Plant Architecture and Its Manipulation

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Plant Architecture and its Manipulation

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Preface

Conventionally, architecture relates to buildings, embracing both art and science, and specifying both form and function. In scope, this closely matches the study of plant architecture. From an artistic perspective, we might marvel at the astonishing diversity of aesthetically pleasing plant structures; yet, as scientists, we know that through natural selection, very little of form is dissociated from function.

The origins of studies of plant architecture and their influences on human existence are steeped in history, but from a twenty-first century perspective, the field has been transformed from a discipline of observation and description into one in which complex networks of genetic, chemical and environmental factors can be directly manipulated and modelled. New insights are emerging on fundamental aspects of the plasticity of development – a phenomenon unique to plants and therefore charting territory not encountered in animal or microbial systems. At the same time, increasingly sophisticated knowledge has provided the foundations for many revolutionary practices in agriculture, horticulture and forestry. Arguably, manipulation of plant architecture has been one of the greatest mainstays of plant improvement, perhaps second only to the discoveries of the nutritional requirements of plants. Whereas science has been only marginally successful at tweaking improvements in the biochemistry of photosynthesis, manipulation of architecture – and hence efficiency, uniformity and ease of plant production – has improved almost beyond recognition. Genetic regulation of stem growth and manual interventions such as pruning are two notable successes. With the advent of the 'gene revolution', there are countless new opportunities for selective modification of plant architecture.

The 10 chapters of this book range from the molecular and cellular through to the whole organism. Chapter 1 explores intracellular processes, focussing on mechanisms of regulation of cell shape, division and directions of growth. Chapters 2–4 detail the structure and regulation of vegetative shoot systems, from the linear extension processes of shoot elongation to the efficient development of planar leaves and three-dimensional branching patterns. Chapter 7 succinctly presents the root version of all these aspects. Chapters 5 and 6 move to the reproductive phases of shoot development, contrasting the highly conserved ordering of floral organs with the diversity of final flower structures and inflorescence arrays. Chapters 8 and 9 cover more complex systems – perennial woody species, along with possibilities for modelling approaches to describe and predict architectures. Chapter 10 rounds off the volume with a perspective on key applications of plant architecture in horticultural and agricultural contexts.

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1 Cellular architecture

Regulation of cell size, cell shape and organ initiation

Andrew J. Fleming

1.1 Introduction

Plants are made of cells. This original observation led to the proposition and eventual acceptance of the cellular theory of organisms – a theory that provides a basis for modern biology. Following on from the initial observation that plant tissue was split up into compartments, it soon became apparent that not all of these compartments were of the same shape and size. Thus was born the science of plant histology, leading to the definition of the numerous cell types described in various plant science textbooks. Obvious questions arising from the observation that cells of different size and shape exist within a plant include: how does this situation arise? To what extent is the particular size and shape of a cell inherent to a specialised cellular function? This chapter will address these questions. In a broader context, one can also ask the question of how cellular architecture is integrated into the whole organism. In particular, a long running question has been to what extent the size, shape and number of cells within an organ determines the size and shape of that organ. Although at first sight possibly a trivial question, research in this area continues to puzzle and intrigue many biologists. This issue will also be addressed in this chapter.

1.2 Growth and cell proliferation are related but separable components controlling cellular architecture

Most higher plants begin life as a single cell – the egg cell. After fertilisation, this cell undergoes a period of growth accompanied by cell division, leading to a multicellular ball of tissue – the globular embryo. The key here is the statement that growth is accompanied by cell division. Although it may be tempting to precis this to state simply that an embryo is formed by rounds of cell division in the fertilised zygote, a focus on cell division ignores an essential developmental process, namely growth. As has been elegantly pointed out (Roberts, 1994), cell division without growth would result in the formation of an organism which was exactly the same size as at the

beginning of the exercise but which simply contained a large number of small cells (Figure 1.1). Moreover, because plant development is characterised by a general lack of cell movement, such an organism would have exactly the same shape as prior to the initiation of cell division. Although the formation of cells without appreciable growth does occur at some stages during plant development, generally growth and cell division are intimately linked. However, the balance between these two processes is not fixed. Indeed, the spatial and temporal control of the balance between these two processes is the tool by which cells of different sizes and shapes are formed, that is, it is the basis of plant cellular architecture (Figure 1.2). An understanding of cellular architecture requires an understanding of cell division, of cell growth, of the inter-relationship of these parameters and of the mechanism by which this inter-relationship itself is controlled. These elements are the focus of the following sections of this chapter.

To start addressing these questions, a reasonable approach is first to ask whether there is a basal cell state from which all the observed varieties of plant cell are derived. The answer to this question is 'yes'. At the tips of shoots and roots (and also at other locations within the plant) are groups of cells associated to form organs termed meristems. These meristems contain cells which undergo repeated rounds of growth and division to generate daughter cells. Some of these daughter cells may retain meristem identity (and so generate more daughter cells), whereas others undergo differentiation. This differentiation process is generally accompanied by the acquisition of a particular cell size and shape. Meristem cellular architecture

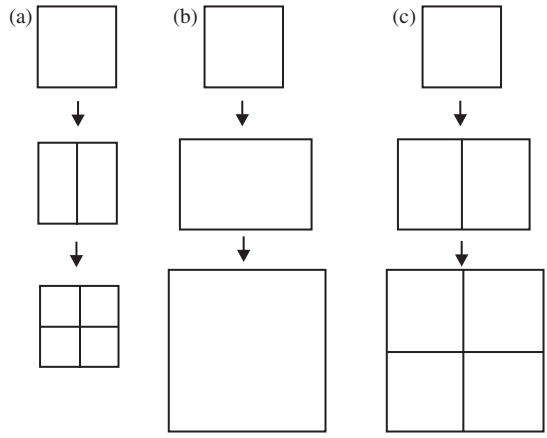

Figure 1.1 Cell division and growth are related but separable processes. (a) A cell can theoretically undergo division without growth. Such a process would, over two rounds of division, lead to a four-celled organism of exactly the same size and shape as at the beginning of the exercise. (b) Increase in size requires growth. This growth does not of necessity involve cell division. (c) During normal plant development, growth and division are intimately related so that increase in size is linked with successive rounds of division. These leads to a multicellular organism of increased size.

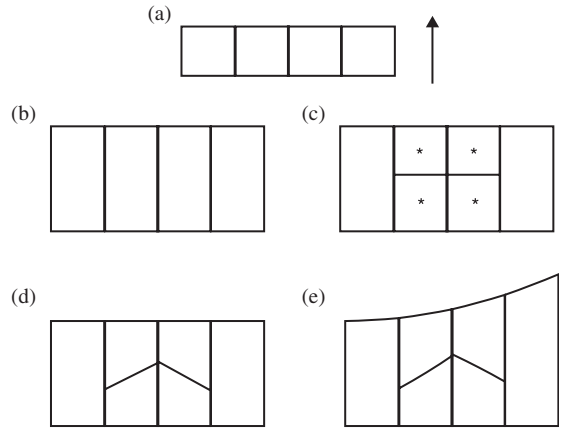

Figure 1.2 Spatial and temporal regulation of the balance of growth and division determines cellular architecture. A theoretical group of four cells undergoes growth in the direction of the arrow (a) If growth is unconnected with division, a form and cellular pattern will be produced as shown in (b). If, however, some of the cells in (a) have a higher balance of cell division to growth, then these cells may undergo a round of cell division to generate daughter cells [* in (c)]. However, the overall growth of the cells that have undergone division and those that have not is identical, that is, the final form in (c) is identical to that in (b). The cellular architecture is, however, distinct. If the orientation of cell division is altered, then, although the total tissue growth and number of cell divisions of the tissue is maintained as in (b) and (c), the cellular architecture is altered (d). If a gradient of tissue growth is imposed across the tissue shown in (d), then morphogenesis occurs (a different tissue shape is formed), as shown in (e). This requires that adjacent cells across the tissue coordinate their individual balance of division to growth to generate a smooth transition in tissue shape. Cell–cell signalling is required.

may thus be thought of as representing a basal state from which all others are derived. What do these cells look like, how are they maintained and how are they instructed to take up alternate cellular fates?

1.3 Meristems as a source of cells in the plant

The shoot apical meristem (SAM) is classically described as a dome structure situated at the distal tip of the shoot, although in many plants the dome is extremely flattened. Early studies revealed a distinction between the outermost layers of the SAM (in which cell division occurred predominantly in an anticlinal orientation) and the inner region in which cell division orientation appeared to be more random (reviewed in Steeves and Sussex, 1989), that is, different regions of the SAM have distinctive cellular architecture. This led to the definition of an outer tunica and inner corpus of cells within the SAM. This differential pattern of cell division orientation within the SAM has a consequence for the organisation of the rest of the

plant. Thus, as shown by clonal analysis, the outer layers of the tissues derived from the SAM are derived from the outer cell layers of the meristem (tunica) whereas the inner body of organs is derived from cells of the corpus. Although this distinctive architecture has the potential to dictate cell fate via cell history, most data indicate that plant cell differentiation is controlled by position. Differentiation based on position implies the presence of an intricate and continual signalling system between cells which allows them to take up an appropriate (genetically planned) architecture. This signalling system will be dealt with later in this chapter.

In addition to the layered nature of the SAM, classical analysis also revealed differences in the cytology of different regions of the SAM. In particular, cells in the central zone (CZ) of the SAM appeared to be slightly larger than those at the periphery. Analysis of the frequency of cell division in these regions revealed that the histological zonation correlated approximately with a gradient of cell division frequency, with cells in the CZ undergoing a lower rate of cell division than the cells in the peripheral zone (PZ) (e.g. Francis and Lyndon, 1978). These observations led to the definition within the SAM of a CZ undergoing a relatively low rate of cell division to supply daughter cells to the surrounding PZ. Estimates of growth rates within the meristem also suggested that tissue at the periphery of the meristem grew at a slightly faster rate than tissue at the centre of the meristem. The observation of different growth and cell division rates within the SAM may, in some sense, simply describe parameters that must exist for an organ with such an architecture to be maintained, that is, if the growth rate of tissue at the tip of a dome structure was higher than that at the periphery, the dome structure could not be maintained. Therefore, the question arises – what is it that controls these differential growth processes within such a small volume to maintain the structure of the SAM? Is this structure essential for SAM function, and how is the overall structure related to the cellular architecture within the SAM? Recent data from experiments using tools of molecular biology have begun to shed novel insights into this essential element of plant development.

A group of cells towards the centre of the meristem (slightly proximal to the classically defined CZ) express a homeodomain transcription factor termed WUSCHEL. If the *WUSCHEL* gene product is rendered inactive, the SAM gradually disappears as an anatomical entity as the cells appear to be used up during the growth of the plant. Once this happens, seedling growth terminates. Thus, the WUSCHEL protein somehow signals to surrounding tissue in the SAM to maintain growth and division (Mayer *et al*., 1998). The cells overlying the WUSCHEL expression domain express a gene termed *CLAVATA3*. This encodes a small extracellular protein which signals to underlying tissue to inhibit growth and cell division (Fletcher *et al*., 1999). The CLAVATA3 protein probably functions via interaction with a family of receptor kinases encoded by genes such as *CLAVATA1* and *CLAVATA2* whose expression domain encompasses that of the *WUSCHEL* gene (Clark *et al*., 1997). Mutation of the *CLAVATA* genes leads to a phenotype in which meristem size increases via a promotion of growth and cell division. Thus, there exists a feedback mechanism by which a positive acting factor for growth

and division in the SAM (WUSCHEL) leads to the stimulation of an inhibitory factor (CLAVATA3) which can decrease expression of the positive acting factor (Figure 1.3: Schoof *et al*., 2000). Thus, local interactions within the meristem act to balance growth rates, tending to maintain SAM size and structure. The exact mechanism of these interactions is still unclear, as are the exact targets of the outputs from the system, that is, whether cell growth or division is the primary target for the downstream signalling elements. It is also unclear whether the observed layered architecture of the SAM is in anyway linked to the molecular mechanism by which cell growth and proliferation is balanced within the tissue. Finally, it is unclear how the system is set up in the first instance to define an appropriate balance between tissue growth (necessary to maintain the SAM) and tissue loss (as a consequence of organ initiation). The setting of this balance defines the size of the SAM and the size and number of the organs derived from it. This has a direct consequence on the architecture of the whole plant.

The situation within the root apical meristem (RAM) appears, at first sight, to be very different. First, the cellular architecture of the root tip appears to be much more organised than that of the shoot, with files of regular cells radiating from a region at the root tip (Dolan *et al*., 1993). This region of the root tip contains the root initial cells. These initials are themselves arranged around a small core group of cells which constitute the quiescent centre (QC). The cells of the QC can be regarded as the basal cell type within the root (although their rate of growth and division is extremely limited) while the initial cells around them undergo markedly higher rates of cell growth and division to generate the observed distinct files of cells. It should be noted that the root initial cells themselves already display distinct cell shapes which presage the distinctive cell shapes seen in different regions of the root apex. The regular cellular pattern in the root apex might suggest that cell fate (and, thus, architecture) was determined by cell history. However, a series of elegant experiments using laser ablation have demonstrated that cell fate (and, thus, cellular architecture) in the root apex is controlled by a complex series of signalling processes by which cells in particular

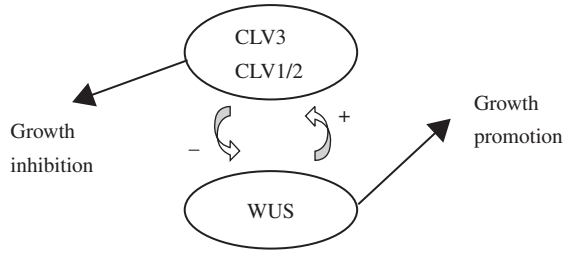

Figure 1.3 Growth in the meristem is controlled by interactions of promoting and inhibitory pathways. The WUSCHEL (WUS) protein promotes growth within the meristem. At the same time, it stimulates the activity of the CLV3, CLV1/2 pathway (CLV) which acts to decrease WUS activity. Thus, any tendency for increased growth via elevated WUS expression is counterbalanced by increased CLV activity which inhibits WUS activity and, thus, growth. The growth inhibitory action of the CLV pathway may be entirely accounted for by its influence on WUS, or may encompass a separate mechanism of growth inhibition.

regions acquire their identity (Van den Berg *et al*., 1995, 1997). Although the nature of a number of these signalling components remains obscure, intercellular movement of transcription factors and hormones appears to play a key role (Sabatini *et al*., 1999; Helariutta *et al*., 2000; Nakajima *et al*., 2001). With respect to the actual maintenance of basal meristem cell identity and the balance of growth and division within the root meristem, the situation was obscure until recently. For example, mutations in the *WUSCHEL* and *CLAVATA* genes (described above to be central to maintaining the cellular architecture in the SAM) did not lead to any obvious phenotype in the root, leading to the possibility that the molecular mechanism controlling basal cell types in the two meristems might be distinct. However, the recent finding in rice that a *WUSCHEL*-like gene (*QHB*) is specifically expressed in the QC, as well as the observation of altered root growth in transgenic plants overexpressing a CLV3-related protein (CLE40), has led to the speculation that, indeed, the maintenance of a basal meristem cell type in both the SAM and RAM might share a similar molecular mechanism (Casamitjana-Martinez *et al*., 2003; Kamiya *et al*., 2003). In this scenario, the lack of an obvious root phenotype in mutations of single *WUSCHEL* or *CLAVATA* genes might reflect a level of functional genetic redundancy. The next few years promise to be an exciting time as this problem is unravelled.

Although the identification and characterisation of the *WUSCHEL* and *CLAVATA* gene products have led to monumental progress in our appreciation of how meristems function, the fundamental question remains of how these proteins work, that is, what are the cellular processes that they target? This question approaches one of the most basic aspects of biology. What is the defining aspect of differentiation? Since differentiation (particularly in plants) is most frequently observed as the acquisition of a specific cell size and shape, to what extent are these parameters intertwined?

1.4 Patterning of cellular architecture

As mentioned at the beginning of this chapter, the shifting balance between cell growth and division dictates directly the size and shape of cells generated within a tissue. Within the SAM and RAM, most cells are characterised by a high rate of division relative to growth. As growth occurs over the whole meristem, daughter cells are left behind the apical tissue, and as a result of this the rate of cell division relative to growth generally decreases. However, this ratio of cell division to growth may be very different in adjacent cells. At the same time, the overall rate and vector of growth in all cells in a slice of tissue must be the same; otherwise, gradients of growth will be generated, leading to distortions of the tissue as a consequence of the biophysical imbalances within it. Such gradients of growth are essential aspects of morphogenesis and of tropisms, but they must be tightly controlled. This aspect of plant morphogenesis has recently been neatly described for the elaboration of lateral organ shape in *Antirrhinum* (Nath *et al*., 2003).

Thus, there are two aspects to the acquisition of cellular architecture as tissue is left behind the growing meristem. First, the decision at the level of the individual

cell of whether to shift the balance from progression through the cell cycle to cessation of cell division and (generally simultaneously) progression to a phase of cell growth. Second, at the whole organ level, growth rate and vector must be spatially and temporally coordinated across the whole field of constituent cells to generate an organ of appropriate size and shape to fulfil organ function. These related but distinct aspects are considered below.

Taking the leaf as an example, initially all cells within a primordium are approximately equivalent in size and shape (although presumptive epidermal cells tend already to be slightly more box-shaped than cells internal to them). As the leaf developmental programme unfurls, cells at different positions take on characteristic shapes. For example, while cells in the epidermis retain a box-like appearance, internal cells destined to form elements of the vasculature undergo a balance of growth and division which leads to cell size remaining rather small, but with an increased polarity along the forming proximal/distal axis of the leaf. Slightly later in developmental time, cells between the presumptive epidermis and vasculature gain specific sizes and shapes (depending on position) which leads to the classical histology of the palisade and spongy mesophyll. Within each of these basic tissues of the leaf, sub-domains may become established; these define specific cell types, again associated with particular local patterns of cell growth and division. For example, within the leaf epidermis, stomatal complexes become established. These require specific cellular architectures to define a functional stomatal pore. Within the root, some cells of the epidermis may form hair-like extensions, whereas on the leaf surface multicellular extensions form the basis of trichomes. All of these elements of functional plant anatomy are associated with local control of the pattern of tissue growth and cell division. What do we understand about the processes controlling these events? How are particular groups of cells blocked out from the basal-cell type and fated to acquire a specific architecture? The creation of spatially distinct domains of transcription factor gene expression has proven to be a paradigm for this process.

The leaves of most higher plants are flattened to generate an abaxial and adaxial side to the organ. This polarity is reflected by the distinctive cellular architectures of the two sides of the leaf, with the adaxial side normally being associated with palisade mesophyll architecture and the abaxial side being characterised by spongy mesophyll. The vascular tissue is also normally polarised into an adaxial xylem tissue and an abaxial phloem tissue. Such polarity in cellular architecture is not apparent at the very earliest stage of leaf initiation, but soon after this, transcripts encoding the homeodomain-ZIPIII transcription factor PHABULOSA accumulate in the presumptive adaxial region of the leaf (McConnell *et al*., 2001). This initial gradient of transcription factor activity then sets in train a differential cascade of transcription factor networks which leads to tissue gaining either adaxial or abaxial identity (Veit, 2004). Interestingly, the generation of the initial gradient of transcripts encoding PHABULOSA involves the action of specific microRNAs which (it has been postulated) might function as a mobile signal within the shoot apex (Kidner and Martienssen, 2004). Similarly, in the root, the acquisition of specific

cell fates (associated with particular histology) is caused by the spatially controlled expression of specific transcription factors (Di Laurenzio *et al*., 1996). The action of some of these transcription factors seems to require movement of the protein between adjacent cells, raising the prospect of local cell–cell communication via transcription factors as a key element in the local coordination of cellular architecture (Helariutta *et al*., 2000). This theme of local distribution of transcription factor activity has also been implicated as a key aspect of epidermal cell differentiation.

In the developing root epidermis, cells become fated to form either hair cells (H) or non-hair cells (N) (Dolan *et al*., 1993). These cell types have very distinct shapes. The acquisition of a specific fate is dependent on the relative activity of two transcription factors, WEREWOLF and CAPRICE (Wada *et al*., 1997; Lee and Schiefelbein, 1999). As a result of signalling from underlying tissue, cells in the epidermis overlying a single cortical cell accumulate WEREWOLF. This favours a transcriptional network leading to N-cell fate. This includes expression of the CAPRICE transcription factor gene. It is thought that the CAPRICE protein can move to neighbouring cells where, if WEREWOLF is not expressed, a transcriptional network is favoured, leading to root hair formation (Lee and Schiefelbein, 2002). Similarly, in the leaf epidermis, local signalling mechanisms lead to neighbouring cells having alternate transcriptional networks which favour either trichome formation or non-trichome formation. Thus, the interaction and patterning of positive regulators (such as GLABRA1 and TTG) and negative regulators (such as TRYPTYCHON and CAPRICE) of trichome formation lead to the accumulation of specific positive regulators in the presumptive trichome cells (Schnellmann *et al*., 2002). Manipulation of the transcriptional networks involved in root hair and trichome formation allows directed manipulation of epidermal cell architecture.

