, which propel them to the bottom of their habitat.

Most adult mosquitoes have well-defined periods of activity for mating in swarms or seeking a blood or sugar meal. *Anopheles* species are usually nocturnal. The periodicity of filarial worms, for example, *Wuchereria bancrofti*, coincides with these activity patterns to maximize transmission. Some culicines are diurnally active, for example, *Ae. aegypti*.

Female mosquitoes use chemical cues (e.g., CO_2, octenol, lactic acid, and the "sweaty feet" chemical methanethiol), infrared radiation and vision to detect their hosts. They fly up gradients of "host odors" in a zigzag manner, measuring changes in the concentrations of these cues.

Once a bloodmeal has been obtained, the female seeks a refuge to reduce the volume of the meal by rapid diuresis and later digestion. Knowing where mosquitoes bite and rest is very important in control programs. Species that feed indoors (endophagic) can be attacked by spraying walls with residual insecticides and the use of insecticide impregnated bed-nets, but for those species feeding and resting outdoors (exophagic) prevention and control are much more limited.

Adults of the two subfamilies are easily distinguished by their resting position: In the Anophelinae, the proboscis and body are in a straight line, the mosquitoes almost appearing to be standing on their heads, in the Culicinae, the abdomen is almost parallel to the surface (Figure 4.22 j,m).

Morphology Most adults (Figures 4.21 and 4.22 j,m) measure 3–6 mm in length and have large compound eyes. The antennal flagellum has 13–14 segments, which bear long setae in the males and make the antennae plumous like a parabolic reflector to detect the species-specific wing beats of females (Figures 4.22 k,l). These are up to 250 times per second making a characteristic whine-like sound to the human ear.

The mouthparts are much longer and thinner than other biting Diptera, and are formed of slender maxillae and mandibles to make a needle-like structure in a protective sheath of the labium (Figure 4.14 c,e). Muscles in the base of the mouthparts can deform either mandible relative to the other to make the feeding

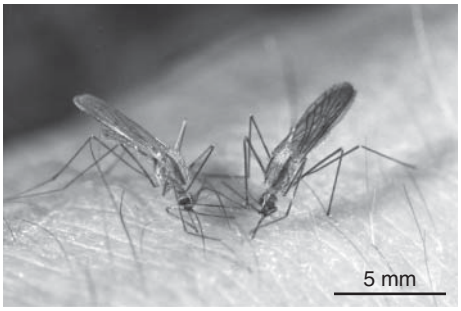


Figure 4.21 Females of *Anopheles* sp. during bloodfeeding. (Image: Courtesy of Heiko Bellmann.)

tube bend and locate a capillary. Only females feed on blood to mature eggs. During bloodfeeding, more than 20 biologically active substances are inoculated into the host in the saliva, including anticoagulants and analgesics. The presence or absence of microspines on the pump that draws blood into the mosquito (hypostomium), blood does not come out under pressure from the host like an oil well, has been correlated to the ability to transmit filariae. The wings and legs are covered in tiny scales, like a butterfly or moth, which vary in color and distribution, and are very important in distinguishing species.

Eggs can be laid as small rafts (e.g., *Culex*), where the eggs are hydrophobic at the narrow end and thus cohere, or singly (Figure 4.22e). *Aedes* scatter the eggs on water margins, but *Anopheles* lay them together forming star-like patterns (Figure 4.22a). In this case, the exochorion (the hardened covering of an egg) is laterally distended as floats (Figure 4.22b–d).

The larva have a conspicuous head with short, slender antennae, compound eyes, and obvious mouth brushes, which they use to feed on microorganisms and particulate matter (Figure 4.22f,i) by setting up circular currents drawing particles onto the brushes and into their mouths. The larvae bear conspicuously long setae on all segments and segmental appendages. The abdomen has nine segments, and segment VIII bears the main spiracle – this is on a short dorsal "siphon" in the Culicinae, which is absent in the Anophelinae. Thus, Anophelinae larvae lie with their body parallel and attached to the water surface by means of the paired, dorsal palmate hairs (Figure 4.22f,g) and the head is rotated 180° so that it can collect food particles from the surface. In the Culicinae, the larvae hang down from the surface at an angle (Figure 4.22i).

The pupae (Figure 4.22h) of all mosquitoes appear almost similar with an enlarged cephalothorax and curved abdomen. The cephalothorax bears a pair of dorsolateral "trumpets," each containing a spiracle. The abdomen terminates in a pair of flattened paddles that enable the pupa to swim in a shrimp-like manner by jerking movements of the abdomen.

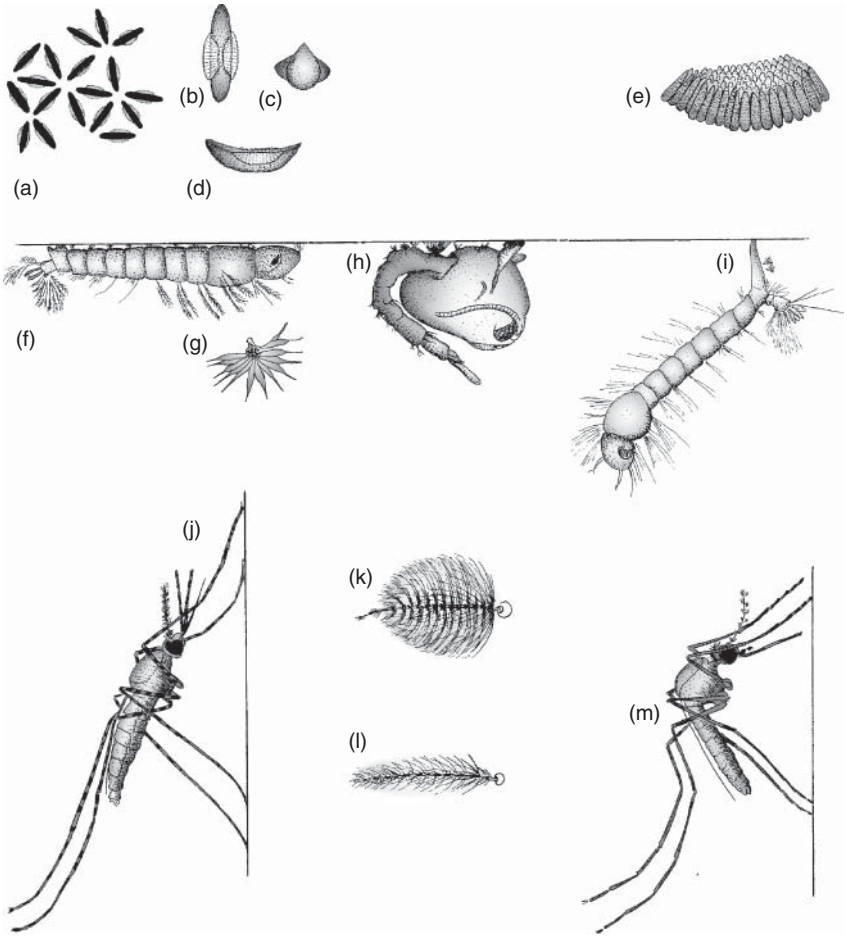


Figure 4.22 Culicidae. (a) Eggs of the genus *Anopheles*, on water surface. (b) Egg of *Anopheles*, top view. (c) Egg, view from the pole. (d) Egg, lateral view. (e) Egg raft of the genus *Culex*. (f) *Anopheles* larvae, attached to the water surface. (g) Palm hair from dorsal side of larva. (h) Pupa of Culicidae. (i) Larva of *Culex* sp., hanging from the water surface. (j) Female *Anopheles* in resting position. (k) Antenna of male Culicidae. (l) Antenna of female Culicidae. (m) Female *Culex* sp. in resting position.

4.4.8.5 **Disease Transmission**

Mosquitoes are doubtlessly the most important vectors of pathogens and parasites to humans; they transmit malaria, arboviruses, and filariasis.

- Of the 450 species of *Anopheles* (Greek: meaning "useless"), about 70 are vectors of *Plasmodium* causing malaria in humans, and of these only 40 are important epidemiologically. Species can be the main vector in one area, but of secondary importance in another, because of differences in abundance, differential susceptibility to local strains of *Plasmodium*, feeding preferences in relation to

alternative hosts (anthropophilic or zoophilic), and mosquito survival times. Some species are restricted to particular habitats, for example, *Anopheles melas*, a saltwater-breeding species is only found in coastal Africa, where it is an important vector (see Section 2.63).

- Rodent malarias (e.g., *Plasmodium yoelii yoelii* and *Plasmodium berghei)*, on which much laboratory work has been undertaken on susceptibility mechanisms and development of parasites in the vector are transmitted in nature by *Anopheles dureni,* although most research is on "nonnatural" vectors, which are easier to rear in the laboratory (e.g., *Anopheles stephensi*).

There are more than 500 arboviruses (=arthropod borne viruses), and mosquitoes are the vectors for probably more than 200 of them, including about 100 to humans. The most important arboviruses are yellow fever virus and Dengue virus.

- Yellow fever is a zoonosis of monkeys with the majority of cases in Africa. An estimated 90% of the infections occur in Africa, but in other parts of the world too. The WHO estimates that yellow fever causes 200 000 illnesses and 30 000 deaths every year in unvaccinated populations. In Africa, several species of *Aedes* are involved in transmission, some in the forest (the sylvatic cycle) and some in rural areas (the rural cycle) and finally, others, including *Ae. aegypti*, in a peridomestic setting (urban cycle), where infection is maintained by human–mosquito–human transmission. In Central and South America, species of forest tree-breeding *Haemogogus* mosquitoes transmit the virus from monkeys. Transovarial transmission is significant in maintaining a reservoir of infection.
- Dengue is widespread in the tropics, with four serotypes of the virus causing a high fever, acute muscle pains (break-bone fever), headache, and prostration. A severe form, dengue hemorrhagic fever, occurs in a minority of patients. The principal vector is the container breeder *Ae. aegypti* worldwide with *Ae. albopictus* in Southeast Asia and recently imported into Central and North America in recycled car tires. *Ae. albopictus* has recently also been found in Europe, which could allow dengue to spread.
- There are many mosquito-borne viruses causing encephalitis in humans and domestic animals, which are zoonoses of wild animals. One of the most important, Japanese Encephalitis is transmitted by *Aedes tritaeniorhynchus*, which breeds in the extensive rice fields of Asia. Humans are considered "dead end" hosts in most encephalites, because the viruses do not multiply enough to infect mosquitoes, that is, humans are not "amplifying hosts." An exception is O'Nyong-nyong fever in Africa, where *An. gambiae* and *Anopheles funestus* are vectors and humans are the only known hosts.
- Several mosquito-transmitted viruses have expanded their range in recent years, including Chikungunya (from East Africa, the Middle East, and Asia to Europe) and West Nile Virus (WNV) with bird reservoirs and *Culex* vectors in Europe. Zika virus (Flaviviridae) has been expanding rapidly over the past few years from its origin in the Zika Forest, Uganda. Following an outbreak in

Polynesia in 2013, it spread to Brazil in 2015 where it was declared a 'Public Health Emergency of International Concern' by WHO. It reached Florida, USA in mid-2016. The virus causes febrile illness but is most notable because of its association with microcephaly in newborns and other neuropathies. Outside Africa, it is transmitted principally by container-breeding *Ae. aegypti* and *Ae. albopictus*.

Filariases: Mosquitoes are the essential intermediate hosts of filarial nematodes, which cause lymphatic filariasis: *Wuchereria bancrofti* and *Brugia malayi*. A wide range of mosquito species is involved in transmission, including both anophelines and culicines, such as *Aedes, Culex,* and *Mansonia*. Other Onchocercidae with mosquitoes as vectors are *Dirofilaria immitis* and *Dirofilaria repens*. For details, see Section 3.3.4.12 – 3.3.4.15.

Control Mosquito control is aimed at both reducing the biting nuisance of mosquitoes and stopping or reducing their role as disease vectors. There have been huge changes in the approach to mosquito control over the past 50 years with the reduction in the use of some insecticides, such as DDT, because of environmental concerns and increasing insecticide resistance. New technologies have been introduced, such as long-lasting insecticide-impregnated mosquito bed-nets (LLINs), pyrethroid insecticides used for larval and indoor residual spraying (and bed-nets), micropolystyrene beads in latrines and cess pits, and biological control with the bacterium *Bacillus thuringiensis israelensis* (Bti) in breeding sites. Long-established methods are aimed at reducing breeding sites for larvae, for example, draining wet areas and intermittent irrigation of paddy fields. One of the most recent developments is control using genetically modified mosquitoes (see Box 4.3).

Box 4.3 Control with Genetically Modified Vectors

In an attempt to improve the control of insect vectors of disease and minimize the environmental and economic costs of existing methods, various alternative control methods are being developed based on a "genetic" approach. There are two main approaches:

1) Reducing the reproductive potential in vector populations using suppression-based strategies, such as the sterile insect technique (SIT) or release of insects carrying a dominant lethal gene (RIDL). SIT requires mass reared insects to be irradiated or chemically sterilized and then released. This requires huge facilities and delivery mechanisms and faces problems with reduced fitness of released insects. It has been very successful with the eradication or control of screwworms (section 4.4.8.14) in North America. RIDL requires the stable transfection of a lethal gene into a laboratory population, which kills the offspring of the released insects. One approach is to use a tetracycline-repressible transcription

factor (tTa) associated with a lethal gene, which enables selection of males to be released and kills the female offspring of released and subsequent modified males.

2) Replacing existing populations with parasite or pathogen resistant strains obtained by genetic manipulation. The main challenge is to drive the desired gene through the target population. One way is to use homing endonuclease genes (HEG's), small selfish genetic elements that can be linked to antipathogen genes. Alternatively, transinfection with appropriate strains of the transovarially transmitted parasite *Wolbachia pipientis* can be used. This bacterium can manipulate its host to induce a form of sterility known as cytoplasmic incompatibility (CI) that reduces the viability of F1's from crosses between infected males and uninfected females or females with a different *Wolbachia* strain. Using this naturally occurring phenomenon is functionally the same at the SIT and is known as the incompatible insect technique IIT.

In addition to technical challenges, there are ethical considerations of releasing transgenic or otherwise modified vectors into the wild.

4.4.8.6 **Simuliidae – Blackflies**

- Females are hematophagous.
- Develop only in running water.
- At least seven larval instars.
- Larvae apode, eucephalic, feeding on microorganisms.
- Pupae in self-spun cocoon, fixed to substrate.
- Intermediate hosts of *Onchocerca volvulus* of humans.

The blackflies or buffalo gnats, are so called because most species are black and the hunched thorax resembles a buffalo (Figure 4.23a,b), but most probably they got their name (from the latin *simulare*, to simulate) from the fact that they look similar to house flies. In addition to being major biting pests in many parts of the world, they are vectors of several protozoans and nematodes, especially *Onchocerca volvulus* of humans causing river blindness in Africa and Central and South America. They only feed on warm-blooded vertebrates and are unique among vectors in that the larvae can only develop in running water.

There are more than 1800 species, occurring worldwide in all climatic regions from deserts to rainforests and tundra in the Arctic. Many species complexes (see Box 4.2) have been discovered in the Simuliidae and doubtlessly many species in the complexes remain to be discovered. Initially, species in complexes were distinguished by banding patterns on their giant polytene chromosomes, but increasingly DNA sequences are used. Many of the most important vector species are members of complexes such as the *Simulium damnosum* complex in tropical

Africa and Yemen, the *Simulium neavei* complex in East Africa, or the neotropical *Simulium metallicum, Simulium ochraceum,* and *Simulium exiguum* complexes.

Biology and Development At oviposition, the females crawl into running water to deposit the eggs onto a carefully selected substrate, the choice of both stream or river type and substrate varying between species. In one group, the *S. neavei* group in East Africa, the larvae are attached to freshwater crabs of the genus *Potamonautes*. The number of larval instars varies quite unusually for Diptera and even within a species from 6 to 11, although mostly there are seven. Immediately on hatching, the larvae secrete a small cushion of silk-like thread from the salivary glands and attach themselves firmly to the substrate by the abdominal circlet of hooks (Figure 4.23e) to prevent being swept away in the often intense water current. The larvae move to optimize their position by either attaching the thoracic proleg to the substrate and looping forward or drifting downstream on a silk thread attached to the substrate. Food particles are caught in the large fan-shaped mouth brushes and periodically combed and food passed to the mouthparts. The larvae (Figure 4.23d) are positioned within the water flow with the posterior abdomen pointing upstream, which ensures a laminar flow over the body and into the head fans. The last instar larva weaves a slipper-like or bag-shaped cocoon (Figure 4.23f,g) from salivary gland secretions with the pointed end of the cocoon pointing upstream. It then pupates in the cocoon with only its gills protruding from the open end of the cocoon and holding itself in place by dorsal spines and hooks on the abdomen.

Adult blackflies are diurnal and exophilic, the females seeking their bloodmeal in the open (exophagous). Biting takes 3–6 min during which they take on their own body weight in blood. Like other poolfeeders, a small droplet of blood remains on the host at the site of the bite. Blood feeding usually takes place every few days. Hourly biting rates of vector species on humans can vary between 30 and 60 in the African savannah to 400–500 in the Amazon and pest species can exceed 1000 per hour. Many blackfly species have feeding site preferences; members of the *S. damnosum* complex prefer the legs, the Central American *S. ochraceum*-complex bite on the head and trunk, and the European species *S. ornatum* bites the belly of cattle. These sites are always the areas where the microfilariae of *Onchocerca* accumulate and thus maximize their chances of transmission.

Adult blackflies can fly considerable distances to find hosts and then oviposition sites. These distances range from 15 to 30 km, through 150–225 km in the arctic up to 400–600 km in the West African savannah by members of the *S. damnosum* complex. The very long-range movements are usually wind-assisted, especially by major weather systems.

In addition to their annoyance, sometimes extreme annoyance due to very high biting rates, the bites of blackflies can result in severe skin reactions lasting several months. Bites can cause disseminated systemic effects in sensitive people leading to headaches, feverish sweating and shivering, nausea, acuting aching joints, lassitude, and psychological depression. Other mammals, especially domestic

animals, can suffer from a similar syndrome, even leading to death, where mass emergence and biting occurs.

Morphology The adults are small (1.4–6.0 mm) and many are black, although several neotropical simuliids, including the vector *S. ochraceum,* are yellowish-red. They have short and stout antennae (Figure 4.23a,b), unlike most other lower Diptera, but they do have seven to nine distinct segments typical of the suborder. The compound eyes are large, often covering most of the head indicating their important role in mating and host detection. Simuliids are telmophagous, that is, poolfeeders and have a short stout proboscis containing the piercing mandibles, laciniae, hypopharynx, and labrum sheathed by the more fleshy labium and labellar lobes (Figures 4.24 and 4.25). The wings are longer than the abdomen and characteristically very broad and triangular. The forelegs are often long and seen testing the substrate.

The eggs are oval-triangular and the distinctive larvae with their well-developed head capsule (eucephalic) and large posterior abdominal segments are 4–12 mm long (Figure 4.23c). The head bears simple eyes, short antennae, a pair of conspicuous, large labral fans (Figure 4.23d), which filter water passing over the

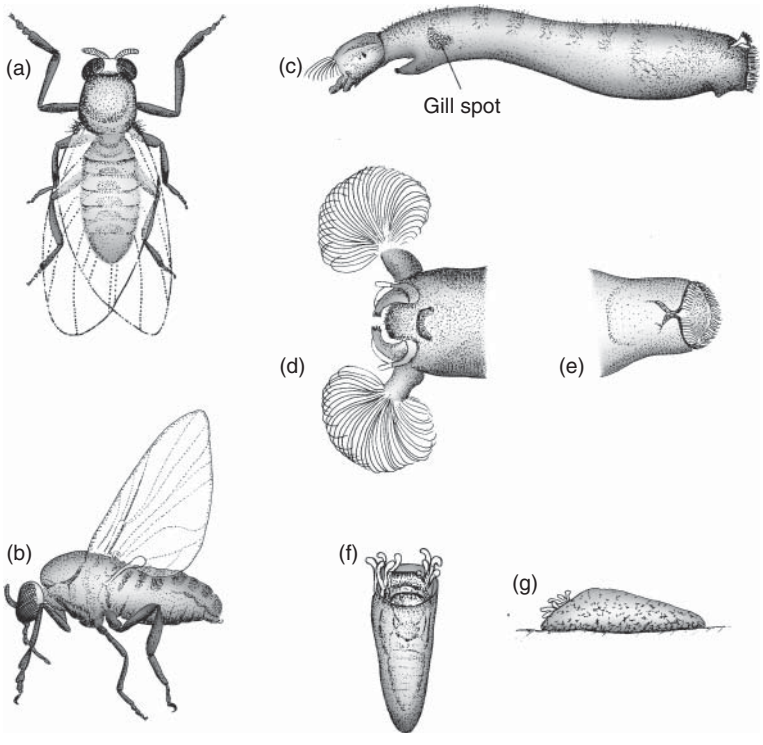


Figure 4.23 Simuliidae. (a) Female *Simulium* sp., dorsal view. (b) Lateral view. (c) Last larval instar. (d) Anterior part of larva with mouth brushes. (e) Posterior part of larva with ring of hooks. (f) Dorsal view of a pupa in its cocoon. (g) Lateral view of cocoon.

larva, and chewing mouthparts. The thorax bears a single anterior proleg with a terminal circlet of hooks. A similar and slightly larger ring of hooks occurs on the posterior end of the body (Figure 4.23e). The last larval instar is recognized by a dark "gill spot" of the developing pupa (Figure 4.23c). The pupa is housed in a cocoon (Figure 4.23f,g). The pupal gills, which can be elongated and branched or short and tubular, are situated on the cephalothorax and are homologous to the respiratory horns of the Culicidae and Ceratopogonidae.

Disease Transmission Simuliids transmit protozoans and filarial worms, but interestingly, there is no evidence yet of significant arbovirus or bacterial transmission, unlike other biting lower Diptera. The main parasites are:

- The protozoan *Leucocytozoon* (Apicomplexa), blood parasites of poultry (*Leucocytozoon smithi*), the cause of the economically important "turkey malaria," and *Leucocytozoon simondi* of ducks.
- The filarial nematode *Dirofilaria ursi,* a parasite of bears, and the human parasite *Mansonella ozzardi,* neither of which are very pathogenic.
- Blackflies are intermediate hosts of the nematode genus *Onchocerca*, inhabiting humans and mammals in temperate and tropical regions of the world. By far, the most important parasite transmitted by simuliids is *O. volvulus,* which causes river blindness in Africa and Central and South America (see Section 3.3.2).

Control – the main focus of control, especially for river blindness in the major foci in Africa and South America is by attacking the larvae in their riverine habitats. Larvae are very susceptible to low doses of chemical insecticides and the biocontrol agent *Bacillus thuringiensis israelensis,* which are applied upstream of major breeding sites. The filter-feeding larvae take up the granular formulation. One of the most ambitious and very successful international vector control programs, the Onchocerciasis Control Programme, was organized to control blackflies in West Africa (Section 3.3.4.14).

4.4.8.7 Phlebotominae – Sandflies

- Hematophagous on reptiles, birds and mammals.
- Small fragile insects.
- Four larval instars.
- Larvae apode eucephalic, in relatively dry habitats.