Clearly, significant progress has been made in the identification of the transcriptional networks involved in defining tissue and cell fate. Since tissue and cell differentiation generally involves altered cellular architecture, these transcriptional networks must control these processes. Identification and characterisation of these target processes remain major research goals in this area. Some elements have begun to be identified but we are still a long way from having a full picture of this process. Progress in this area is described in the next section.

1.5 The cellular decision to proliferate or not to proliferate

As described in the previous section, a key step in the acquisition of a specific cellular architecture is the decision whether to progress through the cell cycle and divide or to enter a phase of differentiation (generally accompanied by an increase in cell growth).

Extensive research over the last decade has led to increasing knowledge and understanding of the plant cell cycle. The basic elements are highly conserved with those described for other eukaryotes (Potuschak and Doerner, 2001). Thus, the cell

cycle is characterised by a sequential process of protein phosphorylation and dephosphorylation which leads to a coordinated progression of a cell through the successive phases of G1, S, G2 and mitosis. A significant body of evidence indicates that a key step in the decision whether to proceed through the cell cycle occurs in the G1 phase. In particular, the activity of a particular class of cyclin-dependent kinases (CDKAs in plants) and their associated cyclins (D-class cyclins) dictates the phosphorylation status of a retinoblastoma-related protein (RBR) (Figure 1.4). In the hypo-phosphorylated state, RBR can interact with a series of transcription factors (E2F-class) leading to the blockage of transcription of a panel of genes required for progression into S-phase of the cell cycle. In the hyper-phosphorylated state (i.e. after phosphorylation by CDK/cyclinD), RBR can no longer inhibit E2F factor activity and so progression through to S-phase can occur. Thus, work in this area has led to the paradigm that the CDK/cyclin/RBR complex acts as a decision point as to whether the cell cycle proceeds or not (Shen, 2002). However, recent data indicate that these components can interact with many cellular partners involved in aspects of both cell proliferation and growth, that is, the situation is not as simple as the paradigm would propose. For example, in insect cells, CDK4/cyclinD can influence growth independently of RB-like proteins via HPH, an enzyme involved in cellular response to hypoxia (Frei and Edgar, 2004). Microarray experiments also indicate that RB-like proteins can influence a wide array of gene expression profiles in a positive and negative fashion. In addition, RBR proteins have the potential to influence chromatin structure, thus raising the possibility that they target the stable expression of sets of genes (Narita *et al*., 2003).

In addition to our incomplete knowledge of the role of the CDK/cyclin/RBR complex in the cellular decision to divide or differentiate, it is also clear that cell

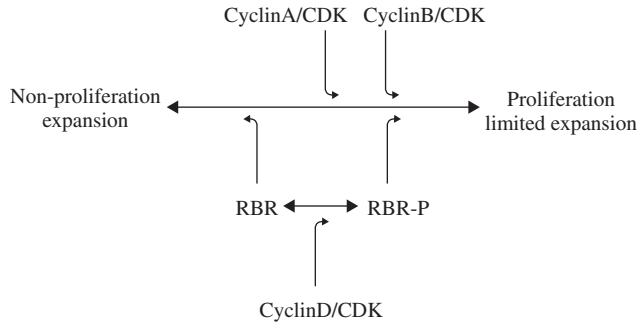

Figure 1.4 The balance of cell proliferation and non-proliferation is controlled by key elements of the cell cycle. A number of cyclin/CDK complexes have the potential to promote a balance in favour of cell proliferation against expansion. A key element is the cyclinD/CDK complex which promotes phosphorylation of the retinoblastoma-related protein (RBR) protein. Phosphorylated RBR (RBR-P) favours cell proliferation. Hypo-phosphorylated RBR (RBR) favours non-proliferation. The various cyclins (and their cognate CDKs) are likely to be targets for the transcription networks which control cellular identity and architecture.

division can be manipulated via altered expression of other components of the cell cycle (e.g. Hemerley *et al*., 1995; Doerner *et al*., 1996; Cockcroft *et al*., 2000; De Veylder *et al*., 2002). To what degree these components play a role in the endogenous programme controlling cell division and differentiation is still unclear, but the data indicate that there are potentially multiple targets by which the transcription networks controlling cellular architecture could impinge on the cell cycle.

Although progress through the cell cycle is normally associated with cell division, it is also possible for cells to undergo repeated phases of DNA replication without cytokinesis (endoreduplication) (Sugimoto-Shirasu and Roberts, 2003). This process leads to nuclei possessing increased amounts of DNA and an increase in nuclear size. A correlation can be made between increased nuclear size and increased cell size, thus switching to endoreduplication is a potential mechanism by which plant cells could promote their own growth, and there are data to support a causal relationship. However, there are also exceptions to this rule (De Veylder *et al*., 2002). The mechanism by which cells switch to endoreduplication is still obscure, but it represents another potential target for transcriptional networks defining cell fate.

This brief discussion of the plant cell cycle demonstrates both the progress that has been made and the limitations that remain in our understanding of this process. However, as pointed out at the beginning of this chapter, consideration of the cell cycle without growth provides only half the story. Recently, this imbalance has begun to be redressed as researchers have turned their attention to the question of the mechanism of growth. Thus, in drosophila, it has been shown that although cdk4/cyclinD normally influences both growth and proliferation, these two outputs are separable. In particular, cdk4/cyclinD can influence growth via modulation of a prolyl hydrolase activity (HPH) which is also involved in cellular response to hypoxia (Frei and Edgar, 2004). Other work, mainly in drosophila, has also highlighted the role of the insulin signalling pathway and nutrient availability in the regulation of growth (separable from the cell cycle) (Bohni *et al*., 1999; Fingar *et al*., 2002). In plants, our understanding of the cellular mechanism controlling growth is seriously limited. This issue is important since, because of the unique structure of plant cells, the machinery involved in promoting (or restricting) growth in plants might be significantly different from that in animals and yeast. Thus, growth in most plant cells is distinguished by the presence of a large vacuole and an extensible but restraining surrounding cell wall. Growth of a plant cell thus requires not only biosynthesis of new cellular material (cytoplasm, membrane, cell wall material), but also involves the generation of an internal hydrostatic pressure (termed turgor) which is counteracted and contained by forces generated within the cellulose-based cell wall. When these two forces are balanced, no growth will occur. Provided sufficient turgor pressure is present, and sufficient metabolic energy and products are available to synthesise the cellular components required for increase in size, the resistance of the cell wall to the forces imposed on it appears to be a key element limiting both extent and direction of cell expansion (Cosgrove, 2000). Thus, regulation of the biophysical parameters of plant cells has been proposed as a major

control point dictating cell size and shape. In particular, interest has focused on the architecture and composition of the cell wall as a major factor influencing cell wall extensibility and, thus, the growth potential and vector of both tissues and component cells. Molecular factors regulating cell wall extensibility are likely to be targets for the transcriptional networks defining cellular architecture.

A number of factors have been implicated in the control of cell wall extensibility, but a significant amount of work has focused on a family of cell wall proteins termed expansins (Cosgrove, 2000). These proteins have been shown to influence cell wall extensibility *in vitro* and, when overexpressed in transgenic plants, to alter aspects of organ growth. In addition, transcripts for certain expansin genes have been shown to accumulate in a polar fashion within certain cells, consistent with the idea that the synthesis of particular cell wall proteins might be targeted to particular regions of the cell (Im *et al*., 2000). However, many other cell wall proteins, carbohydrates and glycoproteins have also been implicated in the control of cell wall extensibility. In the absence of definitive molecular genetic data showing a precise role for most of these gene products, their endogenous function in setting or limiting aspects of cell growth remains open to discussion. Discovering which of these potential effectors of cell growth are indeed targets for the transcriptional networks defining cell identity will shed new light on this important problem.

The properties of the cell wall clearly play an important role in defining the growth rate and vector of cells and tissue. In addition, the positioning of the new cell wall during cytokinesis plays a direct role in determining cellular architecture. Following nuclear division in plants, a new cell plate is formed between the forming daughter nuclei. This new cell wall may be formed equidistant between the daughter nuclei, leading to the formation of two similar sized cells. Alternately, the new cell plate may be positioned asymmetrically and/or slanting between the nuclei, leading to the formation of daughter cells with distinct size and shape. Not only does this lead to an obvious change in cellular architecture of an organ, it can also have a consequence on the potential future preferred orientation of growth of the daughter cells. This differential growth potential is frequently associated with differentiation. The processes involved in new cell plate formation and the preferred orientation of cell growth are intimately linked with the cytoskeleton and interactions between the cytoskeleton and the cell wall. The cytoskeleton, thus, acts as an intermediary between the transcriptional architects and the mechanics of cell proliferation and growth and is likely to be a key target for these transcriptional regulators. This is discussed further in the next section.

1.6 The cytoskeleton as an intermediary in the regulation of cellular architecture

Plant cells contain two primary networks of cytoskeletal elements – the microtubule- and actin-based networks. A vast body of data supports the idea that these networks are fundamental to the processes of cell growth and division (Figure 1.5). However,

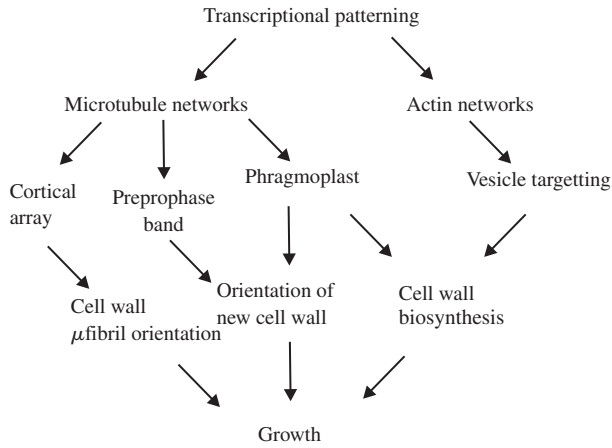

Figure 1.5 The cytoskeleton is a key intermediary in the transduction of transcriptional patterns to differential growth. Both microtubule and actin networks are likely targets for transcriptional networks which orchestrate the pattern of growth and division within a tissue. These networks converge on aspects of cell wall biosynthesis, orientation and architecture. Modulation of the cell wall is a key target for the networks regulating cellular architecture.

the exact contribution made by the separate networks and how these networks are controlled and interact remain matters for discussion.

Microtubules are highly dynamic structures consisting of polymers of alpha and beta tubulin whose biosynthesis requires sequential expression of a precise set associated proteins (Mayer and Jurgens, 2002; Steinborn *et al*., 2002; Hashimoto, 2003). Mutation of at least some of the genes whose products are involved in microtubule biosynthesis is lethal. Interestingly, although the loss of microtubule biosynthesis prevents any semblance of cell division, some degree of cell growth is observed. This general linkage of microtubule function with aspects of cell division is related to the requirement of microtubule arrays for specific elements of cell division. These include the mitotic spindle, the preprophase band (PPB) and the phragmoplast, the latter two being plant-specific. The PPB is of particular interest since it plays a determining role in the positioning and orientation of the new cell wall that is laid down towards the end of cytokinesis (Wick, 1991; Kost *et al*., 1999; Smith, 1999). Early during the commitment to cell division, microtubules in the cell cortex become organised into a ring of parallel bundles, the PPB. Irrespective of the actual orientation of the mitotic spindle (which is formed later and along which chromosomes are separated to the appropriate daughter cells), the position of the PPB seems to mark the mother cell wall so that the nascent cell wall (synthesised within the dividing cell) becomes oriented to fuse with this marked position. The mechanism of this marking remains totally unclear yet is vitally important for a full understanding of cellular architecture. As mentioned in the previous section, the orientation of the new cell wall formed during cell division directly dictates

the shape of the cells formed and may greatly influence the subsequent vector of cellular growth. Understanding the molecular mechanism of PPB formation remains a major challenge.

Greater progress has been made in our understanding of the phragmoplast. This arises as a fusion of microtubules and vesicles between the newly forming nuclei subsequent to nuclear division and which forms the template for the new cell wall (Nishihama and Machida, 2001). The vesicles supply components for the new cell wall which forms as a disc that gradually enlarges until the fusion of the leading edge with the mother cell wall. The greatest insight into phragmoplast formation has come from the work on a family of dynamin-like proteins termed phragmoplastin (Gu and Verma, 1997; Kang *et al*., 2003). This protein accumulates in the developing phragmoplast where it plays a role in the accretion of vesicles. Overexpression of phragmoplastin leads to abnormal orientation of the new cell wall, probably due to prolonged or misdirected supply of cell wall material. This suggests that the fixation of cell wall position by the PPB can be overcome by disruption of the processes involved in cell wall biosynthesis. The outcome of phragmoplastin-induced misorientation of cell division plane is dependent on the extent of phragmoplastin misexpression. Thus, when the protein is constitutively expressed throughout development, a seedling can be formed, but further growth does not occur (Geisler-Lee *et al*., 2002). If overexpression of phragmoplastin is limited in time and space then, although cell division pattern is locally disrupted, further growth and development of the tissue appears normal (Wyrzykowska and Fleming, 2003). This raises the question of the extent to which the normal pattern of cell division (and thus cellular architecture) is required for normal pattern of growth and development at the level of the whole organ and plant (discussed in Section 1.7).

Microtubule arrays are also observed in non-dividing cells, most notably in the formation of parallel cortical arrays which tend to lie perpendicular to the principal axis of extension of a cell. Such arrays have long been hypothesised to influence the orientation of cellulose microfibrils forming in the cell wall, presumably by guiding the movement of cellulose synthesising enzyme complexes along the plasma membrane (Mayer and Jurgens, 2002). Since cellulose is highly inextensible, the orientation of these microfibrils is thought to severely restrict the possible vector of growth of a cell. Thus, control of microfibril orientation provides a direct mechanism by which a cell can regulate its potential size and shape. Recently, the identification and characterisation of plants in which genes encoding particular tubulins are mutated has shed new insight on this hypothesis (Furutani *et al*., 2000; Thatamadee *et al*., 2002). Thus, in *lefty* mutant cells, cortical microtubule arrays have been shown to form predominantly right-handed spirals (in contrast to the *spiral1* mutant in which the cortical arrays take up a predominantly left-handed spiral). Interestingly, the *lefty* mutant plants display a left-handed helical growth whereas the *spiral1* mutant plants display a right-handed growth. Thus, the overall orientation of growth can be related to the overall orientation of the microtubules within the cells.

Further support for the idea that microtubules guide the orientation of cellulose microfibrils has come from the finding that mutation of a katanin-like protein (which causes aberrant microtubule orientation) is associated with an aberrant orientation of cellulose microfibrils (Burk and Ye, 2002). However, despite the various strands of data supporting the original hypothesis, the exact molecular mechanism by which microtubules might guide microfibril orientation remains to be elucidated. Indeed, whether cellulose microfibril orientation always plays an essential role in growth orientation role has been cast in doubt (Sugimoto *et al*., 2003). To summarise, the entire area of cytoskeletal/cell wall interactions is one which is vital to a full understanding of the control of cellular architecture but about which our knowledge remains extremely limited. With respect to the question of what determines cortical microtubule orientation in the first place, specific microtubule-associated proteins are likely to play a key role (Whittington *et al*., 2001). As the identity and function of further proteins associated with the microtubules is elucidated, progress in our understanding of the control of this important cytoskeletal network can be expected.

The other primary cytoskeletal network is actin-based and various lines of evidence indicate that it too plays a significant role in influencing cell architecture. Its role, however, is less linked to the events of cell division and more to directing the components required for cellular growth (Figure 1.5). In this aspect, actin seems to be a key integrator for signalling processes that influence cell size and shape (Vantard and Blanchoin, 2002). Much of the research on the role of actin and its associated proteins has focused (for various experimental reasons) on special cell types, such as pollen, root hairs and trichomes. These cell types display a particular type of growth termed tip growth (i.e. extension of the cell is limited to one specialised region which leads to a single cell taking on a tubular structure). Although this type of growth is widespread in plants, the majority of cells in higher plants undergo some degree of isodiametric growth (i.e. growth occurs to some extent in all directions). Relating the possibly specific roles of the actin network in tip growth to a generalised role in all cell growth may not always be valid, although disruption of the actin network leads to reduced cell elongation throughout the plant (Baluska *et al*., 2001), that is, actin is required for growth of cells growing isodiametrically.

Disruption of the actin network in tip growing cells leads to a cessation of growth which is not observed when microtubule organisation is disrupted. However, the initialisation of tip growth may be microtubule dependent. Thus, the initial formation of root hairs is inhibited by the use of pharmacological agents that interfere with tubulin dynamics, whereas the same inhibitors do not appear to interfere with root hair growth once they have been established (reviewed in Vantard and Blanchoin, 2002). Actin filaments are thought to provide both the physical thrust for tip growth, as well as guides or structures for the polarised delivery or distribution of material required for growth. Their function is intimately linked with families of associated proteins which integrate actin structure with signalling events within the cell. Thus, recent data have implicated Rho-like GTPases in plants (Rops), actin-related

proteins (Arps) and profilins, villin and ADF proteins as factors involved in the transduction of signalling systems (predominantly GTP and calcium) into altered cytoskeletal organisation (e.g. Li *et al*., 1999; Dong *et al*., 2001; Li *et al*., 2003).

A key function of the cytoskeleton in cell growth appears to be as a guidance system for the delivery of vesicles to particular regions of the cell. It is noticeable that a number of mutants in which cell growth is disrupted have been found to be disrupted in elements of vesicle transport and that these disruptions often lead to severe developmental phenotypes. Appropriate targeting and functioning of vesicle transport is required for cell growth and these transport processes represent another potential target for the transcriptional networks that define cell architecture (Lukowitz *et al*., 1996; Assad *et al*., 2000; Waizenegger *et al*., 2000).

1.7 The supracellular organisation of growth

1.7.1 The relationship between cell architecture and organ size and shape

The basic theme of this chapter is that cellular architecture is determined by the balance of cell growth, proliferation and the orientation of cell division. One might suppose that controlling these elements would also lead to the control of the size and shape of the organs to which the cells contribute, that is, that cellular architecture would directly influence organ morphology. Surprisingly, there is a significant body of data indicating that this is not the case. These findings suggest that there is a supracellular regulation of organ size and shape, the nature of which is still very obscure.

Initial insight into this issue came from classical studies on plant morphogenesis which showed that plant cells could take on very complicated forms without cell division (Kaplan, 2001) and that multicellular plants in which cell division was inhibited could still undergo some degree of vectorial growth (Foard, 1971). More recently, the analysis of mutants has indicated that a relatively normal plant morphology can be generated despite quite severe disruption of the rate and orientation of cell division. For example, the *TANGLED1* gene in maize encodes a protein which associates with mitotic arrays of microtubules (Smith *et al*., 2001). Mutation of this gene leads to an altered orientation of the new cell wall from that which normally occurs, most notably in the leaf in which cell division orientation in wild-type maize plants generates a highly ordered pattern (Smith *et al*., 1996). Despite this *TANGLED1*-associated disruption of cell division pattern (and, thus, disruption of wild-type cellular architecture), a plant of approximately normal size and shape is produced. These data both concur with the general importance of microtubule-associated events with the process of cell division (see previous section) and indicate that, at the level of the whole organism, the precise cellular architecture contained within the organism is not of overriding importance.