Phlebotomine sandflies are delicate hairy flies with long slender legs. The flies are easily distinguished from other small Diptera by the way, in which they hold their narrow pointed wings above the body in an inverted V, especially from other members of the family Psychodidae, to which they belong as a subfamily. Their Greek name (*phleps, phlebós* = blood vessel, *tomé* = incision) indicate that they are poolfeeders. Sand flies are vectors of kinetoplastids, most notably *Leishmania* (see Section 2.4). There are about 700 species found throughout the tropics

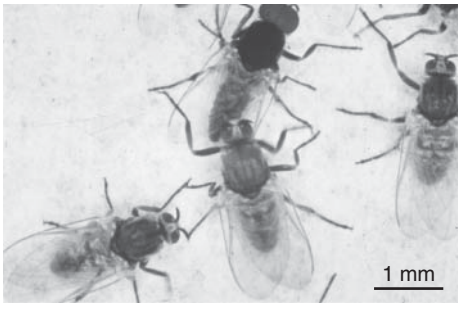


Figure 4.24 Simuliidae. (Image: Archive of the Department of Parasitology, University of Hohenheim.)

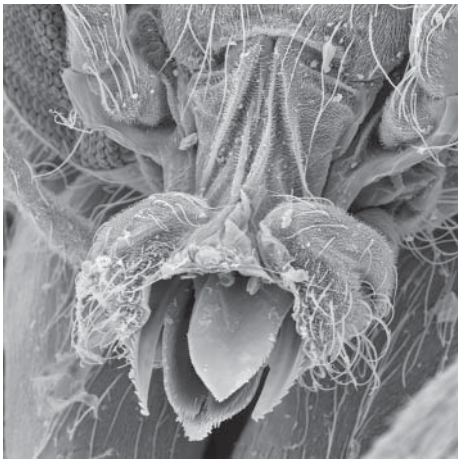


Figure 4.25 Simuliidae, mouth parts. Center (broad): Mandible (the second mandible is not visible). Below mandible (broad): Labium and fused with it hypopharynx (with orifice of salivary duct in the center). At both sides (slender, serrated): Maxillae. Externally (two cushion-like structures). Labrum. (EM image: Courtesy of Eye of Science.)

and subtropics of the Old World and New World, with more than half of the species in the New World. About 70 species are involved in the transmission of parasites to humans and other mammals, particularly *Leishmania* transmitted by species of the genus *Phlebotomus* in Africa, Asia, and Europe and by *Lutzomyia* in the neotropics. The widespread Old World genus *Sergentomyia* feeds mainly on reptiles.

Development and Biology Unlike the mosquitoes, simuliids and ceratopogonids, comparatively little is known about the life history of sandflies, because the immature stages are very difficult to find in nature. Their larvae are terrestrial, feeding on organic material in soil and debris accumulated in crevices, animal burrows

and shelters, caves, or damp leaf litter in tropical forests. Although the breeding sites need to be damp, larvae will migrate away if it becomes too wet. The four larval instars and the nonmotile pupae live in the same site and will diapause in subtropical areas, such as the Mediterranean vector *Phlebotomus ariasi*.

After emergence, the adults seek cool humid, but not wet, places to rest such as caves, tree buttresses in tropical forests, rock cracks, animal shelters, and so on. Mating is usually associated with the host, some species (e.g., *Phlebotomus argentipes* and *Lutzomyia longipalpis*) forming mating leks (a Swedish word for aggregations of animals to find partners), and communication between sexes is by courtship "songs" produced by male wing vibrations together with species-specific sex pheromones.

Only females feed on blood using nutrients to develop eggs. An extremely potent vasodilating peptide is injected into the wound to produce an extravascular pool of blood, which the fly then sucks up. Both males and females feed on sugars. The adults are nocturnal or crepuscular, but will bite during the day if disturbed, in forests or caves for example. They fly toward the host and then advance by a series of little hops, feeding on exposed surfaces only. Most cutaneous leishmaniosis lesions are on exposed areas for this reason. The bite can be painful, one local name translates into "flying ash," which perfectly describes the bite. Blood is taken into the midgut and sugars into the crop before later movement into the gut. Parasites attach to the midgut epithelium before the formation of the peritrophic membrane around the bloodmeal, later migrating to the foregut and forming a bolus before transmission at the next bite.

Morphology Adults (Figure 4.26a) are small, measuring only 1–4 mm, and are usually light or dark brown or occasionally pale gray such as in the major visceral leishmaniasis vector *P. argentipes.* The body, head, and wings are covered in tufts of hairs. The long antennae have 16 segments. The proboscis (Figure 4.25) is broad and short with the labrum, paired mandibles and laciniae, and hypopharynx within the labium. The mandibles have saw-like serrations at their tip and the lacinae have outwardly facing teeth. Sandflies are poolfeeders with the mandibles

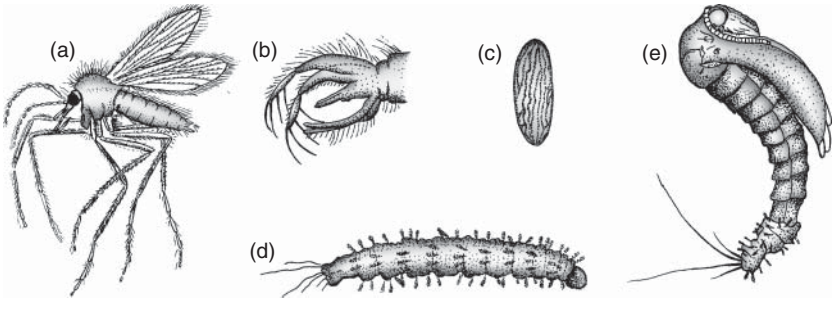


Figure 4.26 Phlebotomidae. (a) Female *Phlebotomus* sp. (b) Copulatory appendage of a male. (c) Egg of *P. papatasi*. (d) Larva. (e) Pupa, standing in upright position fixed on larval exuvia.

cutting a small hole in the host, while the laciniae hold the mouthparts in the host's skin. The long five-segmented maxillary palps bear numerous sensilla on the third segment, which are applied to the host during feeding. The thorax is humped and bear the lanceolate wings with a characteristic venation of several long veins running the length of the wing (Figure 4.26a). Males possess prominent genital terminalia with long claspers (Figure 4.26b).

Eggs are long-oval, somewhat flattened on one side, and have a sculptured surface (Figure 4.26c). The eucephalic larvae have short club-shaped hairs over their body and the last segment bears long bristles, one pair in the first instar and two pairs in subsequent instars (Figure 4.26d). The pupa stands upright, secured to the substrate by the larval exuvia (last cuticle), which is retained at the end of the abdomen (Figure 4.26e).

Disease Transmission Phlebotomines can be a biting pest, such as *Phlebotomus papatasi* in the Middle East, where sensitized persons can experience a long-lasting rash known as "harara" in endemic areas.

Sandflies transmit viruses, bacteria, and Kinetoplast protozoa (*Leishmania*).

- Sandfly fever (3-day fever or papataci fever), is an acute febrile illness of humans lasting 3–4 days caused by a *Phlebovirus* (Bunyaviridae). It occurs in the Eastern Hemisphere, where it is transmitted mainly by *P. papatasi,* but also by *P. perniciosus* and *P. perfiliewi*. No natural vertebrate reservoirs are known and although humans can act as amplifying hosts during epidemics, maintenance of the virus is probably by transovarial transmission. Other viruses are transmitted by sandflies in the Old World, for example, Toscana virus, and the New World, for example, Punta Toro virus.
- Carrións disease (Oroya fever or Verruga Peruana, Peruvian Wart) is caused by *Bartonella bacilliformis*. It is a rare infectious disease found only in the high Andes of Peru, Ecuador, and Colombia between 750 and 2500 m above sea level, its distribution governed by the ecology of its *Lutzomyia* vector.
- The most important parasites transmitted by sandflies are members of the kinetoplast genus *Leishmania* throughout Old and New World tropics and subtropics. Leishmaniases are all zoonoses, except visceral leishmaniasis in India, Nepal, and Bangladesh, transmitted by *P. argentipes* and cutaneous leishmaniasis caused by *Leishmania tropica* in the Middle East, where humans are the only known host. Detailed studies of the natural history of the sandfly vectors, the wild mammal reservoir, and the people infected are required to elucidate the often complex epidemiology (see Section 2.5.9).

Control Vector control is generally restricted to controlling only the adults of those species, which are peridomestic such as *P. argentipes, P. papatasi,* and the *L. longipalpis* complex. Indoor residual insecticides are used in houses and associated cattle sheds in Northeast India, Nepal, and western Bangladesh against *P. argentipes* in large control programs. In many foci, the low infection rate in

humans in relation to the low and sparse populations of sandflies makes vector control unrealistic and other methods, such as reservoir control, are used.

4.4.8.8 Brachycera

Old classifications of the Diptera recognized a narrowly defined group known as the Brachycera (including horseflies and hover flies) and the Cyclorrhapha with two subdivisions: calyptrates (houseflies, blowflies, tsetse, and ectoparasitic Pupipara) and acalyptrates (many families, most plant feeding but also containing the endoparasite botflies). These groups have now been shown to be paraphyletic and replaced with a much more complex classification with a redefined Brachycera (Greek: *brachys* = short, *kéras* = horn (antenna)) incorporating all the above groups, infraorders, and several superfamilies. Unlike in the lower Diptera, few evolutionary trends can be seen in the Brachycera in relation to parasitism or vector transmission, other than the secondary development of bloodfeeding in the adult males and females and the development of a parasitic lifestyle in the larvae, not adults, of botflies (Oestroidea).

Members of this large suborder of the Diptera are usually compact, robust insects in marked contrast to the lower Diptera (former Nematocera). Their larvae are apodous "maggots" with a reduced or no head capsule at all. The adult antennae are not simply short, but also have only three to eight segments. The mouthparts are piercing-sucking in the basal groups (many are predaceous on other insects) and sponging/suctorial in the higher groups (e.g., muscids or houseflies). In general, the larvae are found in moist habitats, in the soil, decaying materials and in plants, although some are free-living hunting other insects.

4.4.8.9 Tabanidae – Horse Flies

- Large, stout, insects with large eyes and strong flight.
- Females suck blood of ungulates and humans.
- 6–13 larval instars.
- Larvae apode and acephalic, living on small invertebrates.
- Obligatory vectors of the filarial nematode *Loa loa* of man.

The horse flies or clegs are large, hematophagous insects (6–30 mm long) with about 4500 species distributed in all tropical and temperate regions except deserts and some oceanic islands. They are adapted for feeding on the blood of large vertebrates, including humans. The genera of medical or veterinary importance are *Chrysops*, *Tabanus, Hybomitra,* and *Haematopota*. They can be a troublesome nuisance for human and animals. Several are mechanical vectors of viruses, bacteria, and protozoa, but they are intermediate hosts of suprisingly few parasites, including the filarial nematode *Loa loa*.

The tabanidae are sometimes arranged as Tabanomorpha to distinguish them from the bulk of all the other Brachycera, the Muscomorpha.

Development and Biology Eggs are deposited in water or damp mud as oval patches (Figure 4.27c) of 200–10 000 eggs. There are 6–13 larval instars, the last can overwinter so the life cycle can take over a year to complete. The larvae of many species are predacious, feeding voraciously on invertebrates, such as snails, earthworms, or even tadpoles. Pupation takes place on dry land.

Adult Tabanidae are diurnal, especially flying in bright sunshine. Only the females feed on blood, seeking hosts by detecting movement in particular with their enormous eyes (Figure 4.27a), rather like tsetse flies, which also feed on large mammals in open habitats. For short distances, their speed can reach 40 km/h. The bite can be painful, typical of poolfeeders, especially given their large size. They fly and land silently and begin to feed before the host detects and swats them. When disturbed, they quickly seek another host, increasing their potential for mechanical transmission of pathogens. Adult tabanids live only for 2–4 weeks. The males feed on nectar or pollen.

Morphology The aquatic larvae are tapered anteriorly and posteriorly (Figure 4.27d) and have a reduced and retractable head capsule. The last segment bears a respiratory siphon posterodorsally. Mature larvae are 15–30 mm long, tapering at both ends. Pupae have legs and wings atteched to the body (Figure 4.27e). Eggs are deposited in damp substrates, as variously layered lumps (Figure 4.27c). The adults, up to 3 cm long, are stout, often large insects (Figure 4.27a,f). They are easy to recognize as their bean-shaped head is broader than long (also broader than the thorax) and seems to be composed of the huge eyes, touching in the males (holoptic) and narrowly separated in females. Eyes, wings, and sometimes parts of the body can be conspicuously colored in stripes or patches. The conspicuous antennae project forward (Figure 4.27g). The

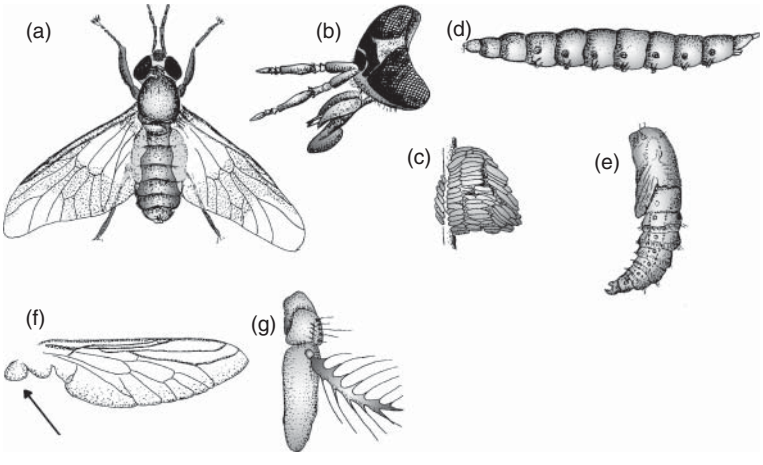


Figure 4.27 Brachycera. (a) Imago of the tabanid *Chrysops* sp. (b) Head of *Chrysops* sp. (c) Clutch of eggs of a tabanid. (d) Tabanid larva. (e) Tabanid pupa. (f) Wing of a higher fly with Calyptra (arrow). (g) Antenna of a higher fly with antennal bristle (Arista).

mouthparts (Figure 4.27b) are telmophagous and combine lapping with biting elements. Six stylets (a broad labrum, a thin hypopharynx, two thin mandibles, and maxillae) slash the skin and cut a bleeding wound. The cushion-shaped labellum is placed onto the skin and blood runs up a series of furrows on the ventral side, "pseudotracheae," into the mouth. Blood-containing pathogens adhering to the pseudotracheae can subsequently be dabbed onto the wound of another host mechanically transmitting an infection. The wings are either folded over each other or spread obliquely away from the body.

Transmission of Diseases The bites of tabanids often elicit severe swelling in humans, including the peripheral lymph nodes. Their abundance can be so high that they affect outdoor working in some areas and impact tourism.

Tabanids transmit several nematodes, protozoa, bacteria, and viruses:

- Loiasis. The filarial worm *Loa loa* is transmitted to humans by at least two species of *Chrysops*, *Chrysops silacea* and *Chrysops dimidiata,* known as mango or deer flies, in forest areas of Central and West Africa. This is a rare instance of a blood-borne nematode transmitted by a poolfeeder. Other *Chrysops* species transmit a related *Loa* species with a nocturnal microfilarial periodicity to monkeys in the same area.
- Tabanidae of the genera *Tabanus* and *Hybomitra* are intermediate hosts of another filariid nematode, *Eleaphora schneideri* of the mule deer *Odocoileus hemionus* in western North America. It is also an important pathogen of the wapiti *Cervus canadensis*, as well as of sheep and moose (*Alces alces*).
- Mechanical transmission of several pathogens by tabanids has been recorded, although evidence is not always very robust: viruses such as Equine infectious anemia virus (EIAV, Retroviridae), bacteria such as *Bacillus anthracis* (Anthrax), *F. tularensis* (Tularemia or rabbit fever), *Leptospira interrogans* (Leptospirosis, Weil's syndrome), *Borrelia burgdorferi* (Lyme disease), and the protozoan *Trypanosoma evansi* (Surra and Mal de Caderas or Dourine).

4.4.8.10 Muscidae – House and Stable Flies

- Larvae live on rotting plant materials or feces of herbivores.
- Three larval instars.
- Only *Stomoxys* as obligatory bloodfeeder, in both sexes.

The Muscidae are a large family of the Brachycera containing house flies and their relatives, which have secondarily developed bloodsucking mouthparts from the sponging/lapping mouthparts found in the majority of "higher" Diptera.

Although most of them are not bloodfeeding like many other vectors or intermediate hosts, several muscids with sponging/lapping mouthparts are intermediate hosts of nematodes and mechanical vectors of viruses, bacteria, fungi, and protozoa. These species lap up secretions from mammals, around the nostrils or eyes, and have common names to reflect this habit such as the face fly (*Musca*

autumnalis) and the sheep head fly (*Hydrotaea irritans),* as well as the familiar house fly *Musca domestica*.

Hematophagous flies with piercing mouthparts are present in genera such as *Stomoxys, Haematobia,* and *Haematobosca*. They feed on large mammals and, rarely, humans. Both sexes feed on blood. *Stomoxys calcitrans*, the cosmopolitan stable fly, is common in and around stables and barns of cattle and pigs, but in agricultural surroundings, it can also attack humans relentlessly. *Haematobia irritans*, the horn fly, is a European species introduced to North America. It lives densely aggregated on cattle, female flies leaving the host only for egg-laying.

Biology Many Muscidae deposit their eggs on feces of herbivorous animals or in decaying organic matter on which the larvae feed before migrating to a drier environment to pupate. Adult flies feed on protein-rich substrates such as eye and nasal secretions and can be remarkably persistent in obtaining a meal. The blood-sucking *S. calcitrans* preferentially feeds on the lower body, especially on the front feet of cattle, taking 16.5 mg of blood twice a day.

Morphology Flies measure 5–8 mm, have prominent eyes, and the antennae consist of three very different shaped segments, the last one bearing a bristle, the arista (Figure 4.27e). The mouthparts of the nonbiting Muscidae (e.g., *Musca*) have a cushion-shaped labellum with pseudotracheae on the underside into which liquids flow by capillary action and then into the mouth. The mouthparts are extended forward when feeding, mopping liquids up as the fly moves forward. In *Stomoxys* and *Haematobia,* the labellum is heavily sclerotized both along its shaft and at the tip. The mouthparts project forward in *Stomoxys* (Figure 4.28a) and can be seen clearly from above. To feed, the fly rasps the host's skin with spines on the tip of the proboscis before the whole rigid structure is inserted and blood and cells are sucked up. The tarsi have a pair of claws and a pad-like pulvillus, which allows the flies to walk on vertical, or even inverted, smooth surfaces. The females of some Muscidae have a telescopic, retractible ovipositor, with which they deposit eggs in wet substrates.

There are three larval instars and the maggots lack legs and a head capsule (Figure 4.28c) but have a pair of long screrotized and curved structures for feeding on soft materials. The spiracles at the end of the abdomen differ between genera and species. (Figure 4.28e–j). The pupae are tightly enclosed in a chemically tanned skin of the last larval instar forming a brown puparium.

Disease Transmission Muscids transmit, viruses, bacteria, and nematodes to large mammals:

- *S. calcitrans* can mechanically transmit the viruses causing Equine infectious anemia (EIA) of horses, mules, and donkeys and the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV). They can also transmit *Trypanosoma brucei evansi*, the causative agent of "Surra" or "Mal de Caderas" in camels, equines, and other animals in South America.

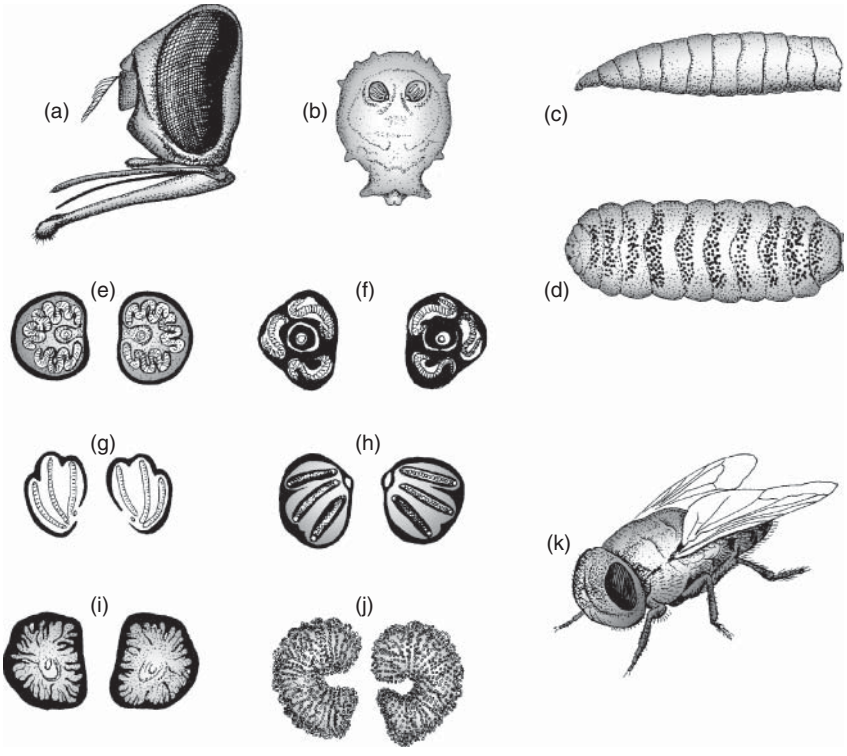


Figure 4.28 Brachycera. (a) Head of *Stomoxys calcitrans* (mouthparts from top to bottom: Maxillary palps, labrum, hypopharynx, labium). (b) Third larval instar of *Lucilia sericata*, rear view. (c) Third larval instar of *Calliphora cuprina* (head: left-hand side). (d) Ventral view of the third larval instar of *Oestrus ovis*. (e–j) Stigmal plates of (e) *Musca domestica*, (f) *Stomoxys calcitrans*, (g) *Sarcophaga* sp., (h) *Lucilia sericata*, (i) *Hypoderma bovis*, (j) *Oestrus ovis*. (k) Imago of *Oestrus ovis*, note the strongly reduced mouthparts.

- Muscidae are intermediate hosts of the nematodes *Habronema muscae*, *Habronema microstoma,* and *Draschia megastoma,* which can cause conjunctival, cutaneous, or gastric disease. The eggs of *Habronema/Draschia* species or their larvae are ingested by maggots and develop in the larvae and pupae emerging from the adult fly when they feed around the lips of horses. In *S. calcitrans*, which also serves as intermediate host for *Habronema/Draschia* species, the infected fly's behavior is modified to feed on moist surfaces of the horse rather feeding on blood thus maximizing transmission.
- Other nematodes transmitted by muscids are: *Musca autumnalis* is the intermediate host of *Thelazia* species (Nematoda, Spiruromorpha) and of the filarial nematode *Parafilaria bovicola. S. calcitrans* is also an intermediate host of the cosmopolitan *Setaria cervi* (Onchocercidae), which inhabits the abdominal cavity of cattle. The horn fly *H. irritans* is the intermediate host of the

common filarial nematode *Stephanofilaria stilesi* of cattle, buffalo, bison, yak, and various deer, but rarely in sheep. In western and southwestern United States and Canada, it can affect up to 80–90% of a herd but is not pathogenic.

4.4.8.11 **Calliphoridae – Blowflies, Screwworms**

- Adults fly-shaped, not bloodfeeding.
- Larvae live on dead or living flesh.
- Larvae of some genera are obligatory parasites, causing Myiasis.

Calliphoridae are stout flies with a metallic and colored appearance, and are slightly larger than house flies. There are about 1100 species and they are notable in that they lay their eggs in vertebrate tissues, usually dead tissues but in a few species, living tissues. Several genera are obligate parasites of land vertebrates, especially the screwworm flies *Cochliomyia*, so called because their larvae have rows of spines in a screw-like manner around their body. The New World screwworm *C. hominivorax* attacks most domestic animals and had a devastating impact on cattle ranching in the Americas until a massive control program using, from the nineteen-sixties onward, sterile male release reduced its impact in the United States and Mexico.