Work from our own group also showed that disruption of the highly ordered pattern of cell division observed in the tunica layer of the SAM (see Section 1.3)

had no overt deleterious outcome on meristem function (Wyrzykowska *et al*., 2002; Wyrzykowska and Fleming, 2003). Similarly, experiments in which progress through the cell cycle was promoted or inhibited led to the observation that overall plant morphology was generally little influenced but that component cell size was adjusted to compensate for there being 'too many' or 'too few' cells, that is, cellular architecture seems to accommodate to the overall parameters of organ growth rather than the cellular parameters of growth and division driving the organ. For example, overexpression of some components of the cell cycle (such as cyclinD) leads to an increased rate of growth and of cell proliferation (Cockcroft *et al*., 2000). However, the final plant size attained is approximately normal. Conversely, decreased activity of some components of the cell cycle (such as CDK) leads to a decreased rate of the cell cycle and fewer cells in the final organs formed (Hemerley *et al*., 1995). However, the average cell size is increased so that the overall size of the organs generated by the plant approach normality, that is, there is a compensatory mechanism by which cell architecture within an organ accommodates to some preset value of organ size. It should be stressed that although such compensatory events are observed following the manipulation of some parameters of the cell cycle, other manipulations do lead to changes of organ size. However, the outcome of these manipulations is not always intuitive. Indeed, a general observation is that promotion of cell proliferation leads to a decrease in organ size. This has been observed following, for example, constitutive overexpression of E2F-like transcription factors as well as local, transient overexpression of cyclinA (De Veylder *et al*., 2002; Wyrzykowska *et al*., 2002). At the same time, inhibition of the cell cycle (via, e.g. overexpression of CDK inhibitors) also tends to lead to a decrease in final plant and organ size, with average cell size being slightly increased (Wang *et al*., 2000; De Veylder *et al*., 2001). With respect to manipulation of cell division orientation, a spectrum of phenotypes has been observed. Some manipulations lead to severe developmental defects (Traas *et al*., 1995) whereas others (which clearly lead to significant changes in cellular architecture) have very little bearing on whole plant and organ growth and size (Smith *et al*., 1996). The overall message is that there appears to be a certain threshold of capability required for cell division and growth without which a viable plant cannot be generated. However, once this basic level is achieved, the system can display extreme flexibility in the balance of cell growth and division to maintain appropriate organ size. The mechanism of this size setting remains a mystery. Theoretical considerations have led to the suggestion that gradients of morphogens could act to regulate growth over space and that discontinuities in the gradient could be sensed, leading to an appropriate growth response (Day and Lawrence, 2000). Alternatively, suggestions have been made that there might be some mechanism for the measurement of tissue mass, with alterations from a set normal level leading to compensatory growth (Potter and Xu, 2001).

Although the molecular machinery underpinning these theoretical mechanisms of size control remains unclear, significant interest in the animal field has focused on the finding that the insulin signalling pathway has a significant impact on the

final size of the organism (Bohni *et al*., 1999). Plant research in this area is much less advanced with the greatest interest being aroused by the characterisation of the *AINTEGUMENTA* gene, overexpression of which is sufficient to induce an increase in organ size (Krizek, 1999; Mizukami and Fischer, 2000). This gene encodes a putative transcription factor, the target processes of which are unknown. Identifying and characterising the mechanism by which *AINTEGUMENTA* exerts its influence promises to provide a significant insight into the mechanism of control of plant organ size. Recently, the ARGOS gene product has been implicated as an intermediary between auxin signalling and regulation of growth by *AINTEGUMENTA* (Hu *et al*., 2003). Overexpression of ARGOS (an auxin up-regulated gene product) leads to an increase in lateral organ size and its activity is dependent on the presence of a functional AINTEGUMENTA protein. AINTEGUMENTA and ARGOS may thus be part of a regulatory system which sets organ size. Further elucidation of the regulation of these genes and the nature of their downstream targets is to be expected.

1.7.2 Cell division and organ initiation

The above discussion indicates that cell division can be disrupted with often only limited outcome on the overall morphology of an organ during its growth. This raises the question of what role cell division plays during the process of organ initiation. Starting with leaf initiation, as described in Section 1.3, the SAM is distinguished by a distinct pattern of cell division in which new cell walls in the outermost cell layers are oriented in an anticlinal direction. At about the time of leaf initiation, a switch to more random or periclinal division orientation is observed, leading to the suggestion that this change in cellular architecture is instrumental in leaf organogenesis (Steeves and Sussex, 1989). Work from our own group has shown that this is not the case. Thus, local promotion of cell proliferation or disruption of the pattern of cell division in the SAM has no influence on leaf formation (Wyrzykowska *et al*., 2002; Wyrzykowska and Fleming, 2003). In contrast, it appears that altered growth characteristics of the tissue destined to form a leaf primordium (e.g. localised expression of expansins, leading to non-uniform cell growth, and/or reorientation of growth direction) is likely to be a key element in the process of leaf initiation (Pien *et al*., 2001). Again, cellular architecture seems to accommodate to the morphology of the organ within which it is formed.

With respect to the initiation of lateral roots, the situation may be different. Lateral organs are derived from a specialised group of cells termed the pericycle (Casimiro *et al*., 2003). Even before overt lateral root initiation, particular groups of pericycle cells located radially external to the protoxylem are distinguished by being slightly shorter than their neighbours, indicating that during their formation, a balance of cell division over expansion was favoured. During lateral root formation, these progenitor cells undergo a series of periclinally oriented cell divisions with only minimal increase in growth. The daughter cells generated by this process then undergo a process of growth in a radial direction associated with more limited

cell division (thus leading to larger cells) and the lateral root pushes through the overlying tissue. During this process, a region at the tip of the emerging lateral root acquires a cellular architecture similar to that of the main root; thus, it appears to recapitulate the processes governing the development of the primary root. A variety of experiments have clearly implicated auxin in the process of lateral root initiation with auxin flux towards the lateral root progenitor cells as a key step in organ initiation (Himanen *et al*., 2002; Benkova *et al*., 2003). However, experiments in which components of the cell cycle have been manipulated in the root have led to various results. For example, manipulation of cdc25 activity led to an increased frequency of lateral roots (McKibbin *et al*., 1998) whereas elevated expression of a cyclinB did not influence rate of lateral root initiation (although cell division and growth rate throughout the root was promoted) (Doerner *et al*., 1996). It is possible that auxin-induced progression through the cell cycle is an essential step for the initiation of lateral roots but that promotion of cell division *per se* is not sufficient for organogenesis to occur. However, it is unclear if the cells fated to alter their growth pattern to form a lateral root also show altered cell wall characteristics analogous to those proposed to occur during leaf initiation.

1.7.3 Coordination of organ initiation

Leaf formation involves coordinated changes in cell growth and division in a defined spatial and temporal pattern within the SAM. This pattern (termed phyllotaxis) indicates that there is a signalling mechanism controlling when and where organ genesis occurs. Recent data indicate that auxin distribution and flux is a key intermediary in this process.

Micro-application of auxin and auxin-derivatives to specific areas on the surface of the SAM showed that exogenous manipulation of auxin levels and transport within the SAM was sufficient to initiate organogenesis and suggested that a high local level of auxin was sufficient to induce leaf formation (Reinhardt *et al*., 2000). These data were initially difficult to reconcile with classical ideas on leaf organogenesis which suggested that leaf formation occurred at the site of minimal accumulation of a positive factor diffusing into the meristem from the leaves previously generated by the meristem. However, the identification and characterisation of markers of auxin transport (the PIN genes) have led to a reconciliation of the modern experimental data with older models of meristem function (Benkova *et al*., 2003). Essentially, the observation of the pattern of auxin tranport marker gene expression suggests that auxin is continuously transported within the epidermal cells from the proximal tissue at the base of the meristem towards the distal tip (Reinhardt *et al*., 2003). Areas where leaf formation has occurred disrupt this distally oriented epidermal flux of auxin. This means that in the regions of the meristem in which leaf formation has not occurred recently, a greater flux of auxin in a distal direction occurs, leading to an accumulation of auxin at the presumptive site of leaf formation. Higher auxin levels initiate organogenesis at this site and this organogenesis leads automatically to a disruption of the upward epidermally located flux of auxin, thus reiterating the system.

The mechanism by which local altered auxin flux might lead to organogenesis is still unclear. However, bearing in mind the data supporting a role for cell wall characteristics in determining morphogenesis in the SAM (Pien *et al*., 2001), a link with the expression of gene products influencing cell wall extensibility seems likely. This pathway remains to be elucidated but the identification of auxin targets via various genetic and genomic strategies promises to shed light on this process in the near future.

1.8 Conclusions

Plant cell architecture depends on the integration of tissue growth and the compartmentation of the tissue via the insertion of new cell walls. This balance of growth and division is tightly controlled over space and time and is subject to both an endogenous genetic programme and to external signals dependent on the situation of the organism. Significant progress has been made over the past few years in our understanding of individual elements of this integrated programme. Thus, the identification of transcriptional networks that presage cell and tissue identities, the characterisation of components of the cell cycle and of cytokinesis, as well as progress in our understanding of cell wall and cytoskeletal architecture and composition, all represent significant strides forward. The challenge over the next few years will be to link these separate components together in a meaningful fashion to identify the chain or network of processes by which specific cellular architectures are achieved. Based on the rate of progress over the last decade, this goal is achievable.

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2 Leaf architecture

Regulation of leaf position, shape and internal structure

Julie Kang and Nancy G. Dengler

2.1 Introduction

The striking morphological diversity in the aboveground architecture of flowering plants is based in the arrangement and form of leaves, in the outgrowth of axillary buds, and in the relative degrees of stem elongation and thickening growth. Leaf architecture and size account for much of this variation: leaves range from the miniscule bracts of the apparently leafless asparagus shoots, to the simple petiolate leaves of *Arabidopsis* and *Antirrhinum*, to the pinnately–palmately compound leaves of the sensitive plant, to the peltate leaves of water lilies and to 10-m-long pinnately compound leaves of the *Raphia* palm. Despite this great range, all leaves share common attributes that reflect mode of development and their function as the photosynthetic organs of the plant. First, leaves are lateral organs that are formed during embryonic and post-embryonic growth from the flanks of the apical meristem. Second, as a consequence of their lateral position, leaves are polar along their dorsiventral axis; even leaves that appear radialized at maturity reveal their fundamental dorsiventrality at early stages of development or by their internal anatomy. Third, leaves are determinate in their growth plan. After initiation, the leaves of most plants expand for a period of a few weeks, but then tissues lose their meristematic properties and cells undergo terminal differentiation. Fourth, all leaves share a common internal architecture with a vascular system that is designed for efficient unloading and loading of water and nutrients to neighboring photosynthetic cells. Thus, leaves are fundamentally different from stems and roots, which are axial in nature, radial in symmetry, indeterminate in growth plan and have a vascular anatomy that is specialized for long-distance transport.

Leaves of diverse form and size share common developmental pathways. They are initiated on the flanks of the shoot apical meristem (SAM) in a precise pattern that is predicted by shoot phyllotaxis. Leaf initiation is marked by an alteration in growth direction, forming a protuberance or leaf primordium, which essentially translates the ‘inside–outside’ symmetry of the SAM into the adaxial–abaxial (dorsiventral) symmetry of leaves. The meristematic potential of the leaf primordium may be limited, or it may undergo a complex pattern of growth suppression and enhancement, producing a diversity of leaf forms during the primary morphogenesis stage. The fundamental leaf architecture produced early in leaf development can

be enhanced or modified during the leaf expansion, or secondary morphogenesis stage. The development of internal leaf architecture, or histogenesis, overlaps the morphogenetic phases in time. While the dermal and ground tissue systems are derived from the L1, L2 and L3 layers of the SAM, the vascular tissue system is formed *de novo* within the ground meristem. Despite the great diversity in leaf venation patterns throughout the vascular plants, they all function to optimize the distances over which water and solutes move between the vascular and other leaf tissues. Cell types within tissues differentiate appropriately within the morphological domains formed by the interacting proximal–distal and adaxial–abaxial axes of the leaf.

Our goals in writing this chapter are to review recent advances in our understanding of the processes that position leaves on the shoot, give rise to distinctive external architectures and coordinate internal with external architecture. We also endeavor to integrate recent findings with selected earlier studies. A number of recent reviews have addressed various aspects of the development of leaf architecture. These include reviews by Byrne *et al*. (2000), Bharathan and Sinha (2001), Dengler and Tsukaya (2001), Kidner *et al*. (2002), Reinhardt and Kuhlemeier (2002), Veit and Foster (2002), Tsukaya (2002, 2003), Tsiantis and Hay (2003), Kessler and Sinha (2004) and Veit (2004).

2.2 Phyllotaxis

The arrangement of leaves on the stem (phyllotaxis) is one of the striking features of shoot morphology. Since axillary buds are positioned in relation to leaves, phyllotaxis is an important determinant of overall shoot architecture. Phyllotactic patterns are characterized by the number of leaves borne at each node and the angle of divergence between leaves at successive nodes. In the most common variant – helical phyllotaxis – shoots bear one leaf per node and the angle of divergence is 137.5°, so that a line drawn through the center of successively formed leaves inscribes a shallow helix (the ontogenetic helix) about the SAM (Figure 2.1). In plants with distichous phyllotaxis, nodes bear single leaves, and successive leaves have a divergence angle of 180°. In decussate species, shoots bear two leaves at each node and successive leaf pairs are oriented at 90°. In whorled phyllotaxis, more than two leaves are borne at each node, with the positioning of leaves at successive nodes rotated by half the angle of divergence. Although a shoot's phyllotactic pattern may be obscured by petiole reorientation or by internode elongation and torsion during development, description of phyllotactic pattern makes it possible to predict the positioning of the next leaves to be formed on the SAM with great accuracy (Callos and Medford, 1994; Jean, 1994; Lyndon, 1998; Reinhardt and Kuhlemeier, 2002; Byrne *et al*., 2003).

Within this broad pattern of consistency and predictability, the specific phyllotactic pattern of an individual may shift during ontogeny, particularly during the transition from the juvenile to adult phase (Poethig, 2003). In many dicots, the opposite positioning of the cotyledons conditions the placement of the next leaves in

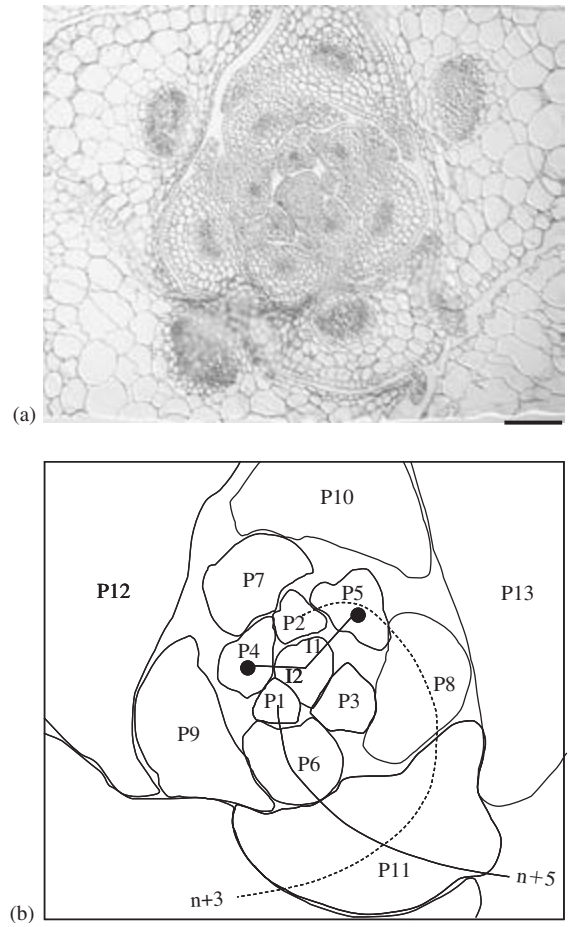

Figure 2.1 Shoot apex transverse section illustrating helical phyllotaxis in *Arabidopsis* (a). In the drawing of the same apex (b), leaves are numbered in order of increasing age (P1, P2, etc.), including the sites of incipient primordia (I1, I2). The angle of divergence, calculated as the angle between the radii of two successive leaves, is indicated for leaves 4 and 5. The plastochron ratio is calculated as the ratio of the lengths of these radii. Three clockwise parastichies connect every third leaf (e.g. n+3, dashed line) and five counterclockwise parastichies connect every fifth leaf (n+5, solid line).

sub-opposite pairs, with a random placement of the leaf initiating either a right- or a left-handed helix. In *Arabidopsis*, placement of floral meristems on the inflorescence continues the ontogenetic helix of the vegetative stage, despite the suppression of bract outgrowth (Long and Barton, 2000; Kang *et al*., 2003; Dinneny *et al*., 2004). In other species, phyllotactic pattern shifts again with the transition from vegetative to reproductive growth (Meicenheimer, 1982; Carpenter *et al*., 1995). In *Antirrhinum*, phyllotaxis is decussate during the vegetative phase and helical during inflorescence

development. Mutations in the meristem identity genes *FLORICAULA (FLO)* and *SQUAMOSA (SQUA)* delay the transition from helical to the whorled phyllotaxis of the flower, resulting in mutant phenotypes with intermediate phyllotactic patterns (Carpenter *et al*., 1995).

2.2.1 Helical phyllotaxis and the Fibonacci series

Helical phyllotaxis has held a fascination for biologists and mathematicians for more than a century. In addition to the ontogenetic helix of sequentially formed leaves, adjacent primordia on the SAM form a series of steeper helices, called parastichies (Esau, 1965; Callos and Medford, 1994; Jean, 1994; Lyndon, 1998; Reinhardt and Kuhlemeier, 2002). Parastichies connect leaves that are formed at regular intervals on the ontogenetic helix. For instance, in *Arabidopsis*, lines passing through the center of every third leaf primordium form three clockwise parastichies (Figure 2.1). Five counterclockwise parastichies connecting every fifth leaf can also be recognized. Helical systems are described by the numbers of intersecting clockwise and counterclockwise parastichies (3 + 5, 5 + 8, etc.). These numbers are members of the Fibonacci series where each number is the sum of the two preceding numbers (1, 2, 3, 5, 8, etc.). Recognition of parastichies depends on the packing of leaf primordia around the meristem; this parameter is summarized by the plastochron ratio – the ratio of the distances between two successive leaf primordia and the center of the meristem (Figure 2.1). These mathematical characterizations of helical phyllotaxis permit predictions of the placement of the next-formed leaf primordium and lead to testable hypotheses about the regulation of phyllotaxis (Lyndon, 1998).

2.2.2 Regulation of phyllotaxis

Theories for the regulation of phyllotaxis fall into two broad categories: (i) those that invoke interactions among primordia on the SAM, and (ii) those that invoke an inductive signal from older regions of the shoot (Larsen, 1983; Jean, 1994; Lyndon, 1998; Reinhardt and Kuhlemeier, 2002). The SAM is a dynamic system, continuously producing new leaf primordia while maintaining its own size. Incipient leaf primordia are placed on the flank of the meristem at the maximum distance from previously formed primordia (Figure 2.2). Such a pattern suggests that primordia might be placed through a reaction–diffusion mechanism in which preexisting primordia are sources of a diffusible inhibitor and new primordia arise in the region of lowest inhibitor concentration (Meinhardt, 1984, 1996). Theoretical models also suggest that phyllotactic patterning on the SAM arises through purely physical, rather than chemical, interactions among preexisting primordia (Green, 1999). The correspondence between the positions of new leaf primordia and the early appearance of the procambial strands that will later become their leaf traces suggests that vasculature in older parts of the shoot could provide an inductive signal for the placement of leaf primordia (Esau, 1965; Larsen, 1983; Lyndon, 1998; Kang *et al*., 2003). In *Populus grandifolia*, leaf traces can be identified at least seven

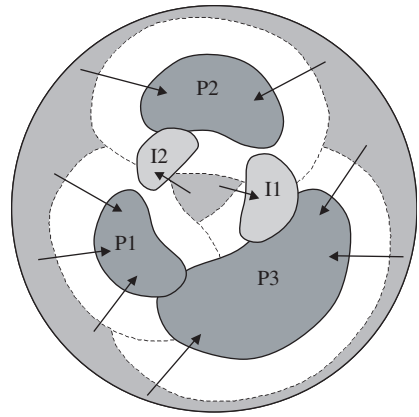

Figure 2.2 Model for regulation of phyllotaxis by auxin [adapted from Reinhardt *et al*. (2003)]. Auxin is transported acropetally toward the meristem (arrows). Primordia (P1, P2, P3) become strong sinks for auxin and deplete surrounding regions of the meristem (white areas). Auxin accumulation at certain minimum distances from earlier-formed primordia induces primordium initiation at the I1 and I2 sites, which in turn become sinks. This model combines the processes of positive feedback (auxin accumulation) and lateral inhibition (depletion of auxin from the surrounding tissue) that are conceptually comparable to the short-range activator and long-range inhibitor of reaction–diffusion systems (Meinhardt, 1984, 1996). A different mechanism presumably prevents primordium initiation at the summit of the meristem.

plastochrons before leaf initiation (Larsen, 1983). In *Arabidopsis*, expression of the *PINHEAD/ZWILLE (PNH/ZWI)* gene occurs in narrow strands of tissue below the site of the incipient primordia (I2 stage, Figure 2.3; Lynn *et al*., 1999), and expression of the preprocambial marker *AtHB-8* reveals the position of the leaf trace procambium at the I1 stage (Kang *et al*., 2003). The strong correlations between the three-dimensional architecture of vasculature within the shoot and the phyllotaxis generated on the SAM suggest, at least, that mechanisms regulating phyllotaxis also organize shoot vascular pattern.