In the Old World, species of *Chrysomya* occupy a similar niche. The females of all screwworms lay eggs on living animals either near wounds or at natural orifices (ears, nose, mouth, urogenital passages). The larvae feed very aggressively and consume much tissue for 3–4 days before dropping off to pupate. The larvae of several genera live in animal nests, for example, bird nests, and feed on the growing young. One species, the delightfully named Congo floor maggot, *Auchmeromyia senegalensis* feeds on humans in huts with mud floors.

In Australia, *Lucilia cuprina*, the Australian sheep blow fly, is a major pest being responsible for over 90% of all "flystrike," where the eggs are laid on soiled wool fleece and the second instar larvae aggravate the skin by feeding on it and causing large red-raw wounds. Secondary infections are common and the sheep suffer from severe discomfort and in some cases die. *L. cuprina* also occurs in Africa and North America. *Lucilia sericata*, the green bottle, normally deposits its eggs on carrion and meat, but occasionally causes fly strike on sheep (Figure 4.28b). Its use in medicine has recently been revived to clean wounds or tissue damaged by trauma in a procedure known as Maggot debridement therapy (MDT). The larvae debride (clean) wounds by consuming the dead (necrotic) tissue and their activity stimulates wound healing.

4.4.8.12 **Oestridae – Bot or Warble Flies**

- Adults colored and haired, reduced mouthparts.
- Larvae are obligatory endoparasites.
- Four subfamilies with larvae under skin, in stomach or nose.

The larvae of bot or warble flies are remarkable internal parasites of mammals. The larvae are highly modified, some have bulging segments with ribbons of ridges or thorns (Figure 4.28d). The adults are large (9–25 mm) and are covered by dense colored hairs and many resemble bumblebees. They have vestigial or lacking mouthparts (Figure 4.28k) and do not feed. The eyes are comparatively small. Little is known of the males.

The larvae of the South and Central American subfamily **Cuterebrinae** are mostly parasites of rodents and lagomorphs, some living in such specialized locations as the scrotum of particular rodent species. The flies are large (12–27 mm). The most well-known species is *Dermatobia hominis*, the human botfly of Central and South America, which parasitizes humans, cattle, dogs, monkeys, and pigs. Female *D. hominis* capture bloodsucking insects such as mosquitoes, other biting flies and even ticks and glue up to 28 fully developed eggs onto them. When these alight on a mammal, the sudden increase in temperature induces the fly larvae to hatch and the first instar larva invades the host's subcutaneous connective tissue forming a small cell, in which it grazes on host tissues. The larva is highly modified with a bulbous anterior section with backward-pointing spines and a slender posterior section with terminal spiracles through which it breathes air. The wound does not often get infected, because bacteria in the larval gut circulate in the wound-producing bacteriostatics that suppress other bacterial growth. After 5–10 weeks, the third instar larva drops from its host and pupates in the ground. *D. hominis* causes considerable financial losses to cattle farmers by leaving holes in the hide reducing their value.

Species of **Gasterophilinae** are parasites of odd-toed ungulates and elephants and are notable because the larvae live in the stomach of their hosts, sometimes forming mats of larvae attached head first to the gut lining. The adults are large (9–35 mm) and yellow or brown, often resembling honey bees. Short-lived adults do not have mouthparts and cannot feed. The females lay eggs on their prey, usually on the legs or on surrounding vegetation, in a species-specific manner. When ovipositing, females of *Gasterophilus intestinalis* curve the abdomen forward under the thorax and dart toward the host and, while still flying, deposit an egg on hairs of the host's legs. When ingested with plant material or licked off from the host's hairs, the eggs hatch rapidly and the young larvae migrate to the mouth, lips, cheeks, or tongue. Subsequent instars move down the gut and attach to the wall of the stomach or the rectum, where they feed on tissue exudates. They do not feed on blood. After being voided with the feces, they pupate in the soil for 20–30 days. The first larval instars cause discomfort in swallowing and chewing. First larval instars of *Gasterophilus inermis* burrow in the cheeks causing a condition known as summer dermatitis. The second and third larval instars in the stomach, duodenum or rectum lead to inflammation, and interruption of peristalsis or secretory functions. If present in large numbers, infestations can be fatal.

The subfamily **Hypodermatinae**, or warble flies, are skin parasites of herbivorous mammals and two species in particular are significant cattle parasites in the Northern Hemisphere. *Hypoderma bovis* and *Hypoderma lineatum* are found

in Europe, Canada, the United States, and northern Mexico. The bumblebee-like adults (0.6–1.1 mm) of *H. bovis* have a characteristic buzzing noise when they are ovipositing, which alarms cattle, causing them to run away frantically ("gadding," the Greek word *oiestro* for rage or fury stands for the family name Oestridae). The eggs of *H. bovis* are glued singly onto a hair on the posterior part of the host's body, those of *H. lineatum,* which oviposits silently, in lines of 3–20 on hairs more anteriorly. After 4 days, the eggs hatch and the first instar larvae penetrate the skin and begin to migrate along muscle, connective tissue or nerve cords. In *H. bovis,* the L1 migrates via the spinal column, where they accumulate in the epidural fat of the thoracic and lumbar vertebrae for 2–4 months, while in *H. lineatum,* the larvae migrate to the submucosal connective tissue of the esophageal wall. Finally, the larvae migrate to the subdermal tissue of the back and a fibrous tissue develops around each larva to form a cyst or "warble." The final instar forms a breathing hole in the host's skin and when fully developed drops from the host to the ground where it pupates. The impact of *Hypoderma* on cattle is from the damage caused during gadding, destruction of muscle tissue, and finally damage to hides from the breathing holes made during the warble stage. The prevalence of both *Hypoderma* species has declined dramatically in North America.

The **Oestrinae**, the fourth subfamily of the Oestridae, are the nasal botflies, which parasitize ungulates and marsupials. *Oestrus ovis*, the sheep bot fly (Figure 4.28k), attacks sheep but also occurs on deer, goats, and sometimes cattle in many tropical and subtropical regions of the world. The females "shoot" the 1-mm-long first instar larvae directly into the nose of a prospective host during flight. The larvae migrate up the nasal passage and enter a nasal sinus. At the completion of development, the third larval instar (Figure 4.28d,j), measuring 30 mm, moves down the nasal passage, drops to the soil and pupates. The larvae cause irritation to the mucosa, with mucous discharge, swelling of the internal membranes of the nose, and possibly impairment of breathing. Sheep may reduce or stop grazing and subsequently lose weight and condition, which can in some cases lead to malnutrition and death.

4.4.8.13 **Glossinidae – Tsetse Flies**

- Obligatory bloodfeeders, in both sexes.
- Viviparous, larvae pupate in soil.
- Present only in tropical Africa, genus *Glossina*.
- Vectors of trypanosomes, which cause sleeping sickness and Nagana.

The Glossinidae or tsetse flies are closely related to three other families, which all share two key features – they are obligate bloodfeeders in both sexes and the female nourishes the larvae in her abdomen giving birth to a third instar larva that instantly pupates. The group used to be called the "Pupipara" but now is referred to as the Hippoboscoidea.

The family Glossinidae contains only one genus, *Glossina*, which is only present in Africa and two small foci in southwestern Saudi Arabia. The distribution of

tsetse in Africa is called the "fly belt," an 11 million square kilometer area north of the Kalahari and south of the Sahara. Here, sleeping sickness caused by trypanosomes prevails and cattle production is severely limited, because the animals are seriously affected or killed by "Nagana."

The genus *Glossina* consists of 23 species, split into three groups, based on a combination of distributional, behavioral, and morphological characteristics (Table 4.5).

Development and Biology The development of the larvae is quite remarkable. After mating, usually only once, a single egg is generated, which is retained in the base of the oviduct, or uterus. The developing larva feeds on a secretion produced by a modified accessory reproductive gland, called the milk gland. This phenomenon is called adenotrophic viviparity. Larviposition takes place on day 10, where upon the larva immediately burrows into the soil and within a few hours forms the hard shell of the puparium. A new larva is generated every 7–11 days so that in her lifetime, a female tsetse fly will produce up to 12 offspring, a reproductive rate similar to mammals. After 30 days, the puparium splits and the young adult crawls to the surface. The site of larviposition and pupation is critical for it has to have precise humidity and consistency, conditions met where the soil is shaded, contains humus and the annual precipitation is at least 500 cm/year. These requirements are key determinants in habitat selection by tsetse, and since they do not fly long distances, they influence the hosts they can feed on, the risk of infection, and targeted control measures.

Table 4.5 Characteristics of the three subgenera of *Glossina*.

Group (subgenus)	Size (mm)	Type of disease	Biotope	Species
Morsitans	7.5–11	Nagana, sleeping sickness	All types of savanna nearly to the margins of deserts	**Savanna flies** *G. austeni, G. morsitans, G. pallidipes, G. swynnertoni*
Fusca	10–13	Nagana	Rain forests and their extensions, tree savannna	**Forest flies** *G. fusca G. nigrofusca, G. fuscipleuris, G. schwetzi, G. longipennis, G. severini, G. medicorum, G. vanhoofi, G. tabaniformis, G. brevipalpis, G. longipennis, G. haningtoni, G. frezili, G. nashi*
Palpalis	6.5–11	Sleeping sickness, Nagana	Rainforests and extensions, wadis and lakeshores far into savanna	**Riverine flies** *G. palpalis, G. tachinoides, G. caliginea, G. fuscipes, G. palicera*

The adults are predominantly diurnal, resting in vegetation until they seek a bloodmeal. As both sexes feed on blood and the larvae only feed inside the female, the whole life cycle is dependent on vertebrate blood. Tsetses are exceptional among Diptera in that they do not require a plant-derived carbohydrate for flight. Flies have to feed every second day and produce an audible, almost intimidating noise in flight.

Hosts are first located by visual stimuli, especially movement, so that all objects moving quickly, whether they are animals, cars, or bikes, are chased. Closer, other visual stimuli, such as shape and color, become important together with olfactory stimuli. Flies of the different species groups (Table 4.5) differ in their response to host odor, those of the savannah dwelling *morsitans* group are very much more sensitive to components of cattle urine (e.g., 1 octen 3-ol) or breath than the scrub-dwelling *palpalis* group, which relies mainly on carbon dioxide cues. Olfactory stimuli can attract flies over long distances, as long as there is a distinct odor plume. The main source of blood is mammals but reptiles are also important in the riverine forest species. Most tsetse species have a well defined host range, determined by availability within a particular habitat as well as by the age of flies; they are less choosy for their first feed than later in life. When large wild mammals become scarce, domestic cattle, pigs, and goats will be attacked. Suprisingly, some large mammals are not attacked (e.g., zebras), even humans are not particularly attractive to tsetse and some are even repelled. The species that feed on humans most frequently are *Glossina palpalis*, *Glossina tachinoides,* and *Glossina fuscipes*. Of them, 80% or more are male flies. Females usually attack larger animals than males.

Unlike many other bloodfeeding flies, tsetses often occur at low densities and therefore do not often constitute biting pests. The main impact of *Glossina* is as vectors of animal trypanosomiasis rather than human sleeping sickness. Indeed, the lack of development, especially agricultural development, in many parts of Africa is constrained by the presence of tsetse flies.