To date, very few bona fide phyllotaxis genes have been identified. Mutations in one of these, the *ABBERANT PHYLLOTAXIS (ABPHYL)* gene, cause a shift from distichous to decussate phyllotaxy in maize (Greyson and Walden, 1972; Jackson and Hake, 1999). Shoot apical meristems of *abphyl* mutants are larger than wild-type, indicating that meristem size might be a causal factor in the regulation of phyllotaxis. In contrast, mutations in *BELLRINGER (BLR)* – a homeobox transcription factor belonging to the *BELL* class – result in phyllotactic defects in *Arabidopsis*, including displacement of the site of floral meristem initiation to a divergence angle of approximately 80–110° (Byrne *et al*., 2003). Shoot apical meristem dimensions of *blr* mutants do not differ from wild type, indicating the *BLR* expression influences the site of leaf initiation independently of meristem size. The relatively small number of mutants may reflect the self-organizing nature of phyllotaxy, in which

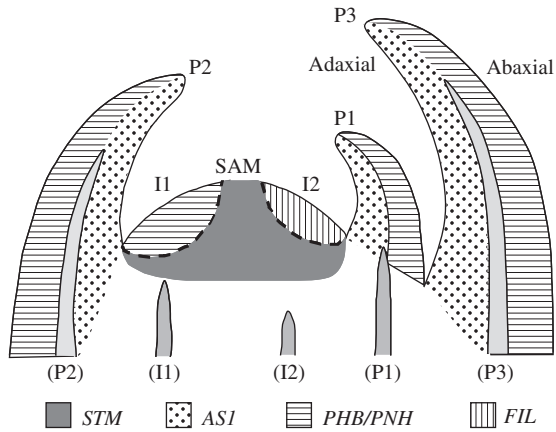

Figure 2.3 RNA expression patterns of genes that mark leaf initiation and the establishment of leaf dorsiventral symmetry in the shoot apical meristem of *Arabidopsis*. The *KNOX* gene *SHOOTMERISTEMLESS (STM)* is expressed throughout the shoot apical meristem (SAM), but is downregulated at sites of leaf initiation (Long *et al*., 1996). The *PHAN* homolog *ASYMMETRIC LEAVES1 (AS1)* is expressed at least by the I1 stage and in leaf primordia until the P4 stage (Byrne *et al*., 2000). A regulator of adaxial domain identity, *PHABULOSA (PHB)* is expressed throughout the site of initiation (I1) and in P1 stage primordia; by the P2 stage, however, *PHB* expression is restricted to the adaxial domain (McConnell *et al*., 2001). *PINHEAD (PNH)* has a similar expression pattern and is also expressed strongly in the locations of leaf trace procambia, including those that will supply the leaves at I1 and I2 (Lynn *et al*., 1999). The *YABBY* gene, *FILAMENTOUS FLOWER (FIL)* is strongly expressed throughout the sites of leaf initiation (I1 and I2) and then becomes restricted to the abaxial domain of the primordium at the P1 stage (Siegfried *et al*., 1999). *AS1*, stippled; *FIL*, horizontal lines; *PHB* and *PNH*, light grey; *STM*, dark grey. Sites of leaf initiation, I1, I2; primordia, P1, P2, P3; procambial strands, (I1) (I2), (P1), (P2), (P3).

mechanistic elements that play other roles in plant development function together to generate phyllotactic pattern (Reinhardt and Kuhlemeier, 2002).

Hormones are logical candidates for signaling molecules that could play a role in the regulation of phyllotactic pattern, and recent pharmacological experiments, use of reporter constructs and immunolocalization studies have identified a pivotal role for auxin in regulating phyllotaxis. Application of the auxin transport inhibitor *N*-1-naphthylphthalamic acid (NPA) to SAMs of tomato specifically inhibits primordium formation, while other meristem properties, such as expression of the homeobox gene *LeT6* and of *histone H4* are unaltered (Reinhardt *et al*., 2000). The resultant shoots have a pin phenotype, with leaf production completely suppressed. Local treatment with indole-3-acetic acid (IAA) induces the formation of leaf primordia on the tomato pins, and pin shoots recovering from NPA treatment establish helical phyllotaxis after a few plastochrons. These results suggest that positioning of primordia requires a localized accumulation of auxin that is dependent on an NPA-inhibitable auxin transport (Reinhardt *et al*., 2000). More recently, the *PIN1*

auxin efflux carrier has been shown to accumulate specifically in young primordia (P1, P2, etc.) and in the sites of incipient primordium formation (I1, I2; Reinhardt *et al*., 2003). Furthermore, the PIN1 protein shows a subcellular localization to the apical-most side of cells in the surface layer of the meristem, which subsequently becomes restricted to a narrow file of cells below the incipient primordium, perhaps corresponding to the zones of expression of *PNH/ZWI* and *AtHB-8* (Lynn *et al*., 1999; Kang *et al*., 2003). The expression of *PIN1* in a pattern that predicts the placement of the next leaf primordium is abolished in *pin1–1* mutants (Reinhardt *et al*., 2003). Together, these observations support a reaction–diffusion model, much like that proposed by Meinhardt (1984, 1996). In such a model (Figure 2.2), preexisting primordia and young developing leaves serve as sinks for auxin, depleting the surrounding field of cells. A new sink for auxin arises at the maximum distance from the previously formed sinks (along the ontogenetic helix at the 137.5° divergence angle from P1). Internally directed auxin could induce formation of the vascular strand that connects the primordium with stem vasculature at the same time. Thus, a single inductive signal could coordinate positioning of a new primordium on the SAM and formation of the predictable vascular links with earlier formed leaves.

2.3 Leaf initiation

The physical process of leaf initiation involves several events that occur more or less simultaneously (reviewed by Lyndon, 1998). The first external indication that organogenesis is underway at the I1 position is the bulging of an externally discernable ledge on the flank of the SAM. This bulge represents a new axis of anisotropic growth that is orthogonal to the prior surface of the meristem flank. Anisotropic growth in plants requires a loosening of the cell wall while turgor is maintained and is typically accompanied by a circumferential orientation of cellulose microfibrils in the cell wall that mirrors the orientation of cortical microtubules in the cytoplasm (Sugimoto-Shirasu and Roberts, 2003). A reorientation of microfibrils that anticipates the actual protuberance of the primordium has been documented for species with helical and with decussate phyllotaxis (Green, 1999). Periclinal divisions in the surface layers of the meristem typically accompany the outward bulging of a new primordium and are followed by other non-anticlinal divisions in deeper layers (Lyndon, 1998). Newly oriented divisions are not restricted to the site of primordium initiation, however, indicating that altered planes or rates of divisions alone are likely to be a causal factor in organogenesis. Moreover, cell divisions without accompanying cell expansion would not result in growth along a novel axis.

2.3.1 Role of expansin in leaf initiation

Recent studies have identified expansin-induced local changes in cell wall extensibility as the key event in leaf initiation (Fleming *et al*., 1997,1999). When beads

loaded with purified expansin were placed on the I2 position of tomato meristems, primordium-like bulges, which later developed some morphological features of mature leaves and expressed molecular markers of photosynthesis, were formed. Further, induction of localized expression of the expansin gene *CsExp1* in tobacco SAMs was sufficient to initiate the entire program of leaf development, from initiation through to mature leaves with a morphology and anatomy indistinguishable from untreated plants (Pien *et al*., 2001). In contrast, use of the micro-induction system to promote local cell division did not result in leaf initiation and organogenesis, thus, providing strong support for the view that cell divisions cannot be causal in leaf initiation (Wyrzykowska *et al*., 2002; Wyrzykowska and Fleming, 2003). It remains to be ascertained what controls the patterns of expansin expression in these meristematic cells, although auxin may have a role here (Reinhardt *et al*., 2000).

2.3.2 Molecular markers of leaf initiation

In addition to the prepatterns formed by expression of the auxin efflux carrier *PIN1* (Reinhardt *et al*., 2003) and of the wall extensibility protein expansin (Pien *et al*., 2001), expression patterns of several transcriptional regulators provide molecular markers for the sites of leaf initiation. The class 1 *KNOTTED*-like homeobox *(KNOX)* family of homeodomain genes, including *knotted-1 (kn-1)* of maize, *SHOOT MERISTEMLESS (STM)* of *Arabidopsis* and *LeT6* of tomato, are normally expressed throughout the SAM, but downregulated at the site of leaf initiation (Jackson *et al*., 1994; Long *et al*., 1996; Janssen *et al*., 1998; Long and Barton, 2000; Figure 2.3). In leaves, downregulation of *KNOX* genes is maintained by the MYB family transcriptional regulator *PHANTASTICA (PHAN)* in *Antirrhinum* (Waites *et al*., 1998) and its orthologs *ROUGH SHEATH2 (RS2)* in maize (Timmermans *et al*., 1999; Tsiantis *et al*., 1999; Theodoris *et al*., 2003), *ASYMMETRIC LEAVES1 (AS1)* in *Arabidopsis* (Byrne *et al*., 2000, 2002) and *LePHAN* in tomato (Kim *et al*., 2003b). In turn, both *STM* and *LeT6* have been shown to downregulate *PHAN* expression in meristems (Byrne *et al*., 2000; Kim *et al*., 2003b), suggesting that the balance between these two antagonistic groups of transcription factors determines whether a region on the flank of the meristem becomes specified as the site of leaf initiation or not (Tsiantis and Hay, 2003; Kessler and Sinha, 2004). Interestingly, alteration of cell division patterns within the SAM by the induced expression of phragmoplastin also alters the expression patterns of *STM, PHAN* and *YABBY* genes in tobacco, suggesting that cell division frequency and orientation feeds back into the expression of transcription factors thought to regulate leaf initiation (Wyrzykowska and Fleming, 2003).

2.4 Development of leaf symmetry

The initiation and development of leaf primordia and of other lateral organs takes place in a unique spatial environment. Unlike radially symmetrical stems and roots,

leaves have dorsiventral symmetry from inception: the adaxial side of the primordium (the part toward the meristem center) arises from the central part of the SAM, while the abaxial side of the primordium (the part away from the meristem center) arises from the more peripheral part of the SAM. The conical geometry of the meristem and the constrained physical environment in the apical region results in a primordium that is flattened on its adaxial side and rounded on the abaxial surface. Enhanced growth of the abaxial side results in curvature of the primordium, and abaxial tissues are enlarged and vacuolated in comparison to adaxial tissues so that the dorsiventrality that is characteristic of mature leaves is expressed both internally and externally from leaf inception. Leaf initiation on the SAM also provides a frame of reference for the development of polarity along the proximal–distal axis, usually expressed as formation of leaf base, petiole and blade domains. Leaves typically display at least a subtle asymmetry along the medial–lateral axis as well and, in some species with horizontally oriented shoots, the right and left halves of the blade may be strongly unequal – an adaptation thought to reduce self-shading, particularly in low light environments (Dengler, 1999; Dengler and Tsukaya, 2001).

Classical experiments have shown that surgical isolation of incipient primordia from the meristem results in loss of dorsiventrality and that a signal emanating from the central region of the meristem is required for its maintenance (Sussex, 1951; Snow and Snow, 1959). More recent molecular studies have identified key molecular players in this putative signaling pathway and show how gene expression patterns in the meristem could be translated into the dorsiventral symmetry of mature leaves (see below).

2.4.1 Adaxial domain

Analysis of mutant phenotypes in the *PHANTASTICA (PHAN)* gene of *Antirrhinum* has identified a key component in the development of dorsiventral leaf architecture (Waites and Hudson, 1995). Leaves of *phan* mutants display a range of phenotypes, but later-formed leaves tend to be radially symmetrical and abaxialized, in that the epidermal, ground and vascular tissues express specific features usually restricted to the abaxial side of the leaf. Other leaves of *phan* mutants are dorsiventrally flattened, but bear ectopic patches on the adaxial surface in which epidermal and ground tissues express abaxial features. The boundaries of these ectopic patches are marked by an outgrowth of laminar tissue (Waites and Hudson, 1995). *PHAN* encodes an MYB transcription factor and is expressed not only at the site of primordium initiation (I1), but also throughout the primordium during the first few plastochrons of development (Waites *et al*., 1998; Figure 2.3). Based on the mutant phenotype, *PHAN* appears to be required for specification of an adaxial domain during post-initiation leaf development, although how expression becomes translated to a signal guiding tissue differentiation on the adaxial side of the leaf is unknown. Waites and Hudson (1995) also hypothesized that the juxtaposition of adaxial and abaxial domains is a prerequisite for the outgrowth of the leaf lamina,

and that the loss of either domain results in formation of a radialized leaf – a hypothesis that has been subsequently supported by phenotypic analysis of other mutants.

In *Arabidopsis*, the *PHAN* ortholog *AS1* primarily functions to downregulate *KNOX* genes at the site of leaf inception (Byrne *et al*., 2000), although severe *as1* alleles result in radialized petioles, indicating a secondary role for *AS1* in establishment of dorsiventrality (Xu *et al*., 2003). Development and maintenance of leaf dorsiventrality in *Arabidopsis* also depends on a mutual antagonism between two other groups of genes, including a newly discovered role for microRNAs. Analysis of mutant phenotypes and of mRNA expression patterns indicates that the adaxial domain is specified by members of a plant-specific class of homeodomain – leucine zipper containing proteins (HD–ZIP), including *PHABULOSA (PHB), PHAVOLUTA (PHV)* and *REVOLUTA* (*REV)* (McConnell and Barton, 1998; McConnell *et al*., 2001). For instance, *PHB* is expressed at I1 and throughout the leaf primordium at the P1 stage; by the P2 stage, however, expression becomes restricted to the adaxial domain (McConnell *et al*., 2001; Figure 2.3). In addition to the HD–ZIP domain, these genes contain the START motif with similarity to mammalian sterol/lipid binding domains (McConnell *et al*., 2001). The semi-dominant mutations in *PHB* and *PHV* result from nucleotide substitutions in the START motif and have adaxialized phenotypes (McConnell and Barton, 1998; McConnell *et al*., 2001). The discovery of two microRNAs (miRNA 165 and 166) with almost complete complementarity to the START coding region of the HD–ZIP genes strongly suggests miRNA-mediated regulation of the restriction of expression to the adaxial domain of leaf primordia (Emery *et al*., 2003; Tang *et al*., 2003). The loss-of-function phenotype is only expressed in triple mutants, such as the *phb-6 phv-5 rev-9* combination, which has a single, radial abaxialized cotyledon, indicating redundant roles for members of this family (Emery *et al*., 2003). *In situ* hybridization using miRNA 165/166 probes show that these accumulate first in the meristem and then in the abaxial domain of the leaf, suggesting that cleavage of the HD–ZIP mRNAs by miRNAs on the abaxial side is required for restriction of the domain of action to the adaxial side of the primordium by P2 (Kidner and Martienssen, 2004). The expression pattern of miRNA 165/166 is influenced by mutations in *ARGONAUTE1 (AGO1)*, indicating that *AGO1* activity influences their regulatory activity (Kidner and Martienssen, 2004). Mutations in *AGO* and in the similar proteins *PNH/ZWI* result in partially abaxialized leaf phenotypes (Bohmert *et al*., 1998; Lynn *et al*., 1999), reinforcing the idea that full expression of leaf dorsiventrality depends on degradation of HD–ZIP mRNA within the abaxial domain.

Recent evidence indicates that at least some of the molecular mechanisms that determine leaf dorsiventrality are conserved between monocots and dicots (Juarez *et al*., 2004). The maize gene *rolled leaf1 (rld1)* encodes a HD–ZIP protein belonging to the same family as *PHB/PHV/REV*. The *rld1-O* mutant has a single nucleotide change in the miRNA 165/166 complementary site and a partially adaxialized phenotype, indicating that miRNAs may normally mediate the post-transcriptional repression of *rld1* expression on the abaxial side of the leaf primordium (Juarez

et al., 2004). The *Leaf bladeless (lbl)* gene also plays a role in the development of dorsiventrality in maize: the strongest mutant phenotypes form radially symmetrical, abaxialized leaf blades, although the molecular identity of *lbl* and how it might interact with other regulators of dorsiventrality are unknown (Timmermans *et al*., 1998).

2.4.2 Abaxial domain

Specification of the abaxial domain of *Arabidopsis* leaves requires activity of two distinct genetic pathways based on the *YABBY* and *KANADI* gene families (Siegfried *et al*., 1999; Eshed *et al*., 1999, 2001; Kerstetter *et al*., 2001; Emery *et al*., 2003). The *YABBY* genes belong to a small family of plant-specific transcription factors (Siegfried *et al*., 1999). mRNA of strongly expressed members of the family such as *FILAMENTOUS FLOWER (FIL)* appears first at sites of leaf inception (I2) and, as the primordium emerges from the meristem, becomes restricted to its abaxial side (Siegfried *et al*., 1999; Figure 2.3). Overexpression of *FIL* or *YABBY3* leads to radialized leaves and the ectopic expression of abaxial characteristics (Siegfried *et al*., 1999). *KANADI* genes belong to the *GARP* family of transcription factors and are expressed on the abaxial side of young leaf primordia (Kerstetter *et al*., 2001). Loss-of-function phenotypes result in the adaxialization of at least some leaf tissue characteristics (Kerstetter *et al*., 2001), while ectopic expression results in abaxialization similar to that induced by *YABBY* genes (Eshed *et al*., 2001). Thus, in *Arabidopsis*, leaf dorsiventrality requires activity of two opposing pathways at the molecular level: an adaxializing function that is mediated through the activity of the *PHB/PHV* and *REV* proteins and their regulators and an abaxializing function mediated by the *YABBY* and *KANADI* gene families (Eshed *et al*., 2001; Kerstetter *et al*., 2001). *YABBY* genes also play a secondary role in suppressing *KNOX* gene expression within leaves (Kumaran *et al*., 2002).

2.5 Development of simple leaf architecture

2.5.1 Dicots

In most dicots, the zone of leaf initiation is spatially restricted on the meristem flank and growth is directed outward, along the proximal–distal axis (Figure 2.4). In some taxa, particularly those with large sheathing stipules borne on the leaf base, the zone of initiation extends laterally, almost encircling the SAM (Hagemann and Gleissberg, 1996). Tissue on the abaxial side of the primordium bulges outward in continuity with thickening growth of the stem below, demarcating a region of enlarged and vacuolated cells along the petiole–midrib axis and thus establishing medial–lateral symmetry (Hagemann and Gleissberg, 1996). Tissues on the lateral sides of the primordium retain their meristematic appearance in continuity with the flanks of the meristem. In simple leaves, the meristematic activity of this marginal strip of tissue is short-lived, as inferred from clonal analyses of contributions of the

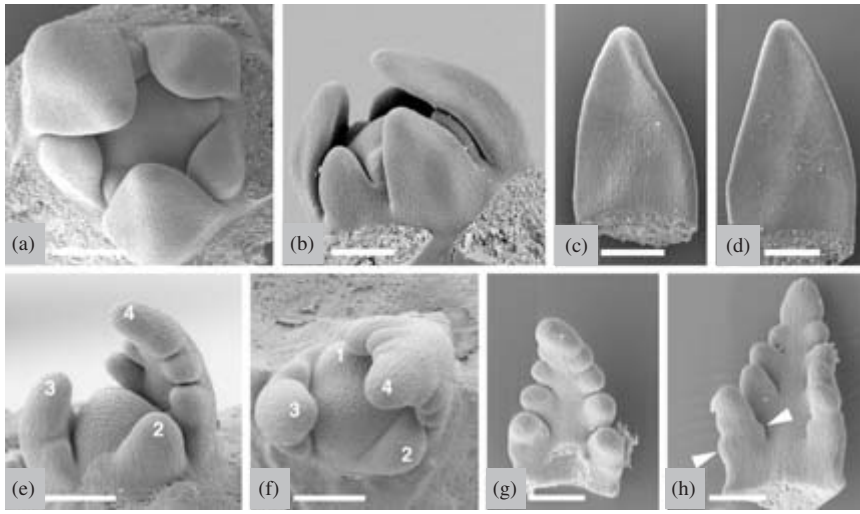

Figure 2.4 Primary morphogenesis of simple and compound leaves in the Papaveraceae. In the simple leaves of *Dendromecon harfordii*, marginal blastozone activity is suppressed at early developmental stages (a)–(d). Thickening growth on the abaxial side along the petiole–midrib axis initiates asymmetry along the medial–lateral axis symmetry (b). In the ternately compound leaves of *Eschscholzia californica*, fractionation of the marginal blastozone gives rise to primary leaflets by the P4 stage (e,f). The direction of primary leaflet formation is acropetal (g), as is the formation of secondary leaflets (arrowheads, h). The petiole is delimited by absence of blastozone activity at the base of the blade (h). Reproduced with permission from Gleissberg, S. (2004) 'Comparative analysis of leaf shape development in *E. californica* and other Papaveraceae–Eschscholzioideae', *American Journal of Botany* **91**, 306–12.

marginal meristem to internal tissues and from analyses of the distribution of dividing cells within the leaf blade (Poethig and Sussex, 1985; Poethig, 1987; Donnelly *et al*., 1999). The zone of marginal growth does not extend completely to the leaf base, and thus defines the boundaries of the petiole region which is intercalated between the blade and leaf base (Figures 2.4, 2.6a,b). In leaves with more complex shapes, the meristematic activity of this marginal strip is prolonged and gives rise to leaf serrations, lobes and leaflets. Since formation of these distinct growth centers parallels that of leaf initiation on the SAM, the term 'blastozone' is used to describe the meristematic margin with organogenic potential, despite its truncated period of activity in simple leaves (Hagemann and Gleissberg, 1996).

2.5.2 Monocots

In maize and other grasses, the site of leaf initiation extends laterally, so that it encircles the SAM (Sharman, 1942; Sylvester *et al*., 1990, 1996; Figure 2.5). Defects in lateral recruitment of cells from the meristem flanks can affect leaf

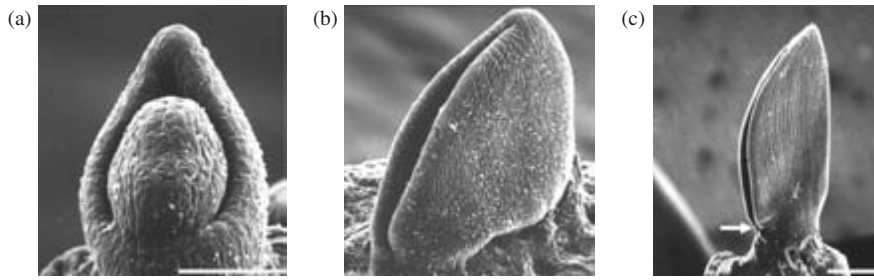

Figure 2.5 Primary morphogenesis of the simple leaves of the grass *Stenotaphrum secundatum*. The zone of initiation extends around the circumference of the shoot apical meristem (a). Proximo-distal patterning into the blade and sheath regions is evident at early plastochrons (b,c). Arrowheads, blade–sheath boundary. Reproduced with permission from Sud, R.M. and Dengler, N.G. (2000) 'Cell lineage of vein formation in variegated leaves of the C_4 grass *Stenotaphrum secundatum*', *Annals of Botany* **86**, 99–112. Scale bars = 50μm (a,b), 500μm (c).

morphology, as seen for the *lbl* and *narrow sheath* (*ns*) mutants of maize (Scanlon *et al*., 1996; Scanlon and Freeling, 1997; Timmermans *et al*., 1998). Early growth of the leaf primordium occurs along the proximal–distal axis, producing an elongated leaf within the first few plastochrons of development. A marginal blastozone is lacking, and all growth along the medial–lateral plane is intercalary. In grasses, the distal region of the leaf primordium differentiates as the blade and the proximal region as the sheath (Figure 2.5). Blade and sheath are typically distinguished by internal tissue architecture: the blade is characterized by photosynthetic mesophyll, high stomatal density and close vein spacing, while the sheath has low vein and stomatal density and a smaller proportion of ground tissues are specialized for photosynthesis (Sharman, 1942; Sylvester *et al*., 1990, 1996; Sud and Dengler, 2000). Most grasses bear a thin flap of tissue – the ligule – on the adaxial side of the leaf at the sheath – blade boundary. Mutations in several *KNOX*-related genes, including *ROUGHSHEATH1 (RS1), LIGULELESS3 (LGL3), LIGULELESS4 (LGL4)* and *GNARLEY (GN)*, induce development of ligule and sheath-like tissues in the more distal leaf blade, suggesting that that normal patterning along the proximal–distal axis of grass leaves is dependent on suppression of *KNOX* activity (Becraft and Freeling, 1994; Fowler *et al*., 1996; Foster *et al*., 1999).

2.6 Development of compound leaf architecture

Unlike simple leaves in which the uniform meristematic activity of the marginal blastozone is curtailed within a few plastochrons of initiation, compound leaves are distinguished by prolonged activity of the blastozone and a complex pattern of suppression and enhancement (Hagemann and Gleissberg, 1996). Although the terms 'compound' and 'dissected' are often used to describe leaves with complex

shape, the process of development is really analogous to an iterative branching process (Kaplan, 2001). Growth centers arise within the marginal blastozone to form leaflets on the main leaf axis. The directionality of leaflet formation can be acropetal, basipetal or divergent – a pattern where new primordia are formed both toward the leaf apex and toward its base (Hagemann and Gleissberg, 1996; Gleissberg, 1998a,b, 2004; Gleissberg and Kadereit, 1999). Some species are periplastic – a pattern in which formation of leaflet primordia occurs uniformly around the entire periphery of the marginal blastozone (Hagemann and Gleissberg, 1996). In species with more complex leaf shape, activity of the blastozone is prolonged, allowing higher-order branching to occur (Figure 2.4h). Cessation of blastozone activity is marked by cell enlargement of marginal tissues and differentiation of trichomes and other specialized marginal cells (Hagemann and Gleissberg, 1996; Gleissberg, 2004).

The generation of complex leaf shape by a reiterative branching process ('fractionation') of the blastozone represents the primary morphogenesis phase of leaf development (Hagemann and Gleissberg, 1996). In some plants, primary morphogenesis involves meristem 'incorporation' in which disjunct blastozones become continuous by localized growth across the adaxial face of the leaf base, forming a peltate leaf (Hagemann and Gleissberg, 1996; Figures 2.6c,d). A complex leaf shape produced through primary morphogenesis can be further altered through the differential distribution of growth during secondary morphogenesis. For instance, the amount of elongation growth along the petiole–rachis axis determines whether a compound leaf is pinnate or palmate: lack of extension of the rachis results in a palmate leaf, while extension results in a pinnate leaf (Hagemann and Gleissberg, 1996; Gleissberg and Kadereit, 1999; Kaplan, 2001, Kim *et al*., 2003a; Figures 2.6e,f,g). The relative timing of blade expansion and secondary morphogenesis also affects final leaf shape. In the *serrate (se)* mutant of *Arabidopsis*, leaf teeth are more prominent because they are formed earlier than in wild type and are enhanced because of subtle differences in leaf expansion (Groot and Meicenheimer, 2000). In *Begonia dregei*, leaf lobes and teeth expand at equivalent rates, but lobes are formed from the marginal blastozone earlier, and therefore reach larger mature sizes (McLellan and Dengler, 1995). In contrast, differences in the depth of the sinuses between lobes among subspecies of *Cucurbita argyrosperma*, do not appear until well after the primary morphogenesis stage and result simply from differential expansion (Jones, 1993).

In contrast to the morphogenesis of most compound leaves through blastozone fractionation, the complex leaf shapes of a handful of monocotyledonous groups arise through a secondary dissection of an initially simple leaf blade. In palms, localized growth during primary morphogenesis results in a series of parallel pleats in a submarginal position (Dengler *et al*., 1982; Kaplan *et al*., 1982). As the leaf expands, a separation zone develops between adjacent pleats and between the pleats and the non-plicate marginal strip, liberating individual pleats as leaflets. Presumably, the separation zone is functionally comparable to an abscission zone, but cellular mechanisms of separation are unstudied. In certain species of *Monstera* and a few related genera, compound leaf shape arises through the programmed cell

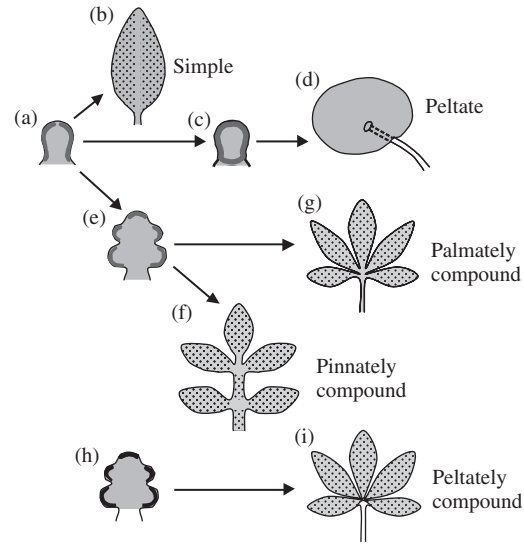

Figure 2.6 Role of marginal blastozone in development of external leaf architecture. A marginal blastozone is present as two lateral strips in most leaf primordia (a), but is suppressed at early developmental stages in simple leaves (b). When the two marginal strips join across the adaxial face of the primordium in the process of incorporation (c), a peltate leaf with the adaxial face inside the funnel or on the flattened side away from the petiole is formed (d). When the activity of the blastozone is prolonged, but its continuity is disrupted by localized areas of organogenic enhancement and suppression (fractionation), a compound leaf is formed (e). Diffuse growth of the rachis results in a pinnately compound leaf (f), while suppression of rachis growth results in a palmately compound leaf (g). Restriction of *PHAN* expression to the distal portion of the primordium (h) results in a peltately palmate leaf (i) [adapted from Kim *et al*. (2003a)]. Adaxial or *PHAN* domain, grey; blastozone activity, thick black lines; expansion and secondary morphogenesis, stippled.

death of discrete patches of cells early in the leaf expansion phase (Melville and Wrigley, 1969; Kaplan, 1984). These initially pinprick sized holes are stretched by leaf expansion and, in some species, the holes tear through the leaf margin, forming a deeply lobed leaf (Melville and Wrigley, 1969; Kaplan, 1984). A comparable mechanism occurs in the distantly related lace plant, *Aponogeton madagascariensis*: here, leaf blades retain a simple outline during expansion, but become perforated with rectangular holes through programmed cell death (Gunawardena *et al*., 2004). In this species, programmed cell death involves the early cessation of cytoplasmic streaming, tonoplast rupture, cleavage of genomic DNA into smaller fragments without laddering and cell wall degradation, followed by the late shrinkage and loss of cytoplasmic density – a sequence that is similar to programmed cell death during tracheary element differentiation (Gunawardena *et al*., 2004). How this diverse assemblage of monocotyledonous groups has co-opted abscission and programmed cell death mechanisms into leaf morphogenesis is a fascinating and, as yet, unexplored aspect of leaf development.

2.6.1 Molecular regulation of blastozone activity

2.6.1.1 KNOX genes

On a molecular level, the prolonged organogenetic activity of the marginal blastozone during compound leaf development is highly correlated with accumulation of KNOX protein (Bharathan *et al*., 2002). This correlation was predicted by the phenotypes of simple leaves in which *KNOX* genes were misexpressed. For instance, overexpression of *BREVIPEDICELLUS (BP)* in *Arabidopsis* results in highly lobed leaves bearing ectopic shoots (Lincoln *et al*., 1994; Chuck *et al*., 1996) The degree of lobing is correlated with the dosage of *BP* and steroid-induced expression of *BP* indicates that leaf tissues are competent to respond to ectopic *BP*, at least between the P2 and P6 stages of development (Hay *et al*., 2003). In *asymmetric1 (as1)* and *asymmetric2 (as2)* mutants of *Arabidopsis, KNOX* genes are not expressed during the I1 and P1 stages of primordium inception and formation, but are misexpressed in older primordia, corresponding to the 'window of competence' observed by Hay *et al*. (2003). These observations suggests that the direct or indirect mechanisms that regulate *KNOX* genes at leaf inception and during leaf growth may be different (Hay *et al*., 2003).

In tomato and a broad phylogenetic sample of other compound-leaved species, *KNOX* genes were found to be up-regulated in leaf primordia after the P1 stage (Bharathan *et al*., 2002). Direct overexpression of *KNOX* genes also leads to a dramatic increase in the level of dissection of mature leaves of tomato (Hareven *et al*., 1996; Chen *et al*., 1997; Janssen *et al*., 1998). The dominant mutations *Mouse ears (Me)* and *Curl (Cu)* result in the misexpression of the tomato *KNOX* gene *TKn6* and have highly dissected phenotypes, suggesting that *KNOX* genes are directly involved in the prolongation of the primary morphogenesis phase of leaf development (Hay *et al*., 2002). In tomato, the effects of *KNOX* genes on leaf development may act, at least in part, through mediation of the hormone gibberellic acid. For instance, misexpression of *KNOX* genes in the *Me* and *Cu* mutants is accompanied by reduced gibberellic acid biosynthesis, and application of exogenous GA decreases the degree of expression of *KNOX* genes in wild-type tomato and the *Me* and *Cu* mutants (Hay *et al*., 2002). Therefore, *KNOX*-induced suppression of GA biosynthesis may be necessary to maintain activity of the marginal meristematic zone during the development of compound leaves (Hay *et al*., 2002).

Characterization of *KNOX* expression also highlights the importance of secondary morphogenesis in determining mature leaf architecture. KNOX protein accumulation is conspicuous in the leaf teeth of young developing leaves of the crucifer *Lepidium oleraceum* and the basal angiosperm *Amborella trichopoda*, but in both cases, growth of marginal serrations is suppressed, resulting in simple leaves with almost entire margins at maturity (Bharathan *et al*., 2002).

2.6.1.2 Phantastica

Expression of *PHAN* also plays an important role in compound leaf development, and juxtaposition of adaxial and abaxial domains appears to be required to leaflet

formation and certain aspects of secondary morphogenesis in tomato and other compound leaved species (Kim *et al*., 2003a,b). In wild-type tomato, *LePHAN* RNA is expressed on the adaxial face of P3 and P4 primordia, as well as in the SAM and in leaf and stem vascular traces (Kim *et al*., 2003a,b). Expression of antisense *LePHAN* under control of *CaMV 35S* promoter results in abaxialized cup-shaped or needle-shaped leaves that resemble the mutant phenotypes of *as1* or *phan* in *Arabidopsis* and *Antirrhinum* (Kim *et al*., 2003a). Some transgenic lines have reduced numbers of leaflets and/or palmately compound leaves that are peltate with leaflets formed around the entire circumference of the petiole (Kim *et al*., 2003a). Leaves of tomato *wiry (w)* mutants have similar radialized phenotypes (Kessler *et al*., 2001), and expression of *LePHAN* is modified in developing leaves: in completely abaxialized needle leaves, *LePHAN* is not detected at all and, in mutants with cup-shaped or peltate palmate leaves, expression is limited to the distal region of primordia (Kim *et al*., 2003a). Comparison of *PHAN* expression in nine species, representing at least five independent evolutionary origins of compound leaves, indicates that the tomato pattern is a general one. *PHAN* is expressed throughout the adaxial domain in pinnate and non-peltately compound species, but is restricted to the distal region of the leaf in peltate species (Kim *et al*., 2003a; Figures 2.6h,i). This perfect correlation between *PHAN* expression and leaf morphology supports a model in which the boundary between adaxial and abaxial domains is required for leaflet formation along the marginal blastozone (Figure 2.6). Furthermore, in tomato, the expression patterns of *LePHAN* and the KNOX gene *LeT6* suggest that balanced dosages of each are required for compound leaf development (Kim *et al*., 2003b).

2.6.1.3 Floricaula, Leafy, Unifoliata and Falsiflora

In addition to a *KNOX*-mediated pathway, a range of compound-leaved species have been shown to utilize homologs of the meristem identity genes *FLORICAULA (FLO)* and *LEAFY (LFY)* of *Arabidopsis* and *Antirrhinum* (Hofer *et al*., 1997; Gourlay *et al*., 2000; Bharathan *et al*., 2002; Busch and Gleissberg, 2003). In pea and at least some other legumes, *KNOX* genes do not appear to function in compound leaf development (Hofer *et al*., 1997; Gourlay *et al*., 2000; Hofer *et al*., 2001; Bharathan *et al*., 2002). Instead, the *FLO/LFY* homolog *UNIFOLIATA (UNI)* gene appears to play an equivalent role in prolonging the organogenetic activity of the blastozone. In wild-type pea plants, *UNI* RNA is expressed not only in the SAM, but also in the blastozone regions of leaf primordia. Organogenic activity of the blastozone is acropetal in pea, with proximal stipules and leaflets formed before the more distal leaflets and tendrils; organogenesis continues through the P4 stage and is highly correlated with the expression of *UNI* (Hofer *et al*., 1997, 2001; Gourlay *et al*., 2000). *uni* loss-of-function mutants have a simple leaf shape phenotype, and activity of the marginal blastozone is terminated by P2 (DeMason and Villani, 2001; Hofer *et al*., 2001). Developmental analysis of single and double mutants of the *AFILA (AF)* and *TENDRIL-LESS (TL)* genes indicates that these genes negatively regulate *UNI*. Higher order branching of the leaf primordium continues until P7/P8 in *af* and *tl* mutants, and expression of *UNI* is

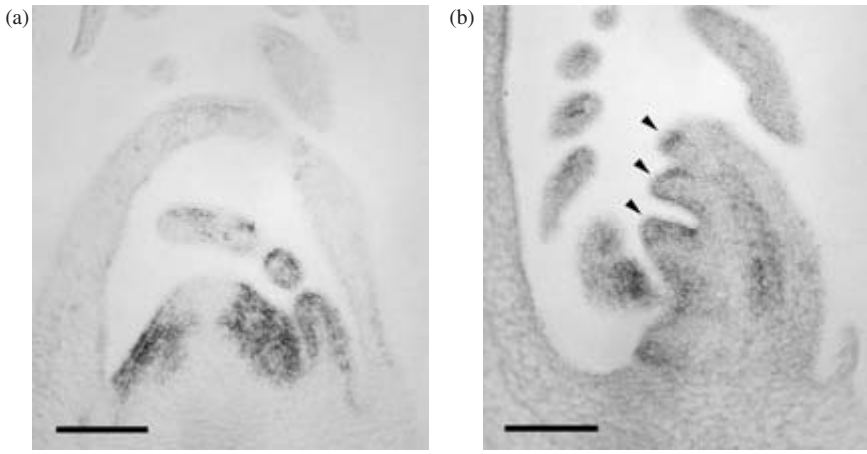

Figure 2.7 RNA expression of *EcFLO* in the shoot apical meristem (a) and during formation of leaflets from the marginal blastozone (b) in developing leaves of *Eschscholzia californica*. Reproduced with permission from Busch, A. and Gleissberg, S. (2003) '*EcFLO*, a *FLORICAULA*-like gene from *E. californica* is expressed during organogenesis at the vegetative shoot apex', *Planta* **217**, 841–8.

prolonged in mutant primordia (Gourlay *et al*., 2000; DeMason and Villani, 2001; Hofer *et al*., 2001). The KNOX gene *Pskn1* is also expressed in the SAM and shoot vascular strands in pea, but is not detectable in developing leaf primordia, even in the highly branched *af tl* mutants (Hofer *et al*., 2001). The role of *UNI* and its interacting genes in maintaining blastozone activity in this group of legumes presumably represents an evolutionary loss of this specific function of *KNOX* genes, since *KNOX* expression is strongly correlated with compound leaf architecture across a broad sample of vascular plants (Bharathan *et al*., 2002).

FLO/LFY homologs function in compound leaf development in other flowering plants. In the ternately compound leaves of *Eschscholzia californica, EcFLO* is expressed in the peripheral zone of the vegetative SAM (in contrast to *UNI*), downregulated at I1, and expressed throughout higher order branching of the leaf primordium (Busch and Gleissberg, 2003; Figure 2.7). In tomato, the *FLO* homolog *FALSIFLORA (FA)* is expressed in the marginal blastozone during leaflet formation, and *fa* mutants show a slight decrease in higher order dissection (Molinero-Rosales *et al*., 1999). In *Vitis vinifera*, the *FLO* homolog *VFL* is strongly expressed in the blastozone during lobe formation (Carmona *et al*., 2002). The broad phylogenetic distribution of compound leaves expressing *FLO* homologs during primary morphogenesis indicates that this pathway is also an ancient one in the evolutionary diversification of flowering plants (Busch and Gleissberg, 2003). Thus, it appears that the *KNOX* and *FLO* pathways can function either in concert or separately to regulate compound leaf development.