Morphology The adults are slightly larger than a housefly (Table 4.5) and show two important characteristics when the fly is at rest (Figure 4.29a): the wings are folded over the abdomen one on top of the other, looking like a tongue (Latin: *glossa)* and the distinct proboscis points forward. The antennal arista has branched hairs with numerous chemo-, thermo-, and hygro-receptors (Figure 4.29d) and the wing venation shows a characteristically shaped cell, the hatchet cell (Figure 4.29c). The mouthparts are identical in both sexes and at their base is a bulb-like swelling called the thecal bulb (Figure 4.29b), which contains muscles to manipulate the mouthparts. The piercing stylets are the labrum, hypopharynx, and labium, the latter with tiny teeth at the tip for cutting into the skin, protected by the pair of spiky maxillary palps. When the fly feeds, the mouthparts are lowered from the palps (not the labium as in other biting calyptrate flies) into a vertical position for piercing the host's skin. Tsetse flies are capillary feeders, either piercing a capillary directly or cuting capillaries to form a blood pool. The sexes are very similar, but

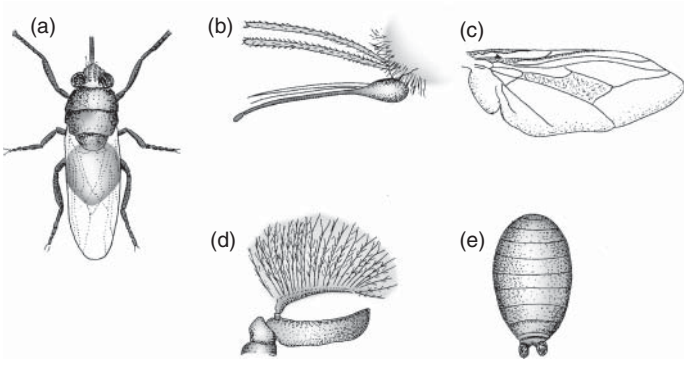


Figure 4.29 Tsetse flies. (a) Dorsal view of *Glossina* sp. (b) Mouthparts of *Glossina* sp. (shown splayed out, from top to bottom: The two maxillary palps, labrum, hypopharynx, labium). (c) Wing of a tsetsefly (shaded: the hatched-shaped discoidal cell). (d) Antenna with dorsally pilose arista. (e) Pupa.

can be distinguished by the hypopygium of the male, a knob-like structure folded underneath the last two segments and clearly visible with the naked eye.

The puparium, which is the shape of the third larval instar, has a conspicuous pair or large black swellings at the posterior end (Figure 4.29e). These are the polypneustic lobes, which carry many small holes through which the larva had been breathing. The fully developed puparium appears dark brown, but is rarely seen in nature as it is buried in soil.

Control Disease control relies mainly on vector control, because (i) vaccines are extremely difficult to develop because of trypanosome antigenic variation and (ii) drugs are relatively expensive and have to be administered each time an animal is ill and drug resistance is developing. In the past, vector control relied on extensive clearance of *Glossina* habitats, especially riverine forests and widespread spraying, including resting sites. Nowadays, vector control is achieved by (i) aerial spraying of nonresidual insecticides, using the sequential aerosole technique (SAT) with reduced volumes and lower concentrations; (ii) odor baited traps and screens impregnated with insecticides; (iii) sterile insect technique (SIT) by releasing laboratory-reared, gamma-radiated male flies, which prevent a female giving birth to offspring, since females mate only once in their lifetime. Genetic transformation of bacterial symbionts (*Wigglesworthia glossinidae, Sodalis glossinidius, Wolbachia pipientis*) to produce a hostile environment for transmission of the trypanosomes is an interesting research perspective.

4.4.8.14 Hippoboscidae, Nycteribiidae, Streblidae – Louse Flies, Keds and Bat Flies

- Adults bloodsucking, parasitic.
- Streblidae and Hippoboscidae with short flight periods.
- Mostly wingless, with highly modified appearance.

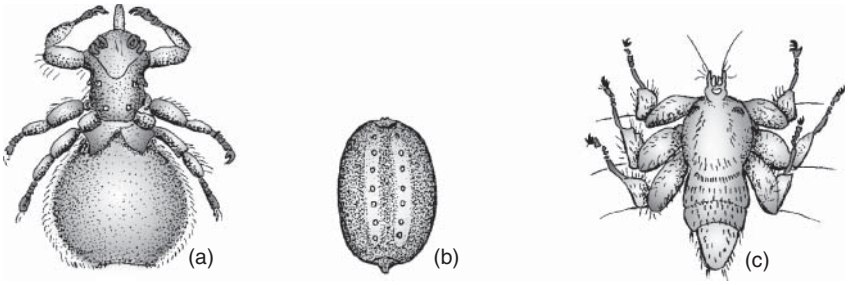


Figure 4.30 "Pupipara." (a) Imago of *Melophagus ovinus* (Hippoboscidae). (b) Puparium of *M. ovinus*. (c) Imago of *Lystropodia* sp. (Nycteribiidae).

These three families also have adenotrophic vivipary as in the Glossinidae, and are some of the most highly modified Diptera for their parasitic lifestyle on birds and mammals. The adults are dorsoventrally flattened, spiderlike, and the majority do not have wings (see also Figure 1.12). Those that are winged shed them when they locate a host. The best known member of the Hippoboscidae is the sheep ked *Melophagus ovinus* (Figure 4.30a,b), a wingless parasite of sheep. Other members of the family parasitize large mammals such as deer and are common on birds. Members of the Hippoboscidae are intermediate hosts of the protozoans *Trypanosoma* and *Hemoproteus*, and of a nematode, *Dipetalonema* sp.

The Nycteribiidae and the Streblidae, the bat flies, are permanent, flattened, and spiderlike ectoparasites (Figure 4.30c), exclusively of bats (Microchiroptera).

Further Reading

Aksoy, S. (2003) Control of tsetse flies and trypanosomes using molecular genetics. *Vet. Parasitol.*, **115**, 125–145.

Alphey, A., Benedict, M., Bellini, R., *et al.* (2010) Sterile insect methods for the control of mosquito borne disease: an analysis. *Vector Borne Zoonotic Dis.*, **10**, 295–311.

Bruce, W.N. and Decker, G.C. (1958) The relationship of stable fly abundance to milk production in dairy cattle. *J. Econ. Entomol.*, **51** (3), 269–274.

Colwell, D.D., Hall, M.J.R., and Scholl, P.J. (2006) *The Oestrid Flies: Biology, Host-Parasite Relationships, Impact and Management*, CABI Publishing, Cambridge.

Dittmar, K., Porter, M.L., Murray, S., and Whiting, M.F. (2006) Molecular phylogenetic analysis of nycteribiid and streblid bat flies (Diptera: Brachycera, Calyptratae): implications for host associations and phylogeographic origins. *Mol. Phylogenet. Evol.*, **38**, 155–170.

Giribet, G., Edgecombe, G.D. (2013) *Phylogeny of Arthropods. In Arthropod Biology and Evolution: Molecules, Development, Morphology* (eds A. Minelli, G. Boxshall, G. Fusco), Springer, Heidelberg, pp. 17–40.

Ishiwata, K., Sasaki, G., Ogawa, J., *et al.* (2011) Phylogenetic relationships among insect orders based on three nuclear protein-coding gene sequences. *Mol. Phylogenet. Evol.*, **58** (2), 169–180.

Johnson, K.P., Yoshizawa, K., and Smith, V.S. (2004) Multiple origins of parasitism in lice. *Proc. R. Soc. London, Ser. B*, **271** (1550), 1771–1776.

Kettle, D.S. (1995) *Medical and Veteriary Entomology*, 2nd edn, CAB International.

Kutty, S.N., Pape, T., Pont, A., *et al.* (2008) The Muscoidea (Diptera: Calyptratae) are

paraphyletic: evidence from four mitochondrial and four nuclear genes. *Mol. Phylogenet. Evol.*, **48**, 639–652.
Lane, R.P. and Crosskey, R.W. (eds) (1993) *Medical Insects and Arachnids*, Chapman & Hall, xv + 723 pp.
Lehane, M. (2005) *The Biology of Blood-Sucking in Insects*, Cambridge University Press, 321 pp.
Lyal, C.H.C. (1985) Phylogeny and classification of the Psocodea, with particular reference to the lice (Psocodea: Phthiraptera. *Syst. Entomol.*, **10** (2), 145–165.
Maroli, M., Feliciangeli, M.D., Bichaud, L., *et al.* (2013) Phlebotomine sandflies and the spreading of leishmaniasis and other diseases of public health concern. *Med. Vet. Entomol.*, **27**, 123–147.
Marquardt, W.C. (ed.) (2004) *Biology of Disease Vectors*, 2nd edn, Elsevier, 785 pp.
Misof, B., Liu, S., Meuseman, K. *et al.* (2014) Phylogenomics resolves the timing and pattern of insect evolution. *Science*, **346**, 763–767.
Pampiglione, S. *et al.* (2002) (Insecta, Siphonaptera), the two species of the genus *Tunga* parasitic in man. *Parasite*, **11**, 51–57.
Reinhardt, K. and Siva-Jothy, M.T. (2007) Biology of the bed-bugs (Cimicidae). *Annu. Rev. Entomol.*, **52**, 351–374.
Rothschild, M., Ford, B. (1964) Breeding of the rabbit flea (Spilopsyllus cuniculi Dale) controlled by the reproductive hormones of the host, *Nature*, **210**, 103–104.
Service, M. (2012) *Medical Entomology for Students*, 5th edn, Cambridge University Press, 317 pp.
Tian, Y., Zhu, W., Li, M. *et al.* (2008) Influence of data conflict and molecular phylogeny of major clades in Cimicomorphan true bugs (Insecta: Hemiptera: Heteroptera). *Mol. Phylogenet. Evol.*, **47** (2), 581–597.
Veracx, A., Raoult, D. (2012) Biology and genetics of human head and body lice. *Trends in Parasitology*, **28**, 563–571.
WHO (2007) Anopheline Species Complexes in South and South-East Asia, WHO. ISBN: 978-92-9022-294-1, NLM Classification No. QX 515.
Yeates, D.K., Wiegmann, B., Coartney, G.W., *et al.* (2007) Phylogeny and systematics of Diptera: two decades of progress and prospects. *Zootaxa*, **1668**, 565–590.
Yoshizawa, K. and Johnson, K.P. (2006) Morphology of male genitalia in lice and their relatives and phylogenetic implications. *Syst. Entomol.*, **31** (2), 350–361.

Test Questions

1. How many tagmata (body parts) have insects, how many wings, how many legs?
2. What have insects to do with parasitology?
3. Which two general types of development are there in insects?
4. Which are the hosts of the former "Mallophaga" (now Amblycerca and Ischnocera)
5. Which lice occur on humans?
6. Which of these lice transmit diseases?
7. Which diseases are transmitted by this louse? (Name, disease, and pathogen.)
8. Which bugs (Heteroptera) are parasites? (Name, family, or species)
9. What is the vector of Chagas' disease?
10. Which are (almost) never hosts of fleas?
11. Which fleas occur on humans?
12. Describe the development of fleas.
13. Which diseases are transmitted by fleas?

14. Which parasites are transmitted by mosquitoes?
15. Describe the development of mosquitoes.
16. Where do simuliids live?
17. What do simuliids transmit?
18. Where do tsetse flies occur?
19. Describe the development of tsetse flies. Which stage is bloodsucking?
20. What is transmitted by tsetse flies?
21. Which group of insects contains obligatory endoparasites of vertebrates?
22. What is the difference between Nematocera and Brachycera? Name at least three families belonging to each of them.

Answers to Test Questions

Chapter 1

Section 1.1

1. Parasites are organisms living in or on a foreign host, obtain their nutrition from it, and damage it.
2. Protozoa, helminths, and arthropods.
3. In symbiosis, both partners gain benefit from the relationship; in parasitism, the benefit for the parasite prevails.
4. Temporary parasites: mosquitoes and tabanids. Permanent parasites: helminths, mange mites, and larvae of warble flies.
5. Monoxenous parasites have only one host, whereas heteroxenous ones have several hosts.
6. The sexual phase takes place in the final host.
7. In a paratenic host, stages of parasites accumulate to reach a final host.
8. A parasite with narrow host specificity is adapted only to a single or few host species.
9. Detectable parasite stages occur in a host during patency.
10. Zoonoses are infectious diseases transmitted from animals to humans.
11. Horizontal transmission occurs between members of the same species that are not in a parent–child relationship (malaria, lice). Vertical transmission takes place from mother to offspring (toxoplasmosis).