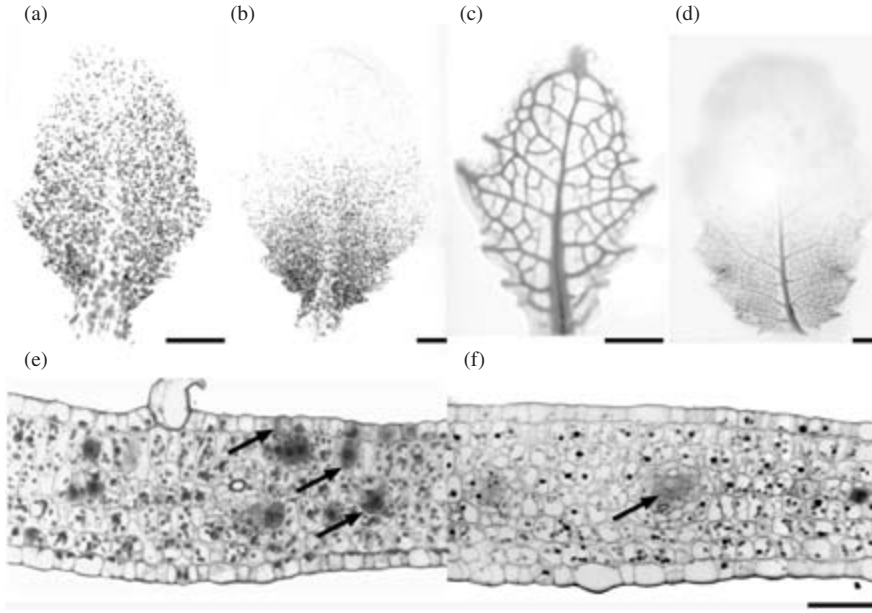

Figure 2.8 Formation of internal architecture and vascular pattern in the leaves of *Arabidopsis*. Spatial pattern expression of a B-type *cyclin::GUS* reporter at days 4 and 8 of leaf expansion (a,b). Spatial pattern of procambium-specific marker *AtHB-8::GUS* in leaves of comparable age (c,d). Cross-sections of leaves expressing the *cyclin::GUS* marker (e) and the *AtHB-8::GUS* marker at day 8. Note diffuse distribution of cycling cells in ground, dermal and vascular tissue system precursors (arrows) and predominance of anticlinal division planes giving rise to tissue layers in (e). *ATHB-8::GUS* expression is restricted to undifferentiated procambium (arrow, f). Reproduced with permission from Kang, J. and Dengler, N. (2002) 'Cell cycling frequency and expression of the homeobox gene *AtHB-8* during leaf vein development in *Arabidopsis*', *Planta* **216**, 212–19. Scale bars = 500μm (a)–(d), 20μm (e)–(f).

2.7 Leaf expansion

Following primary morphogenesis, leaves expand several-thousand-fold in surface area. This dramatic growth requires coordination of overall tissue expansion with cell division, so that cellular units of the appropriate size and shape for mature function are produced at the same time as mature leaf size is realized. In *Arabidopsis*, cell divisions are initially diffusely distributed throughout the leaf but, as the blade enlarges, the zone of cycling cells gradually becomes restricted to the more basal portions of the blade and then to the petiole (Donnelly *et al.*, 1999; Kang and Dengler, 2002; Figure 2.8). Two levels of control of this orderly basipetal suppression of cell proliferation have been identified recently. The *CINCINNATA (CIN)* gene of *Antirrhinum* is a member of the *TCP* family of DNA binding proteins, and leaves of mutant plants are rounder and larger than wild type, with a crinkly surface

and an overall downward curvature (Nath *et al*., 2003). During blade expansion, mutants show a delay in the basipetal arrest of cell proliferation, and the shape of the arrest front is altered from weakly convex to strongly concave, allowing marginal regions to continue growth longer than the medial regions of the blade (Nath *et al*., 2003). *CIN* is hypothesized to act by sensitizing tissues to the cell cycle arrest front (Nath *et al*., 2003). *CIN*-like genes have been shown to be regulated in turn by a microRNA in *Arabidopsis* (Palatnik *et al*., 2003). Dominant mutations in an independent locus, *JAW*, result in serrated leaves with a downward curvature that is reminiscent of *cin* mutants in *Antirrhinum*, and expression of *CIN* is decreased in *jaw-D* mutants (Palatnik *et al*., 2003). Introduction of a miRNA-resistant *TCP* gene into *jaw-D* mutant rescues the mutant phenotype, indicating that miRNA mediated control of *CIN* is required for the proper transition between cell proliferation and differentiation (Palatnik *et al*., 2003). While *CIN* appears to act by promoting cell cycle arrest during blade expansion, other genes such as *JAGGED (JAG)* appear to have an antagonistic effect. *jag* mutants have leaves and petals with saw-tooth-like distal margins and an early arrest of cell cycle activity in the distal portion of the blade, indicating that wild-type *JAG* functions to suppress cell cycle arrest (Dinneny *et al*., 2004; Ohno *et al*., 2004). The auxin regulated gene *ARGOS* also functions to maintain cell proliferation during leaf expansion (Hu *et al*., 2003). Expression of *ARGOS* is induced by auxin, and *ARGOS* in turn transduces this signal to the indirect and direct regulators of the cell cycle, *AINTEGUMENTA1 (ANT1)* and *CycD3* (Hu *et al*., 2003).

During wild type development, mean cell size does not change in regions of active cell division, indicating that cell cycling must also mark zones of active cell growth (Francis, 1998). As leaf tissues gradually exit the cell cycle, mean cell size increases dramatically, particularly so when endoreplication of nuclear DNA is a component of cell differentiation (Melaragno *et al*., 1993; Donnelly *et al*., 1999; Mizukami, 2001). Perturbation of cell division through misexpression or mutation of cell cycle regulators such as cyclin-dependent kinases (CDKs), cyclins or CDK inhibitors, indicates that a suite of overlapping and redundant mechanisms compensate for defects in either cell division or expansion (Hemerly *et al*., 1995; Smith, 1996; Mizukami and Fischer, 2000; Tsukaya, 2003). For instance, when a dominant-negative mutation in an A-type CDK was used to suppress cell divisions, leaves had fewer cells at maturity, but were of normal size and shape, indicating that cell expansion had compensated for reduced cell division (Hemerly *et al*., 1995). Similarly, overexpression of the *INHIBITOR OF CYLIN-DEPENDENT KINASE 1 (ICK1/KRP)* gene results in leaves with fewer, but larger, cells (Wang *et al*., 2000; DeVeylder *et al*., 2001). Loss-of-function of the *ANT1* or *STRUWWELPETER (SWP)* genes results in observable decreases in cell number and compensatory increases in cell volume (Mizukami and Fischer, 2000; Autran *et al*., 2002). Leaf expansion can also be robust to perturbations of the planes of cell division. In the *tangled1 (tan1)* and *warty1 (war1)* mutants of maize, leaves undergo a disrupted pattern of cell divisions during expansion, yet, are able to maintain a wild-type-like overall leaf shape (Smith *et al*., 1996; Reynolds *et al*., 1998).

In other cases, overall leaf expansion does not compensate for perturbation of cell cycling, planes of cell division or the directionality of cell expansion. Suppression of the cell cycle through overexpression of *ICK1* or *KRP2* has dramatic effects on leaf shape (lobed or strongly toothed), suggesting that some regions of the leaf might be more susceptible to cell cycle suppression than others (Wang *et al*., 2000; DeVeylder *et al*., 2001). Overexpression of the cyclin gene *CycD3;1* results in abnormally shaped leaves with incompletely differentiated cells (Dewitte *et al*., 2003). Perturbation of the directionality of cell expansion occurs in the *angustifolia (an)* and *rotundifolia3 (rot3)* mutations of *Arabidopsis* without any compensatory adjustment in cell proliferation (Tsuge *et al*., 1996). *an* mutants have narrow leaves, and cells undergo reduced expansion in the medial–lateral plane, while *rot3* mutant leaves are rounded and cells undergo reduced expansion in the proximal–distal plane (Tsuge *et al*., 1996). So, while there is often a compensatory interaction between the cell-based processes of cell division and cell expansion, misexpression of cell cycle regulators or mutations in indirect regulators of the cell cycle (see later) often results in abnormally sized or misshapen leaves, indicating that there are limits to this mutual interaction (Mizukami, 2001; Beemster *et al*., 2003; Tsukaya, 2003).

2.8 Development of internal leaf architecture

Despite the great diversity in external leaf architecture, internal leaf anatomy displays common features across a range of leaf shapes and sizes. As in other organs, leaf tissues are organized into three tissue systems: the dermal, ground and vascular tissue systems (Figure 2.8). Within each tissue system, cells differentiate in a spatial pattern that is appropriate for the context provided by external leaf architecture. For instance, dorsiventral symmetry is expressed through differences in stomatal density and trichome distribution on the adaxial and abaxial epidermal layers. In the photosynthetic ground tissue of the leaf (mesophyll), palisade parenchyma cells are specialized for light capture on the adaxial side of the leaf, and spongy parenchyma cells are specialized for gas diffusion. A collateral arrangement of vascular tissues, with xylem positioned toward the adaxial side of the leaf and phloem toward abaxial side, is the most common pattern (Esau, 1965; Dengler and Kang, 2001). Many variations on these themes occur, of course: in vertically oriented leaves such as those of most grasses, the mesophyll is homogeneous and in some families, leaf veins are bicollateral, with both abaxial and adaxial phloem. Nevertheless, the context-dependent differentiation of specialized cells is sufficiently consistent to provide anatomical markers of the adaxial and abaxial domains in the analysis of mutant phenotypes (e.g. Waites and Hudson, 1995; McConnell and Barton, 1998; Timmermans *et al*., 1998; Lynn *et al*., 1999; McConnell *et al*., 2001; Emery *et al*., 2003; Juarez *et al*., 2004). In addition, the organization of internal leaf tissues into these three tissue systems is highly robust to perturbation of cell divisions and other developmental processes: in both mutants and misexpression lines, the fundamental

organization of dermal, ground and vascular tissues remains, despite disruption of leaf size and shape and/or cell size and shape (e.g. Hemerly *et al*., 1995; Smith *et al*., 1996; Reynolds *et al*., 1998; Mizukami and Fischer, 2000; Wang *et al*., 2000; DeVeylder *et al*., 2001; Dewitte *et al*., 2003).

2.8.1 Cell division and tissue patterning

Differential patterns of cell division and enlargement are among the first visible markers of differentiation of the three tissue systems. In *Arabidopsis*, cell division is prolonged and net cell expansion is delayed in the palisade as compared to the spongy mesophyll layers (Donnelly *et al*., 1999). Cell cycling associated with stomate formation (see later) continues long after the cessation of divisions in the ground tissue, but enlargement of other epidermal cells overtakes that of mesophyll cells quickly (Donnelly *et al*., 1999). Cell cycling in the procambium – the precursor to the vascular tissue system – continues throughout the period of leaf expansion and within the largest veins even after blade expansion is complete (Donnelly *et al*., 1999; Kang and Dengler, 2002). Although the leaf venation system is characterized by a hierarchical pattern of vein orders, most veins are equivalent in diameter at the time of origin, and it is the duration of cell divisions that essentially remodels the venation system during leaf expansion (Kang and Dengler, 2002, 2004).

A conspicuous feature of internal leaf architecture is the layered arrangement of dermal and mesophyll tissues. These layers arise from the regularity of anticlinal cell divisions in the precursor tissues – the protoderm and ground meristem. Observation of conspicuous two-dimensional ‘plates’ of cells that arise from exclusively anticlinal division planes gave rise to the term ‘plate meristem’ to describe the diffuse pattern of growth that perpetuates cell layers during expansion of dicot leaves (Esau, 1965; Maksymowych and Wochok, 1969). Although development of internal leaf architecture is characterized by predictable patterns of cell division, clonal analyses have shown that positional context takes priority over lineage in guiding cell differentiation (Poethig, 1987; Marcotrigiano, 2001). Genetic mosaic analysis has also provided evidence for communication among cell layers and for the influence of specific internal layers on overall leaf shape (McHale and Marcotrigiano, 1998; Marcotrigiano, 2001). Recent work has identified a role for *PHAN* as a molecular component in the translation of domains of adaxial and abaxial identity into tissue-specific cell proliferation patterns (McHale and Koning, 2004). In tobacco, *NTPHAN* is first expressed throughout the leaf primordium, but expression becomes restricted to the middle mesophyll layer in expanding leaves. In antisense transgenic tobacco plants, the adaxial mesophyll layer appears suspended in an immature state, showing disorganized patterns of proliferation and delayed differentiation of the palisade parenchyma (McHale and Koning, 2004). This phenotype is correlated with ectopic expression of the class I KNOX gene *NTH20*, suggesting that *NTPHAN* normally functions to downregulate *NTH20* in the adaxial mesophyll, thus promoting the determinate state and an orderly pattern of anticlinal cell divisions (McHale and Koning, 2004).

In the leaves of maize and other grasses, distinctive patterns of cell division are associated with leaf expansion and the formation of tissue layers (Sylvester *et al*., 1990, 1996; Sud and Dengler, 2000). Genes that function to maintain these regular patterns of cell division have been identified by mutant analysis. For instance, mutations in the *Extra cell layer1 (Xcl1)* gene disrupt the pattern of anticlinal divisions in the protoderm, so that additional protoderm layers are produced by both oblique and periclinal divisions (Kessler *et al*., 2002). Clonal analysis of this mutant supports the conclusion that the multiple-layered epidermis is derived through periclinal divisions of the protoderm, since all layers express epidermis-specific features (Kessler *et al*., 2002). Similarly, in *tan1* mutants of maize, the longitudinal anticlinal cell divisions that increase the numbers of cell files in the ground and dermal tissue systems are disrupted (Smith *et al*., 1996). In the leaf blade, the photosynthetic bundle sheath layer that surrounds the vascular bundles is normally one cell layer thick, but in *tan1* mutants, aberrant cell divisions give rise to multilayered bundle sheaths (Janovsky *et al*., 2001). As with the *xcl1* mutant, the extra bundle sheath cell layers maintain the same identity as their clonally related cells, suggesting that specific cell fates may become determined, even when precursor cells are competent to respond to cell proliferation signals (Janovsky *et al*., 2001).

2.8.2 Vascular pattern formation

Leaf vein pattern is characterized by a hierarchy of vein size orders, continuity between veins and regular spacing (Nelson and Dengler, 1997; Dengler and Kang, 2001; Scarpella and Meijer, 2004; Figure 2.8). Although great diversity in leaf venation exists among flowering plants, these common features function to maintain an even flow of water and nutrients throughout the vein system (Roth-Nebelsick *et al*., 2001). In dicots, the leaf trace procambial strand is continuous with stem vasculature and extends acropetally into the leaf primordium, establishing the course of the primary vein (Nelson and Dengler, 1997; Kang *et al*., 2003). Second-order veins usually appear as branches of the primary vein and extend toward the leaf margin, although in *Arabidopsis*, early formed secondary veins appear as continuous loops (Nelson and Dengler, 1997; Candela *et al*., 1999; Mattsson *et al*., 1999; Sieburth, 1999; Dengler and Kang, 2001; Kang and Dengler, 2004; Scarpella *et al*., 2004). In leaves with a complex blade shape, second-order vein formation coincides temporally with formation of leaflets or lobes from the marginal blastozone and the sequence of vein formation reflects the directionality of primary morphogenesis (reviewed in Nelson and Dengler, 1997). The higher order minor venation is formed during the diffuse expansion of the leaf blade, and minor veins usually arise from a middle layer of the ground meristem. Procambial strands appear simultaneously along their length, delimiting polygonal regions of ground tissue – the areoles. Mutant phenotypes with vascular pattern defects and experiments using auxin transport inhibitors point to a strong connection between the polar transport of auxin and

development of vein pattern (reviewed in Turner and Sieburth, 2002; Reinhardt, 2003; Scarpella and Meijer, 2004).

In some monocot groups, leaf venation forms a reticulate pattern comparable to that of most dicots (Bharathan, 1996). In the grasses and other advanced groups, however, the larger major and small minor veins form a longitudinal striate pattern with equidistant spacing (Nelson and Dengler, 1997). Adjacent veins, particularly small minor veins, join at the blade apex and base, so that only the midvein and other larger veins extend through the sheath in continuity with the stem vasculature. Small transverse veins interconnect adjacent longitudinal veins forming a closed reticulum. The ontogeny of grass leaf vein pattern differs from that of dicots in one significant aspect: vein procambium is initially isolated within the primordium and develops basipetally to connect with the stem vasculature (Bosabalidis *et al*., 1994; Dengler *et al*., 1997, Nelson and Dengler, 1997). In maize, large numbers of procambial strands extend basipetally from the developing leaf and 'capture' stem procambial strands within the stem node (Pizzolato and Sundberg, 2001, 2002). This initial discontinuity between stem and leaf vascular systems suggest that the initial signals, or at least their signaling pathways, might differ between the dicot and monocot modes of vascular pattern formation.

Advancement of understanding of leaf vein pattern formation depends on the recognition of pattern at its earliest stages. The precursor to vascular tissues – procambium – is identified anatomically as a strand of cytoplasmically dense, narrow cells that divide parallel to the direction of leaf expansion (Esau, 1965; Nelson and Dengler, 1997; Scarpella and Meijer, 2004). As might be predicted from mutant phenotypes and auxin transport inhibition studies, molecular markers of auxin signaling are expressed prior to the appearance of the distinctive anatomical features of procambium (Mattsson *et al*., 2003). The homeobox gene *AtHB-8* is also expressed in narrow strands of ground meristem cells prior to the appearance of procambium and, surprisingly, is expressed in a progressive pattern, unlike the simultaneous appearance of procambium (Kang and Dengler, 2004; Scarpella *et al*., 2004). As a member of the the *HD–ZIP* Class III group of homeodomain proteins, *AtHB-8* is closely related to *PHB, PHV* and *REV* (McConnell *et al*., 2001; Emery *et al*., 2003). Unlike other members of the group, the function of *AtHB-8* is likely restricted to vascular development as it is expressed only as procambial prepatterns in undifferentiated procambium, and in differentiating xylem precursors (Baima *et al*., 1995; Kang and Dengler, 2002, 2004; Scarpella *et al*., 2004; Figure 2.8). Interestingly, the expression of *AtHB-8* in xylem precursors suggests that it functions in establishing dorsiventrality within vascular tissues, much as related genes establish broader domains of dorsiventrality.

2.8.3 Epidermal cell pattern

The differentiation of specialized cell types begins during the primary morphogenesis stage and continues throughout leaf expansion. The small number of cell types and accessibility of the leaf epidermis makes this tissue highly suitable for studying

cell pattern formation (Larkin e*t al*., 2003). The spacing of stomates and trichomes within the epidermal layer is not random, indicating that patterning mechanisms ensure an optimum spacing for function (Larkin *et al*., 1997; Nadeau and Sack, 2002a, 2003; Larkin *et al*., 2003). These mechanisms have been hypothesized to be either lineage-based or to involve a lateral inhibition mechanism, but it is only with the identification of genes involved in epidermal cell patterning that strong evidence to distinguish between these hypotheses has been provided (Nadeau and Sack, 2002a, 2003; Larkin *et al*., 2003). Patterning mechanisms must also interact with determinates of leaf dorsiventrality, since densities of stomates and trichomes differ on the adaxial and abaxial sides of the leaf.

2.8.3.1 Stomate pattern

Stomatal spacing pattern depends on a series of stereotypical cell divisions that result in clonally related guard cells and neighboring epidermal cells (Yang and Sack, 1995; Geisler *et al*., 2000; Serna and Fenoll, 2000; Nadeau and Sack, 2002a, 2003; Bergmann, 2004). In *Arabidopsis*, the pathway begins with the selection and asymmetric division of a meristemoid mother cell, followed by two additional asymmetric divisions of the meristemoid cell – a lineage pattern that gives rise to three epidermal cells surrounding the meristemoid. The meristemoid is converted to a guard cell mother cell and divides symmetrically to give rise to two guard cells (Geisler *et al*., 1998). Loss-of-function mutations in two genes, *TOO MANY MOUTHS (TMM)* and *FOUR LIPS (FLP)*, result in clustered stomates (Yang and Sack, 1995; Geissler *et al*., 1998, 2000). *TMM* encodes a receptor-like protein and is expressed strongly in meristemoids and weakly in some neighbor cells, particularly those with the greatest likelihood of dividing asymmetrically (Nadeau and Sack, 2002b). The *STOMATAL DENSITY AND DISTRIBUTION (SDD)* gene encodes a subtilisin-like protein and is expressed in meristemoids and guard cell mother cells; *sdd* mutants also have increased stomatal density and clustering (Berger and Altmann, 2000; Von Groll *et al*., 2002). These mutant phenotypes, molecular identities and expression patterns all indicate that *TMM* and *SDD* play a role in cell-to-cell signaling, particularly in generating the signals that regulate the timing and plane of cell divisions in neighbor cells. Additional evidence for signaling between cell layers comes from the placement of stomatal complexes away from major veins and adjacent to the intercellular spaces of the mesophyll (Serna and Fenoll, 2000; Nadeau and Sack, 2002a).

Stomatal patterning also responds to environmental cues, including ambient CO_2 concentration (Woodward and Kelly, 1995). While wild-type plants generally have reduced stomatal density under elevated CO_2, loss-of-function mutants in the *HIGH CARBON DIOXIDE (HIC)* gene have increased stomatal density (Gray *et al*., 2000). *HIC* encodes a fatty acid elongase enzyme that may be involved in the synthesis of cutin and waxes and is hypothesized to act through its effect of the movement of an inhibitory molecule from the guard cells to neighboring cells (Gray *et al*., 2000).

2.8.3.2 Trichome pattern

Like stomates, trichomes display a nonrandom spacing pattern, but clonal analyses and mis-expression studies indicate that the spacing pattern is not lineage-based, but is derived from a lateral inhibition mechanism (Larkin *et al*., 1996; Schnittger *et al*., 1999). Over twenty genes that are required for trichome morphogenesis have been identified (Hülskamp *et al*., 1994), and, as indicated by the complete loss or reduction of trichomes in mutants, at least three of these are required for the initial specification of trichome precursors. *GLABRA1 (GL1)* encodes an MYB transcription factor and is strongly expressed in developing trichomes, but weakly expressed throughout the developing protodermal tissue (Oppenheimer *et al*., 1991; Larkin *et al*., 1993). *TRANSPARENT TESTA GLABRA1 (TTG)* encodes a small protein with WD repeats (Walker *et al*., 1999), and *GLABRA3 (GL3)* encodes a basic helix-loop-helix motif (Payne *et al*., 2000). There is strong evidence that these three proteins interact and are required to act in concert for trichome formation (Payne *et al*., 2000). *GL1, TTG* and *GL3* appear to be negatively regulated in turn by two MYB-related transcription factors, *TRYPTYCHON (TRY)* and *CAPRICE (CYC)* (Wada *et al*., 1997; Schellmann *et al*., 2002; Schiefelbein, 2003). *TRY* and *CYC* are expressed most strongly in developing trichomes, but it has been hypothesized that their proteins move from the trichomes to neighboring cells to suppress the action of *GL1*/*TTG*/*GL3*and thus prevent trichome formaton (Schellman *et al*., 2002; Larkin *et al*., 2003).

2.9 Concluding remarks

Several families of transcriptional regulators clearly play a central role in many aspects of development, including specification of founder cells at the site of leaf initiation, growth along the main axes of leaf symmetry, maintenance of the meristematic activity of the marginal blastozone and patterning of internal leaf architecture. Modifications of the regulatory regions of these genes are key to evolutionary diversification of plant form (Doebley and Lukens, 1998). While some molecular mechanisms, such as the downregulation of *KNOX* genes during leaf inception, are highly conserved, other mechanisms, such as prolongation of blastozone activity by *KNOX* or *FLO* genes, illustrate how different groups of transcriptional regulators can be co-opted for the same function in different phylogenetic clades. In addition to transcriptional regulators, hormones and their signaling pathways play important roles in the development of phyllotaxis, leaf initiation and expansion, and vascular patterning. The interplay between plant hormones and these transcriptional regulators will be an important field of inquiry in the next decade and should lead to a better understanding of the fundamental nature of plant development with its combination of predictability and flexibility (Mattsson *et al*., 1999; Berleth and Mattsson, 2000).

Despite sharing the common architectural attributes of lateral position, dorsiventral symmetry, determinate growth plan and internal tissue patterning that reflects the requirements of photosynthesis, evolutionary diversification has resulted in

dramatic variation in leaf architecture. Molecular mechanisms that regulate this diversity presumably also regulate within-individual variation. For instance, almost all species display heteroblastic variation in leaf shape, size and cellular features that is correlated with overall shoot phase change (Tsukaya *et al*., 2000; Poethig, 2003). In species with complex leaf architecture, such as pea, heteroblastic variation is conspicuous, since the first-formed juvenile leaves are simple, while later-formed leaves are compound (DeMason and Villani, 2001). Other species display considerable phenotypic plasticity, where the development of external and internal leaf architecture responds to environmental cues such as light or submergence (e.g. Granier and Tardieu, 1999; Kuwabara *et al*., 2001). Additionally, specific characteristics such as stomatal density are finely tuned to the ambient conditions during leaf expansion (Woodward and Kelly, 1995). Significant challenges for future research will be to identify how the fundamental processes of leaf development are modified by whole plant phase change or by external signals. We also need a more integrated view of the development at the levels of external and internal leaf architecture, and it will be important to place discoveries made using genetic model organisms in a broader phylogenetic context, much as been done by Bharathan *et al*. (2002) and Kim *et al*. (2003a).

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3 Shoot architecture I
Regulation of stem length

John J. Ross, James B. Reid, James L. Weller and Gregory M. Symons

3.1 Introduction

The term 'architecture' normally applies to man-made structures, but is entirely appropriate in the context of plant development. There are, in fact, some interesting parallels between the architecture of buildings and plant stems. Both often consist of modules – floors in the case of high-rise buildings and the internode/node unit for caulescent plants. And just as the height of a building is an integral part of its overall shape, so too is stem length a critical determinant of plant architecture. However, while the modules (stories) comprising a building are often of a standard height, modules differ enormously within the plant kingdom, and variation in internode length is a major contributor to variation in stem length. The other major contributor is variation in internode number.

The stem performs essential functions as a supporting structure for the leaves and as a conduit for water and nutrients moving from one part of the plant to another. It is, therefore, a crucial factor in the agronomic success of crop plants, and stem architecture is a vital consideration for plant breeders. Accordingly, much research has been conducted on the regulation of stem length and great advances have been made. In this chapter, we review that progress, beginning with the growth hormones involved, and then discussing the environmental regulation of stem elongation. We also highlight the role of mutants in advancing our knowledge. Finally, we tackle the contentious question of whether hormones actually regulate stem growth or are merely permissive factors necessary for elongation to occur.

3.2 Plant growth hormones and genes regulating their levels

3.2.1 Auxin, gibberellin and brassinosteroid

The late 1800s and early 1900s saw the emergence of evidence for the existence of hormone-like substances that affect plant growth. The initial evidence came mainly from experiments on phototropism, or the bending of plant stems towards a light source. These early studies eventually led to the discovery of the first growth-promoting plant hormone, auxin, in the mid-1930s. It turned out that the main auxin

present in important model species such as pea and *Arabidopsis* is indole-3-acetic acid (IAA). The discovery of auxin was followed by the isolation of a second growth-promoting hormone, gibberellin (GA). Active GA was isolated in Japan in the 1930s, and characterised further by Western scientists in the 1950s (Phinney, 1983). GA, but not auxin, spectacularly stimulated stem elongation when applied to dwarf plants (Figure 3.1; Brian and Hemming, 1955). This dramatic effect led to a synthesis of physiology and genetics that yielded key information about the hormonal regulation of plant growth (Phinney, 1961).

One GA-responsive mutant in particular, Mendel's dwarf, had already played a key role in the study of inheritance (Mendel, 1865). The difference between normal (tall) and dwarf peas (Figure 3.2) was one of the seven discrete differences upon which Mendel formulated his famous laws. It is perhaps less well-known that dwarf types also played a role in the 'rediscovery' of Mendel's principles at the beginning of the twentieth century. The main early proponent of Mendelian genetics in Britain, William Bateson (Bateson, 1909), published on the inheritance of the dwarf trait in the sweet pea, *Lathyrus odoratus* (whereas Mendel worked on the garden pea, *Pisum sativum*). Bateson found that dwarfism in sweet pea, as in garden pea, is

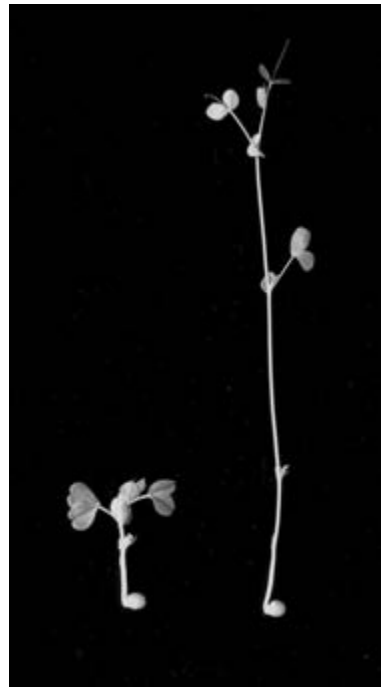

Figure 3.1 Applied gibberellin (GA) stimulates stem elongation of GA-deficient dwarf pea plants. Left, control plant; right, plant with 5 μg bioactive GA (GA_1) applied to the seed. Photo taken 11 days after sowing.

Figure 3.2 The difference between tall (*LE*, left) and Mendel's dwarf pea plants (GA-deficient *le-1*; right).

a simple recessive trait. Interestingly, research conducted nearly 90 years later showed that the same biochemical step is affected in the two dwarf types.

It turned out that Mendel's dwarfing allele, *le-1*, blocks the final activation step in the biosynthesis of the active GA in pea, GA_1 (Figure 3.3). Shoots of the *le-1* mutant contain only 5–10% the GA_1 found in wild-type plants, while levels of GA_{20}, the immediate precursor of GA_1, are elevated in the mutant. After supplying tritiated GA_{20}, much less tritiated GA_1 is produced in the mutant compared with the wild type (Ingram *et al*., 1984). The dwarfing mutation *l* in sweet pea also impairs conversion of GA_{20} to GA_1 (Ross *et al*., 1990). Other mutations block early steps in GA biosynthesis (Figure 3.3), again resulting in dwarfism (Reid and Ross, 1993). Yet another mutation, *sln*, in pea, blocks GA deactivation, leading to an accumulation of GA_{20} in mature seeds. This GA_{20} is converted to GA_1 during germination, resulting in elongated, slender seedlings (Reid *et al*., 1992; Ross *et al*., 1995; Lester *et al*., 1999). From a plant architectural point of view, it is interesting to note that virtually all the GA-related stem length mutants are mainly affected in internode length rather than internode number. Furthermore, GA deficiency appears to reduce both the length and number of cells in the internodes (Reid *et al*., 1983).

Since the early 1990s, nearly all the genes encoding GA biosynthesis and deactivation enzymes have been cloned, and this has enabled us to understand much

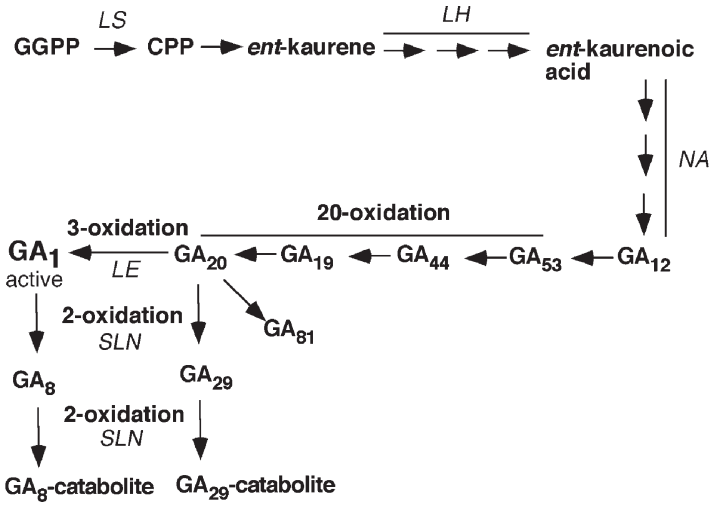

Figure 3.3 The gibberellin (GA) biosynthesis and deactivation pathway in pea shoots. Gene symbols indicate the steps affected by known mutations. This pathway is common to many species (e.g. maize, rice and barley). In other species (e.g. *Arabidopsis*), the main bioactive GA is GA_4, produced from GA_{12} by a pathway parallel to the one shown.

more about how GA biosynthesis is regulated. Bioactive GAs negatively regulate their own biosynthesis in a feedback mechanism that tends to maintain a constant level of active GA, and, therefore, a constant degree of GA-induced growth (Hedden and Croker, 1992). As will be discussed in the subsequent sections, environmental factors exert strong effects on GA biosynthesis (sometimes disrupting negative feedback) as does the level of auxin. All these factors affect GA biosynthesis by altering the mRNA level of key GA biosynthesis genes, usually from the later stages of the pathway (20-oxidation, 3-oxidation and 2-oxidation; Figure 3.3).

The synthesis of genetics and physiology, which proved so fruitful in the case of the GAs, was invoked again in the 1990s to provide greater insight into a third group of plant growth-promoting hormones, the brassinosteroids (BRs). The BRs had been discovered in the 1970s (Mitchell *et al*., 1970; Grove *et al*., 1979) and their growth-promoting properties were soon well-documented. It was not until the mid-1990s, however, that certain mutants were shown to owe their phenotype specifically to BR deficiency (Kauschmann *et al*., 1996; Fujioka *et al*., 1997; Nomura *et al*., 1997). In particular, the dwarf stature of BR-deficient mutants indicated that the normal level of active BR found in wild-type plants was necessary for normal stem elongation. Well-known BR-deficient mutants are *det-2, cpd, dwf1* and *dwf4* in *Arabidopsis* (Figure 3.4; Li *et al*., 1996; Szekeres *et al*., 1996; Fujioka *et al*., 1997; Choe *et al*., 1998; Klahre *et al*., 1998), *lkb* and *lk* in pea (Figure 3.5; Nomura *et al*., 1997, 2004) and d^x in tomato (Figure 3.6; Bishop *et al*., 1996, 1999). All these mutants are phenotypically short.

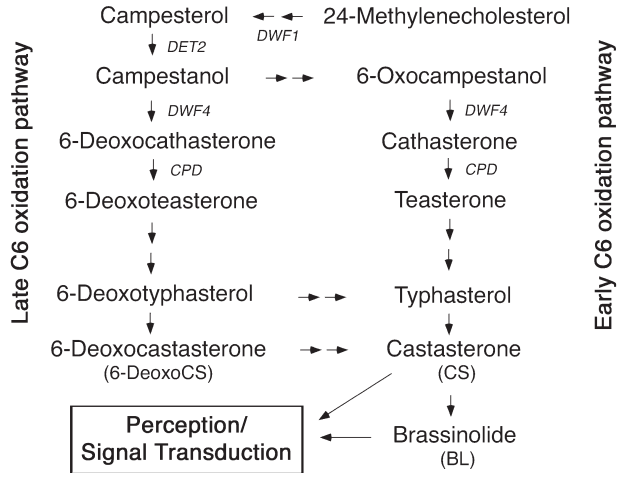

Figure 3.4 The brassinosteroid (BR) biosynthesis pathway in *Arabidopsis* shoots. Gene symbols indicate the steps affected by mutations.

Figure 3.5 The pea mutant *lkb* deficient in brassinosteroid (BR) (right pot), shown with wild-type plants (left). The *lkb* mutation blocks an early step in BR biosynthesis.

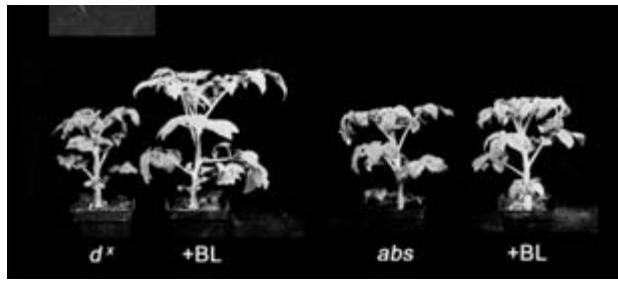

Figure 3.6 Response of tomato brassinosteroid (BR) mutants to applied BR. From left to right: the BR-deficient mutant d^x, control; d^x with applied BR; the BR response mutant *abs*, control; *abs* with applied BR. The *abs* mutant shows a much reduced response. [From Montoya *et al*. (2002). Copyright of the American Society of Plant Biologists; reprinted with permission.]

An important difference between the physiological–genetic scenarios played out for the BRs and GAs is that for the latter there are mutations (the *le* mutant alleles; Lester *et al*., 1997) that block the final step in the biosynthesis of active GAs. The dwarf stature of Mendel's *le-1* mutant, for example, showed that GA_1 is the key bioactive GA in pea, and the same was discovered in other species, such as maize and rice (Spray *et al*., 1984; Reid and Ross, 1993). For some time, the key bioactive BR was thought to be brassinolide, which is produced from castasterone. However, no mutation that blocks the conversion of castasterone to brassinolide is presently known and both compounds strongly promote elongation of BR-deficient dwarf plants. Consequently, in the past, it has not been clear which is the more important BR for elongation. It now appears that while both BRs are intrinsically bioactive – as both can interact with the BR receptor (Wang *et al*., 2001) – castasterone may be the more important endogenous BR for the simple reason that in many systems, brassinolide is undetectable by even the most sensitive physico-chemical methods, and castasterone is much more abundant (Nomura *et al*., 1999, 2001; Shimada *et al*., 2003; Symons and Reid, 2003).

As with GAs, most BR biosynthesis genes have been cloned and again their expression has been shown to be regulated by active BRs in a negative feedback system (Fujioka and Yokota, 2003). However, there is little information on other factors affecting BR biosynthesis.

Unlike studies on GAs and BRs, auxin research has not been dominated by mutants deficient in the hormone. In fact, there are still very few (if any) mutants which specifically owe their phenotype to auxin deficiency; rather, auxin-deficient systems have been created for study by removing parts of the plant in which auxin is synthesised and/or loaded into the specialised basipetal auxin transport system that supplies the elongating internodes. Mostly, these systems comprise excised stem segments incubated on a liquid growth medium. These segments show a very strong response to auxin added to the medium. Excised sections of pea stems, for

example, were shown to almost double their elongation in response to auxin. Another method of depleting auxin in elongating internodes is to remove the apical bud (decapitation). It is via the apical bud and/or young leaves that auxin enters the basipetal transport stream. Interestingly, in contrast to auxin, there is strong evidence that the biologically active GAs and BRs are not mobile within the pea shoot system (Reid *et al*., 1983; Symons and Reid, 2004). This indicates that attempts to create systems specifically deficient in these hormones, by excising parts of the shoot, would not be successful.

Although unequivocal auxin-deficient mutants with a phenotype that can be rescued by auxin application are rare, there are, nevertheless, some striking auxin-related mutants. The *pin1-1* mutant of *Arabidopsis* is a prime example (Figure 3.7). Okada *et al*. (1991) reported evidence that the *pin1-1* mutation reduces auxin transport, and this was subsequently confirmed later in the 1990s when four separate laboratories cloned the *PIN1* gene, which encodes an auxin efflux carrier (Jones, 1998). The 'chemi-osmotic' theory, which states that auxin transport is polar because an auxin efflux carrier is situated primarily at the base of cells, was borne out by studies on the localisation of the PIN1 protein (Jones, 1998; Friml and Palme, 2002). The most obvious aspect of the *pin1-1* phenotype is a 'pin-shaped' structure that develops in place of the normal inflorescence stem: the *pin1-1* mutant is unable to produce flowers (Figure 3.7). This is thought to be due to deficiency of auxin at localised sites near the shoot apex at which flowers are initiated.

Figure 3.7 The *pin1-1* mutant of *Arabidopsis* (right), shown with a wild-type plant (left).

Application of a miniscule amount of auxin (in lanolin paste) to this site stimulates flower production in the mutant (Reinhardt *et al*., 2003).

Auxin overproducing mutants have also been characterised. Mutations at the *SUR1* and *SUR2* loci of *Arabidopsis*, for example, result in auxin accumulations of up to 20-fold, compared with the wild type (Boerjan *et al*., 1995; Delarue *et al*., 1998; Barlier *et al*., 2000). These mutants are characterised by an increase in hypocotyl length (as well as other phenotypic alterations suggestive of auxin). It was discovered that *SUR2* encodes the cytochrome P450 CYP83B1, which converts an IAA precursor, indole-3-acetaldoxime, to precursors of indole glucosinolates (Figure 3.8; Barlier *et al*., 2000; Bak *et al*., 2001; Hoecker *et al*., 2004). *SUR1* also catalyses a step in the glucosinolate pathway (Mikkelsen *et al*., 2004). Thus indole-3-acetaldoxime is a key branch point in the IAA biosynthesis pathway: it can be converted to IAA (via several steps) or to the indole glucosinolates.