Section 1.2

1. Organisms with a small genome usually multiply quickly. With increasing size of the genome, multiplication and thus reproduction slow down.
2. Crowding effect. Concomitant immunity (= premunition).
3. Reduction of wings in hippoboscids. Reduction of legs in the crustacean *Sacculina carcini*.
4. In the adult stage, *Sacculina* is reduced to a sac-like externa and fibrous network (interna), with no resemblance to a free-living crustacean.
5. Large parasitic worms can produce masses of eggs.
6. Because of missing sexual exchange, no new alleles can be acquired.

The Biology of Parasites, First Edition. Richard Lucius, Brigitte Loos-Frank, Richard P. Lane, Robert Poulin, Craig W. Roberts, and Richard K. Grencis.

Section 1.3

1. Reduced condition and increased mortality of offspring.
2. Because of intensive transmission of the intestinal nematode *Trichostrongylus tenuis*.
3. Parasites (or other pathogens) are not present in a small founder population ("missing the boat"), and introduced species are not susceptible to parasites of resident species (e.g., common shore crab in North America).
4. The European eel is hardly resistant to the parasite.

Section 1.4

1. The pathogenic effect of parasites forces their hosts to invest energy in defense reactions.
2. Hosts exert selective pressure on parasites, thereby forcing them to build up evasion mechanisms.
3. Intraspecific competitors not only compete for the same ecological niche (e.g., habitat or food), but also for sexual partners.
4. Parasites depend on their hosts, as they cannot live without them, whereas hosts perform better without parasites.
5. Parasites are genetically more flexible than their hosts, as they are more in number and genetically less complex hence they replicate quicker and have more chances to introduce mutations or pick up new alleles.
6. It implies that pathogens and hosts exist in a permanent evolutionary arms race, in which both constantly develop new strategies to defeat the adversary, but none of them can gain a definite victory.
7. The honey bee mite changed from the Eastern honey bee to the European honey bee.
8. Resistances are most effectively spread by resistance alleles in populations.
9. Because these alleles with their particular features may protect against pathogens specialized in prevalent types of hosts.
10. Genetic disease causing resistance against malaria are, for example, sickle cell anemia, thalassemia, glucose-6-phosphate dehydrogenase deficiency, and ovalocytosis.

Section 1.5

1. Ornaments signal genetic fitness to the sexual partner.
2. The handicap principle states that a male is valued as particularly attractive if it is adorned with elaborate ornaments that are a disadvantage for survival.
3. Infection with infective agents; poor brood care; low genetic qualities.
4. With its colors, it shows health and good genes to the female.
5. By analysis of odors.

Section 1.6

1. Innate immune responses are triggered by recognition of conserved molecular structures of pathogens by receptors (e.g., toll-like receptors).

2. Intracellular parasites can be killed by their host cell after its activation by external cytokines.
3. Intestinal helminths can be expelled by the "rapid expulsion mechanism" similar to an allergic response.
4. In case of sparse exposure, insect saliva typically at first induces a hypersensitivity of the delayed type; in cases of more frequent exposure, this reaction shifts toward immediate hypersensitivity ("allergic response") or even to tolerance.
5. Myocardial inflammation in Chagas disease.
6. Kidney.
7. By development of nodules as, for instance, in *Onchocerca volvulus*.
8. Example for disguise with host antigens: adult schistosomes; examples for antigen variation: Trypanosoma brucei, *Plasmodium*, and *Giardia*.
9. Reactivation of dormant brain cysts, leading to local lesions with fatal prognosis if not treated.
10. Both conditions induce IgE and high levels of eosinophils. Many helminths have the capacity to modulate inflammatory immune effector mechanisms to increase their chances of survival, thus leading to reduced responses to environmental allergens as a spillover effect.

Section 1.7

1. Extended phenotype indicates alteration of the host by the parasite, for example, of host behavior, immune response, or morphology. Leads to an advantage for the parasite.
2. The nurse-cell of the first larva in *Trichinella spiralis* and the tissue cyst of *Toxoplasma gondii*.
3. Hormonal castration (by interference with the hormonal balance) or mechanical castration (by eating the gonads).
4. Trematode-infected snails may grow larger, because energy is diverted from reproduction to growth by parasitic castration.
5. *Taenia crassiceps* feminizes males by interference with the hormonal metabolism (conversion of testosterone to estrogen).
6. Damage of the mouthparts of bloodsucking insects can result in inefficient blood uptake, leading to repeated attempts to feed, thus increasing transmission.
7. Metacestodes of *Taenia multiceps* develop in the brain of sheep as a plum-sized coenurus that causes the host to walk around in circles.
8. *Toxoplasma gondii* causes loss of aversion against cat urine odor and disorientation in mice, thus increasing chances of contact with the cat final host.
9. Cystacanth larvae of certain acanthocephalan species manipulate their intermediate hosts, copepods, by inducing them to frequent upper layers of the water columns and alter their escape behavior, which increases the chances of being eaten by ducks.
10. An unencysted metacercaria in the ant's brain.

Chapter 2

Section 2.1

1. Four pairs of flagella.
2. *Giardia lamblia* attaches to intestinal cells by an adhesive disk.
3. *G. lamblia* swirls food particles into a ventral slit, where they are ingested by its cytostome.
4. The variant surface protein of *Giardia* (VSP) covers the surface of the parasite as a uniform layer, contains many cysteines, binds metal ions, and occurs in variants of very different size.
5. *G. lamblia* evades antibody responses by antigen variation.
6. *G. lamblia* is eliminated by IgA antibody responses.
7. By feco–oral transmission of cysts.

Section 2.2

1. *Trichomonas vaginalis* is transmitted by sexual intercourse.
2. *T. vaginalis* lives in the genital mucosae of humans.
3. *T. vaginalis* moves by four leading flagellae and a trailing flagellum.
4. *T. vaginalis* has hydrogenosomes.
5. *T. vaginalis* is usually associated with Chlamydia.
6. *Trichomonas gallinae*.
7. *Tritrichomonas foetus* potentially induces infertility in cows.

Section 2.3

1. Amobae move by pseudopodia, namely lobopodia, filopodia, or acanthopodia.
2. *Entamoeba histolytica* lives in the human colon.
3. *E. dispar* is not pathogenic because of the absence of factors enabling tissue invasion, especially a CP5 protease.
4. Via the bloodstream, *E. histolytica* is transported to the liver. The lytic process is largely due to the attraction and killing of neutrophil granulocytes, whose constituents lyse the tissue.
5. The infective stage of *E. histolytica* is a cyst with four nuclei.
6. The reduced organelle of *E. histolytica* is the mitosome.
7. The ability of *E. histolytica* to invade tissue is based on lectins on the surface of trophozoites, pore-forming peptide ("amoebapore"), and cysteine proteases.
8. Acanthamoebae may harbor *Legionella* bacteria.

Section 2.4

1. Kinetoplastea can occur as trypomastigote, epimastigote, promastigote, and amastigote forms.
2. The genome of *Trypanosoma brucei* is organized in 11 chromosomes and about 100 medium-sized and mini chromosomes. It has a size of about 36 Mb and codes for about 9100 proteins.

3. A kinetoplast is an accumulation of mitochondrial DNA consisting of maxicircles and minicircles.
4. In trypanosomatids, transsplicing occurs during the processing of transcripts, which involves the attachment of a spliced leader sequence of 39 bp on to the 5′-end of individual transcripts.
5. In vertebrates, trypanosomatids use sugars as the source of energy, and prolin in insects.
6. *Trypanosoma brucei* causes the disease Nagana in cattle and sleeping sickness in humans.
7. *T. brucei* occurs as long slender, intermediate and short-stumpy forms in the bloodstream.
8. The cause for central nervous system disorders in *T. brucei* infections are perivascular inflammations induced by complement-activating immune complexes.
9. The surface coat of *T. brucei* consists variable surface glycoproteins (VSGs) anchored with a GPI-anchor in the surface membrane.
10. *T. brucei* evades the immune response by antigen variation.
11. *T. vivax* can (also) be transmitted by the mouthparts of insects.
12. *T. evansi* causes surra in ungulates.
13. *T. equiperdum* is transmitted by copulation between horses.
14. *T. cruzi* is transmitted by reduviid bugs ("kissing bugs").
15. Bedbugs cannot transmit *T. cruzi* as they defecate away from their blood donor.
16. During infection with *T. cruzi,* the heart muscle may be damaged by chronic inflammation caused by the presence of low levels of parasites.
17. On the surface of *T. cruzi,* GPI-anchored trans-sialidases and gp63-proteases are found.
18. *T. cruzi* invades the host cell by induced phagocytosis.
19. Sand flies of the genus *Phlebotomus* (Old World) and *Lutzomyia* (New World).
20. Promastigote stages of *Leishmania* are transmitted by the saliva of its intermediate host.
21. *Leishmania* invades a host cell by its phagocytosis.
22. The principal host cell of *Leishmania* is the macrophage.
23. *Leishmania* survives in the host cell by blocking its oxidative burst, neutralizing its reactive oxygen products with detoxification enzymes, increasing the pH of the phagolysosome, and destroying proteolytic host enzymes.
24. *L. tropica* causes cutaneous leishmaniasis.
25. Persons with *L. donovani* die of trivial diseases as their hematopoiesis and immune responses are severely disturbed.

Section 2.5

1. The apical complex is composed of conoid, rhoptries and micronemes.
2. The pellicle of the Apicomplexa consists of a complex of three elementary membranes and microtubules under them.

3. The apicoplast is a plastid organelle, acquired by double phagocytosis.
4. Most Apicomplexa have in their life cycle the phases schizogony, gamogony, and sporogony.
5. The infective stage of Apicomplexa is the sporozoite.
6. Cell invasion of the Apicomplexa is based on gliding motility.
7. After invasion, the Apicomplexa lie in a parasitophorous vacuole.
8. The oocyst of Coccidia harbors several sporocysts, each one harboring several sporozoites.
9. The *Eimeria*-like pathogen is *Isospora belli*.
10. Felidae are final hosts of *Toxoplasma gondii*.
11. The early stages of development of *Toxoplasma gondii* in the intermediate host are the tachzoites, the late ones bradyzoites.
12. In fresh feces, the oocysts of *T. gondii* are not yet sporulated.
13. An unborn child is not at risk by *T. gondii* if the mother already had an earlier infection.
14. Reactivated *Toxoplasma* cysts can lead to development of necroses in the brain being fatal if not treated.
15. Main host cells for resting stages of *T. gondii* are neurons.
16. *Neospora caninum* causes aborts in cattle.
17. *Surcocystis suihominis* at first develops schizonts in endothelial cells in pigs and then tissue cysts in muscle cells.
18. *Plasmodium vivax* and *Plasmodium ovale* cause malaria tertiana, *Plasmodium malariae* gives rise to malaria quartana, and *Plasmodium falciparum* causes malaria tropica.
19. Hepatocytes and erythrocytes are colonized by species of *Plasmodium*.
20. Exoerythrocytic schizogony is the asexual division of *Plasmodium* in hepatocytes.
21. Schizogony stages of *Plasmodium* found in erythrocytes are ring, trophozoite, schizont, and merozoites.
22. Malaria does not exist anymore in Central Europe, because transmission has been interrupted by draining breeding areas of *Anopheles*.
23. *Plasmodium vivax* is specialized on young erythrocytes (reticulocytes).
24. *P. falciparum* evades the spleen passage by cytoadherence, using PfEMP1 proteins present in knobs on the erythrocyte surface.
25. The major surface antigen of the sporozoite of *Plasmodium* is the circum sporozoite protein.
26. Potentially fatal pathomechanisms in Malaria tropica are cerebral malaria (caused by cytoadherence of infected erythrocytes), with hyperacidity of the blood, metabolic stress, and/or anemia. A combination of these symptoms is called "severe malaria."
27. Long-lived stages of *Plasmodium vivax* are the hypnozoites.
28. *Babesia divergens* is transmitted by the castor bean tick *Ixodes ricinus*.
29. *Babesia* can remain a long time in tick populations because of transovarial transmission.

30. *Theileria* species cause tropical theileriosis and East Coast Fever in ruminants, diseases proceeding similar to malaria.
31. *Theileria* exploits at first lymphocytes as host cells, later erythrocytes.
32. The main host infected by *Balantidium coli* is the pig.
33. Free-swimming ciliated daughter organisms (called theronts) attach to and invade the skin of fish.

Chapter 3

Section 3.1.1

1. Flat worms, namely the usually free-living planarians, parasitic trematodes (Digenea), and parasitic cestodes (Eucestoda).
2. Parasites with asexual reproduction in mollusks (usually snails) and sexual reproduction in vertebrates.
3. Oral and ventral sucker, bipartite blind-ending intestine, two testes, one ovary, paired vitellarium, and long unbranched uterus.
4. Life cycle is water-bound. Free-swimming stages: miracidium, cercaria, and asexual propagation in first intermediate host. The presence of second intermediate host, which harbors the metacercaria. Adult in final host.
5. Egg, miracidium, sporocyst (giving rise to daughter sporocysts or redia), cercaria, metacercaria, and adult.
6. The asexual reproduction in the first intermediate host (usually a snail).
7. Intestine.
8. An animal being part of the food chain of the final host, rarely (*Fasciola*) a plant.
9. Humid biotopes, for example, moist meadows and banks of drainage channels, where *Galba truncatula* lives.
10. Yes, in the worst case, cancer of the bile ducts can develop.
11. By forcing ants to expose themselves to grazing animals.
12. Lung fluke of the Far East, final hosts are crab-eating mammals and humans, freshwater life cycle. Lungs are affected. Dangerous are "ectopic" localizations.
13. Metacercariae in eye of second intermediate hosts and fish render them blind. Cercariae migrating in body of fish may cause damage.
14. (i) Dioecious, (ii) living in bloodstream, (iii) no second intermediate host, (iv) eggs not operculated.
15. Eggs, they lodge in human tissues, particularly in the liver.
16. *Schistosoma mansoni* in Africa and parts of South America, *Schistosoma haematobium* in Africa, and *Schistosoma japonicum* in Southeast Asia.
17. *S. mansoni*: mesenterial veins of large intestine, *S. haematobium*: veins of the lesser pelvis and bladder, and *S. japonicum*: mesenterial veins of intestine.
18. *S. mansoni*: *Biomphalaria*, stagnant or slowly running water. *S. haematobium*: *Bulinus*, water bodies such as *S. mansoni*. *S. japonicum*: *Oncomelania*, amphibious mode of living, especially in paddy fields.

19. Only a minor role, as climate too cold for snails of human pathogenic species. Some bird schistosomes may affect humans by causing swimmer's itch.

Section 3.1.2

1. White, long, flat with many segments (proglottids). Scolex at anterior end with holdfast devices for the adherence in the host's intestine. Hermaphrodite, lacking an intestine.
2. The adult worm, producing masses of eggs.
3. Egg and coracidium (in water), procercoid (in copepod), plerocercoid (in fish), and adult (in human intestine).
4. The adult worm may (in rare cases) settle in the ileum and then deprive the intestine of vitamin B_{12}, resulting in pernicious tapeworm anemia, which can be fatal if not treated.
5. Egg with oncosphere, metacestode, and adult.
6. *Rodentolepis nana*, Hymenolepididae.
7. A "normal" life cycle via cysticercoids in beetles that are ingested by a final host, or via eggs developing into cysticercoids within villi of the definitive host.
8. *Taenia*, *Hydatigera*, *Versteria*, and *Echinococcus*.
9. Carnivores, humans in very few species.
10. Prey animals of the carnivore final hosts.
11. *Taenia multiceps*, *Taenia serialis*, *Taenia crassiceps*, and all *Echinococcus* sp.
12. The tapeworm, that is, the adult worm, does not occur in cattle. It is the metacestode (cysticercus), which lives in cattle.
13. *Taenia saginata*, *Taenia solium*, and *Taenia asiatica*.
14. *Taenia solium*, because humans can become infected by cysticerci settling in the brain or eye.
15. Humans may harbor the metacestodes of all species, causing cystic, alveolar, or polycystic disease.
16. *Echinococcus granulosus* and *Echinococcus multilocularis*.

Section 3.2

1. Smooth body, unsegmented. Conspicuous proboscis at the head, armed with spines.
2. Vertebrates, mainly fish.
3. Intestine.
4. The proboscis is anchored in the host's gut wall like a dowel.
5. Arthropods.
6. Punctiform damage of the wall of the intestine.

Section 3.3

1. In water and soil, as pests of plants and parasites of animals and humans.
2. Circular cross section, elongated, more or less tapered at both ends, tough cuticle, no organs allowing anchorage to the host.

3. Oval, both poles similar.
4. Eggs, juvenile stages (incorrectly) called larvae 1–4, dioecious adults.
5. None, because holdfast organs are missing.
6. Definitive and intermediate host are the same individual.
7. The muscle-stage larva.
8. Two different ones: in a parasitic cycle, parthenogenic females may generate offspring potentially leading to autoinfection and hyperinfection, and a free-living cycle, in which males and females develop.
9. By eating infected intermediate hosts such as the giant African snail *Achatina fulica* or other snails.
10. It causes parasitic gastroenteritis. Masses of L4 in the abomasums, mainly of sheep, cause anemia. Hypobiosis can prevent detection of eggs.
11. There is none.
12. Humans as inappropriate hosts can suffer from a *larva migrans visceralis*, which may cause damage of the eye or neuropathology.
13. By intrauterine or lactogen infections of the bitch.
14. Fourth-stage larvae of *Anisakis* and related species in the stomach of humans. Eating raw fish may cause distress or, if they break through the stomach wall, even severe gastric symptoms.
15. Herring worms.
16. At contact with water, a blister forms above the anterior end (the vulva) of the pregnant female. The blister opens and the larvae are released.
17. Humans are infected only by ingesting the intermediate host (small crustaceans).
18. The nocturnally active female worms move to the anus and lay eggs, causing severe itching, which deprives children from sleep.
19. There is none. Humans are infected by the eggs they shed.
20. In large lymphatic vessels of humans.
21. The first-stage larvae of filariae, sheathed or unsheathed.
22. A human disease, mainly in Africa, caused by dead microfilariae of *O. volvulus* in the eye.
23. In Africa south of the Sahara, also small areas in Yemen, and in Latin America.
24. Because the nematode cannot live without its symbiont *Wolbachia*, which is killed by the drugs.

Chapter 4

Section 4.1

1. Advantages: protection from external environment, mechanical advantage, enables morphological plasticity. Disadvantages: constraint on maximum size, requires molting for growth, sensory organs needed on outside of exoskeleton.

2. Thin, impermeable, nonchitinous layer (epicuticle) and a thick, elastic, permeable, laminated inner layer (endocuticle). Composed mainly of chitin with some protein.
3. Four main groups – Eucheliceratа (arachnids, scorpions, pseudoscorpions, etc.), hexapods ("insects"), crustaceans (crabs, shrimps), and myriapods (centipedes, millipedes).
4. The number of secondary cases arising per day from one infective case in a susceptible population of hosts transmitted by a vector.
5. *Trypanosoma cruzi* (in triatomine bug) and *Rickettsia prowazekii* in body lice.

Section 4.2

1. Mites and ticks.
2. Chelicerata.
3. Body consisting of one unit, not divided into prosoma and opisthosoma. A special structure, the gnathosome, bears the mouthparts. A six-legged larva is present.
4. They are not only terrestrial predators, but live in fresh and saltwater as pests of plants and true parasites.
5. Prelarva (stays inside the egg), six-legged larva, three eight-legged nymph forms (proto-, deuto-, and tritonymph), adult.
6. Skin, hairs, feathers.
7. *Varroa destructor* (Mesostigmata).
8. *Demodex folliculorum*, *Demodex brevis*.
9. A heteromorphic deutonymph in the astigmatic mites, often phoretic, nonfeeding, without mouthparts.
10. Infestation with mites, causing damage of hairs, feathers, and skin.
11. *Sarcoptes scabiei*.
12. Mite lives in the skin. Female digs burrows. Eggs are deposited in them. Larvae have to bore to the outside.
13. The hypostome is a special, conspicuous structure. One prominent dorsal shield. Peritreme conspicuously large, roundish.
14. Ticks are exclusively parasites.
15. Ixodidae (hard ticks), Argasidae (soft ticks), Nuttalliellidae (no vernacular name).
16. Rocky Mountain spotted fever, Lyme disease, Babesiosis, Anaplasmosis, tick-borne relapsing fever.
17. *Dermacentor*, *Ixodes*, *Ixodes*, *Ixodes*, and *Ornithodoros*.
18. Vector of Lyme disease or Borreliosis and of tick-borne encephalitis.
19. Ixodidae (hard ticks) transmit babesiosis and theileriosis of cattle in the tropics and subtropics.
20. Argasidae in dry tropical and subtropical climates and biotopes in caves and burrows of animals.

Section 4.3

1. An ectoparasite of freshwater fish.
2. Blood.
3. The nauplius (first larval stage).
4. *Carcinus maenas*, the European shore crab.
5. The "externa," part of the female.

Section 4.4

1. 3, 2 or 4, 6 (wings are secondarily lost in many parasitic species).
2. They can be parasites themselves (fleas, lice, bot flies) and they are vectors of parasites (malaria, trypanosomes, filariae) and other pathogens (viruses, bacteria).
3. Hemimetabolic, holometabolic.
4. Birds and mammals.
5. Head louse *Pediculus humanus capitis*, body louse *Pediculus humanus humanus*, pubic louse *Pthirus pubis*.
6. Only the body louse.
7. Epidemic typhus (*Rickettsia prowazekii*) Trench fever (*Rochalimea quintana*), Louse-borne relapsing fever (*Borrelia recurrentis*).
8. Cimicidae (*Cimex lectularius*), Reduviidae Triatominae (*Rhodnius prolixus*).
9. South American reduviids/triatomines, for example, *Triatoma infestans*.
10. Hosts (birds and mammals) without permanent resting places.
11. *Pulex irritans*, *Ctenocephalides felis*, *Tunga penetrans*.
12. Holometabola with three larval stages, pupa, adult. Larvae living on organic material, including dried blood emitted by the adults.
13. Plague (*Yersinia pestis*), murine typhus or endemic typhus (*Rickettsia typhi*), a flea typhus (*Rickettsia felis*), cat scratch disease (*Bartonella henselae*).
14. Malaria (*Plasmodium* ssp.), nematodes *Wuchereria* and *Brugia*.
15. Holometabolous, four larval instars, pupa (all in water), adults, only females suck blood.
16. Larvae and pupae in flowing water.
17. *Onchocerca volvulus*, river blindness.
18. In the so-called fly belt of Africa, that is, north of the Kalihari and south of the Sahara.
19. Three larval instars develop and are nourished in the maternal uterus. The third larva burrows into the soil immediately after being laid. Both adult sexes are bloodsucking.
20. Trypanosomes, sleeping sickness of humans, Nagana of animals.
21. Oestridae, bot flies, or warble flies.
22. Nematocera: development bound to water; antennae with many segments; if bloodsucking, then only females. Ceratopogonidae, Phlebotominae, Simuliidae, Culicidae. Brachycera: development not bound to water; antennae with few segments; if bloodsucking, then both sexes: Muscidae, Calliphoridae, Oestridae, Glossinidae.

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Page numbers **in bold** indicate passages covering a parasite or a topic in depth
Words **in bold** indicate important taxa or topics

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