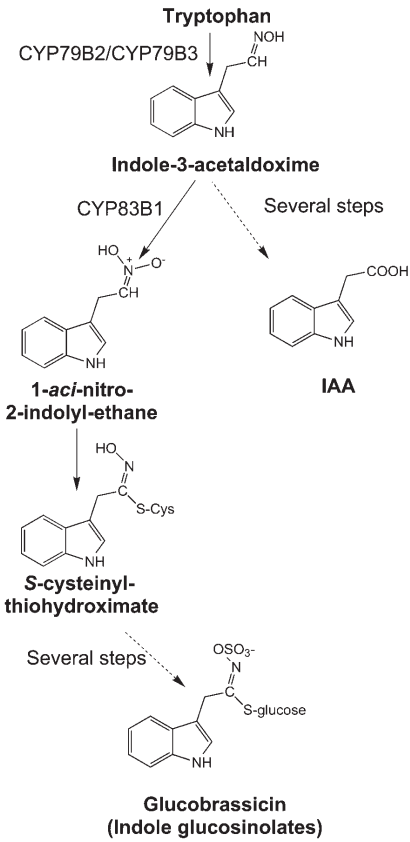

Figure 3.8 IAA biosynthesis. Indole-3-acetaldoxime is a key branchpoint intermediate that can be converted either to indole glucosinolates or to IAA. [From Bak *et al*. (2001). Copyright of the American Society of Plant Biologists; reprinted with permission.]

Disruption of this last branch by mutations, such as *sur2* increases the level of indole-3-acetaldoxime available for conversion to IAA.

The *bushy* mutant of pea contains up to 12-fold less auxin than the wild type, and its phenotype is entirely consistent with the roles traditionally ascribed to auxin deficiency, including short internodes and a branching habit (Symons *et al*., 2002a). However, the primary effect of the mutation may not be to block IAA synthesis, because the mutant phenotype cannot be fully rescued by auxin application.

3.2.2 Ethylene and cytokinin

Ethylene typically inhibits elongation in light-grown plants (Reid, 1995), but is a promotory factor in submerged plants, as discussed in a subsequent section on the effects of flooding. One of the more striking ethylene over-producing genotypes in pea is the double mutant *phyA phyB*, the internodes of which are twisted and thickened, and often split (Weller *et al*., 2001a). Application of the ethylene synthesis inhibitor, AVG, restores the internodes to near-normal (John Ross, unpublished). In the same conditions, internodes of wild-type plants are unaffected by AVG. An important implication of this last observation is that in light-grown wild-type pea shoots, ethylene production is too low to inhibit internode elongation.

As with auxin, the identification of roles played by cytokinins (CKs) in intact plants has been hampered by a lack of CK-deficient mutants. Recently, however, CK-deficient plants have been obtained by genetic engineering. Genes encoding CK deactivating enzymes have been introduced into tobacco (Werner *et al*., 2001) and *Arabidopsis* (Werner *et al*., 2003). In the transgenic tobacco plants, moderate reductions in CK levels were associated with a dwarf habit with shortened internodes, while in *Arabidopsis*, the flowering stems were thinner than in the wild type. However, there was no evidence of whether exogenous CK was able to rescue the phenotype of the over-expressing plants. Therefore, it remains unclear whether the phenotype observed was primarily due to CK deficiency.

3.3 Hormone signal transduction

Considerable advances have been made in understanding the mechanism of action of plant growth-promoting hormones, mainly downstream of hormone reception. Reviews on GA, auxin and BR signalling have been published by Sun and Gubler (2004), Ward and Estelle (2001) and Wang and He (2004), respectively, and only a brief outline is presented here. All three hormones appear to affect the stability of key signalling proteins. GA destabilises growth-inhibitory proteins belonging to the 'DELLA' family (Figure 3.9). DELLA proteins, such as RGA in *Arabidopsis*, SLN in barley (Gubler *et al*., 2002), SLR1 in rice (Itoh *et al*., 2003), RHT in wheat and D8 in maize (Richards *et al*., 2001) are growth repressors, and GA is therefore seen as a 'repressor of a repressor' (Brian and Hemming, 1958; Richards *et al*., 2001). The importance of these proteins has been demonstrated by mutant phenotypes.

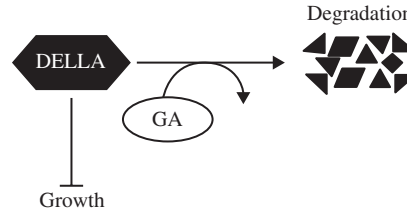

Figure 3.9 Gibberellin (GA) destabilises growth-repressing DELLA proteins, thereby promoting elongation growth. [From Achard *et al*. (2003). Copyright of the American Society of Plant Biologists; reprinted with permission.]

The *rga* mutation, for example, reverses the dwarfing effect of GA deficiency, because the encoded protein is rendered incapable of repressing growth (Silverstone *et al*., 1997).

Auxin also destabilises signalling proteins; the best-studied examples are members of the Aux/IAA family (Figure 3.10). Aux/IAA proteins appear to prevent other proteins, the auxin response factors (ARFs), from up-regulating auxin-responsive genes involved in growth responses (Figure 3.10; Tiwari *et al*., 2001). The destabilising effect of IAA on the Aux/IAA proteins, therefore, enables ARFs to up-regulate auxin-responsive genes. Again, mutations demonstrate the critical role played by Aux/IAA genes. A mutation in one such gene, *IAA17*, also known as *axr3-1*, gives rise to a dwarf phenotype. Interestingly, this dwarfism is mainly due to reduced internode number rather than internode length (Leyser *et al*., 1996).

In contrast to GAs and auxin, the BRs appear to *stabilise* key signalling molecules. These proteins, BES1 and BZR1, accumulate in response to reception of the BR signal (Nemhauser and Chory, 2004), and this appears to trigger expression of downstream genes associated with growth (Wang and He, 2004).

Much effort has been invested in identifying receptors for growth-promoting hormones (Napier, 2004). Progress appears to be the greatest on the BR receptor identified from *Arabidopsis* (Wang *et al*., 2001), pea (Nomura *et al*., 2003) and barley (Chono *et al*., 2003). In *Arabidopsis*, the receptor is encoded by the gene *BRI1*, and in pea, by *LKA*. As expected, mutations in these genes cause phenotypes similar to those of BR-deficient mutants, apart from their inability to respond normally to BR application (Nomura *et al*., 1997). An interesting feature of the BR receptor is that in tomato, the same receptor functions for both BRs and systemin – a compound involved in wound responses (see Wang and He, 2004).

3.4 Dwarfism not mediated by hormones

The cell wall is usually the ultimate site of hormone action on stem elongation, and it is not surprising that mutations that directly affect cell wall synthesis (rather than hormone levels or signal transduction) can also lead to dwarfism. Recent examples are the dwarf cellulose-deficient *kobito1-1* and *kobito1-2* (*kob1-1* and *kob1-2*)

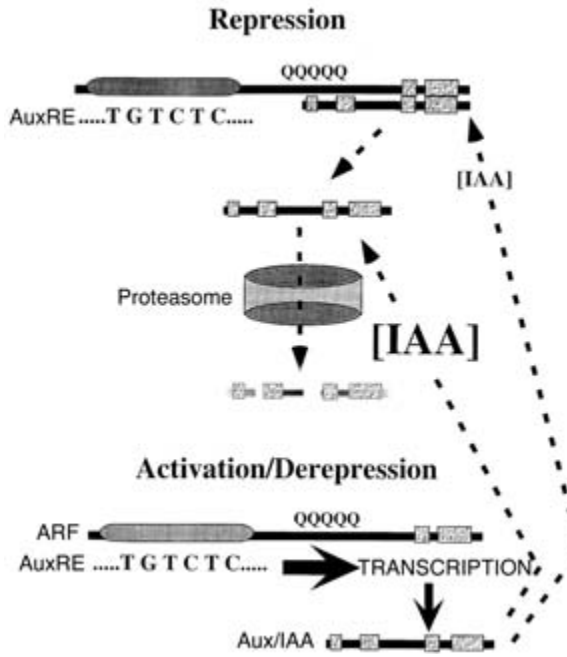

Figure 3.10 Interactions between Aux/IAA proteins and auxin response factors (ARFs) regulate the transcription of key auxin response genes. When IAA levels are low (small bracket), Aux/IAA proteins are bound to ARFs, preventing the latter from stimulating gene transcription. When IAA levels are high (large bracket), the proteasome-mediated degradation of Aux/IAA proteins is enhanced, thereby enabling ARFs to up-regulate gene transcription. ARFs bind to the AuxRE sequence TGTCTC of the auxin response gene promoter. [From Tiwari *et al*. (2001). Copyright of the American Society of Plant Biologists; reprinted with permission.]

mutants of *Arabidopsis* (Pagant *et al*., 2002). Another *Arabidopsis* cell wall mutant, *parvus*, also exhibits a dwarf phenotype, but only under low humidity conditions (Lao *et al*., 2003). Again, in the L-fucose-deficient *Arabidopsis mur1* mutant, borate ester cross-linking of cell wall constituents is reduced, resulting in a dwarf phenotype (O'Neill *et al*., 2001). Of course, cell wall constituents controlled by these genes are not regulators of growth in the same sense that plant hormones are thought to be. They are, typically, much more abundant in the plant, lack mobility and have no corresponding receptors.

3.5 The green revolution

The 'green revolution' of the 1960s and 1970s was based on introducing dwarfing genes into two of the world's key crops, wheat and rice, along with improved agronomic practices, especially discovery of roles of inorganic nutrients that led to

the increased use of fertilisers. This allowed the amount of food produced per person to increase between 1960 and the present, even though the world's population has more than doubled to 6 billion over this period (Hedden, 2003a). The dwarfing genes change plant architecture so that fewer resources are directed to stem (straw) growth, leaving more resources for seed development. The short stature of dwarf varieties also reduces lodging (and hence losses) that can be caused by wind and rain (Evans, 1998).

The use of dwarf varieties to achieve these benefits was not completely new, as such traits had been used in crops such as peas for over 500 years (Blixt, 1972). However, it is only in the last few years that the molecular and biochemical effects of the genes used have been discovered, and that the reasons for the success of the green revolution have become clear.

In rice, the gene used to induce dwarfism is *sd1* (Sasaki *et al*., 2002). This mutation originated in a Chinese cultivar, Deeyeo-woo-gen, and has been introduced into *INDICA* cultivars to produce high-yielding dwarf cultivars suitable for growth in tropical and subtropical regions and into *JAPONICA* cultivars for more temperate areas (Hedden, 2003b). While the original work was carried out in Taiwan and at the International Rice Research Institute in the Philippines, high yielding rice cultivars involving the recessive *sd1* allele have been independently selected in Japan, the United States and China (Hedden, 2003b). An interesting question is why the *sd1* gene has been selected as the gene-of-choice when there are many other dwarfing genes available in rice? The *SD1* gene has been shown by Sasaki *et al*., (2002) to encode a GA 20-oxidase, a key enzyme in the later part of the GA biosynthetic pathway (Figure 3.3). This enzyme catalyses the three step oxidation from $GA_{53} \rightarrow GA_{44} \rightarrow GA_{19} \rightarrow GA_{20}$ (Figure 3.3). GA_{20} is the immediate precursor of GA_1 – the bioactive GA in rice.

The *sd1* mutation results from a 383 base-pair deletion that results in a frameshift resulting in a stop codon (Sasaki *et al*., 2002), and is therefore presumably null. However, *sd1* plants elongate reasonably well, and are semi-dwarf rather than dwarf. This can be explained by the fact that rice contains at least two GA 20-oxidases (Sasaki *et al*., 2002) with partially overlapping expression patterns, which ensures that the stem of *sd1* plants is not totally deficient in GAs. Perhaps of even more importance is that another GA 20-oxidase, *OsGA20ox1*, is preferentially expressed in the reproductive organs, ensuring that GA levels (and consequently flower and fruit development) are relatively normal (Sasaki *et al*., 2002). Hence, the yield of *sd1* plants is unaffected by the partial GA deficiency of the stems. Small gene families have been identified for many of the 2-oxoglutarate-dependent dioxygenases (such as the GA 20-oxidases) that catalyse the later steps in the GA biosynthetic pathway.

An analogous situation has occurred with Mendel's *le-1* dwarfing allele in peas. The *LE* gene is expressed strongly in shoots and *le-1* causes a reduction in GA_1 levels in this tissue (Ross *et al*., 1992) but does not affect GA_1 levels in developing seeds or roots (MacKenzie-Hose *et al*., 1998; Yaxley *et al*., 2001). Hence, *le-1* plants have a dwarf stature but are not limited for GAs in the developing seeds or the roots.

Thus, they have been used for over 500 years as a crop with improved plant architecture, featuring reduced lodging and increased disease resistance and yield.

The second major crop involved in the green revolution was wheat. Bread wheat is a hexaploid and hence single recessive dwarf mutants such as *sd1* in rice or *le-1* in pea are unlikely to be found because of the multiple loci present for each gene. Instead, semi-dominant gain-of-function dwarfing genes at the *RHT* loci have been selected for the development of dwarf wheat cultivars. Over 70% of current commercial wheat cultivars worldwide now contain these dwarfing genes (Evans, 1998). They have been derived from a variety called Norin 10, which resulted from an early Japanese crossing programme. Norman Borlang at the Centro International de Mejoramiento de Maiz Trigo (CIMMYT) in Mexico led a group that bred the dwarfing genes into highly productive cultivars, suited to subtropical environments, and able to produce two crops per year (Hedden, 2003b). He was awarded a Nobel Peace prize for his work.

The two *RHT* genes used were *Rht-B1* and *Rht-D1*, homoeologous genes on chromosomes 4B and 4D in the B and D genomes of wheat, respectively (Silverstone and Sun, 2000). Peng *et al*. (1999) showed that these genes encode mutant GA response modulators. The conserved collinearity of sections of cereal genomes allowed it to be shown that *Rht* was likely to be similar to *D8* in maize, and both were phenotypically similar to *GAI* in *Arabidopsis*. Dominant, dwarf mutants at the *Rht*, *D8* and *GAI* loci are all insensitive to applied GA. A rice expressed sequence tag related to the *GAI* sequence was therefore used to isolate a cDNA from wheat. The DNA from two *Rht* mutant alleles showed differences from wild-type DNA, confirming that *Rht* is an othologue of *GAI* (Peng *et al*., 1999).

It is interesting that to date the dwarfing genes used to change crop architecture have all acted through the GA synthesis or response pathways, even though many dwarfing mutants influence elongation through other pathways. This can be explained by two main factors. First, plants moderately deficient in GA levels or sensitivity have reduced elongation of shoots but otherwise appear largely normal in terms of leaf, root and reproductive development. Hence, there is no major penalty in yield, disease resistance or resource acquisition. Second, and partly explaining this situation, is the presence of small gene families for the GA synthesis genes. This genetic redundancy explains why even null alleles can affect the stem without affecting other tissues. Therefore, mutants which have a minimal effect on other aspects of development, as has occurred in rice and peas, can be selected.

Recently, however, Chono *et al*. (2003) have shown that the *uzu* mutation in barley causes a semi-dwarf phenotype (80–90% the height of wild-type plants) through a missense mutation in the BR receptor gene *HvBRI1*. This gene was used extensively in barley varieties grown in Japan and the Korean peninsular during the 1930s and is currently being bred into hull-less barley cultivars in Japan. Similarly, the *dwarf3* mutant in sorghum has been used in combination with other genes to reduce shoot height. This mutant has recently been shown to lack a P-glycoprotein that modulates polar auxin transport (Multani *et al*., 2003). Hence, weak mutant

alleles at BR- or auxin-related loci may also provide agronomic benefits in some circumstances or in particular crops.

Genetic engineering has great potential for modifying plant height for agronomic purposes. Indeed, several trials have been reported, the most promising of which have involved modification to the GA biosynthetic pathway either by altering synthesis or deactivation of bioactive GA (Hedden, 2003b). For example, Sakamoto *et al*. (2003) successfully dwarfed rice by the ectopic over-expression in the shoot of a GA 2-oxidase gene that inactivates biologically active GA (Figure 3.3). Within the next few years, we will, no doubt, be able to dramatically restructure the shoot architecture of any given crop to suit its environment, providing solutions for plant breeders in their pursuit of increasing yields and/or adaptation to new technologies or adverse environments.

3.6 Interactions between hormones

Efforts to genetically engineer plants by altering hormone pathways will stand a better chance of success if interactions between the growth-promoting hormones are better understood. In fact, studies on hormone interactions date back to the early days of hormone research. When the GAs were discovered, plant physiologists hypothesised about whether, and how, auxin and GA might interact to regulate stem elongation. Many theories were advanced (e.g. Brian and Hemming, 1958), but the issue was not resolved until the late 1990s. Then, Jocelyn Ozga and colleagues reported that an auxin, 4Cl-IAA, up-regulated the expression of a key GA synthesis gene, GA 20-oxidase, in pea pods (van Huizen *et al*., 1997). Following that advance, Ross and colleagues showed that in pea stems, IAA from the apical bud is necessary to maintain normal GA_1 biosynthesis (Ross *et al*., 2000). When the apical bud is excised, IAA levels fall in the internodes, and this in turn leads to a reduction in GA_1 levels. Application of IAA to the stump of decapitated plants restores the GA_1 content and elongation rate to at least that of intact plants.

The growth response to auxin has mainly been studied in excised stem segments (e.g. Brian and Hemming, 1958). Recently, it was shown that auxin promotes GA_1 biosynthesis in excised pea stem segments (O'Neill and Ross, 2002; Ross *et al*., 2003), and it is interesting to note, therefore, that in at least some early studies on auxin–GA interactions, researchers may have unknowingly increased GA levels by adding auxin to the system. At that stage, techniques for quantifying GAs were in their infancy, and it was not known that GA_1 is the main bioactive GA in pea stems.

In pea, wild-type *(LE)* stem segments show a stronger growth response to auxin than do mutant *le-1* segments (Ockerse, 1970; Ross *et al*., 2003). This appears to be because *LE* segments readily convert GA_{20} to GA_1 (in response to auxin), whereas the rate of this conversion is dramatically reduced in *le-1* segments. Therefore, the auxin-induced GA_1 in stem segments appears to mediate part of the auxin response, and GA_1 can be viewed as a component of the auxin signalling pathway (Figure 3.11). Thus, the auxin–GA interaction is of paramount importance in

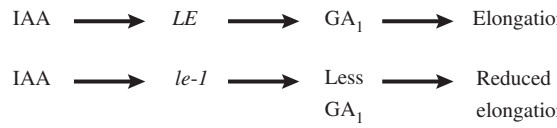

Figure 3.11 In pea stems, IAA up-regulates *LE* expression, increasing GA_1 content and therefore elongation. GA_1 can, therefore, be viewed as part of the auxin signalling pathway. The pathway is disrupted by the *le-1* mutation.

physiological terms. We can now assign clear roles to auxin and GA in the intact plant: auxin moves as a messenger from the apical bud into the elongating internodes, and stimulates the production of GA_1 – arguably, the final hormonal effector of elongation in those internodes. Consistent with this model is evidence that endogenous GA_1 itself (in contrast to applied GA_1) does not appear to be mobile within the shoot system of the garden pea (Reid *et al*., 1983).

In wild-type pea plants, auxin promotes GA_1 biosynthesis by up-regulating the expression of the *LE* gene, as indicate by mRNA levels (Lester *et al*., 1997; O'Neill and Ross, 2002). Interestingly, in pea, auxin also inhibits GA_1 deactivation, by downregulating the expression of a GA 2-oxidase gene (*PsGA2ox1*).

Auxin also stimulates GA biosynthesis in tobacco (Wolbang and Ross, 2001) and barley (Wolbang *et al*., 2004), despite the radically different growth habit of the latter. In grasses such as barley, the true stem is encased by the leaves until relatively late in the plant's life. Then, stem extension can proceed rapidly, and very long internodes are often produced. The elongation zone of extending grass internodes is restricted almost entirely to the base of the internode. In peas, however, as the internodes mature, elongation occurs mainly in the apical section of the internode. The finding that auxin promotes GA biosynthesis in both peas and barley indicates that the interaction might well be an ancient one, evolving before the divergence of the monocots and dicots.

In *Arabidopsis* roots, another major auxin–GA interaction has been described (Fu and Harberd, 2003). In this case, auxin is thought to be necessary for normal GA signalling (Fu and Harberd, 2003). When the shoots of GA-deficient *Arabidopsis* seedlings were decapitated, root elongation was reduced, as was the ability to respond to GA application. Application of auxin to the shoot at the decapitation site, restored GA responsiveness to the roots (Fu and Harberd, 2003). Fu and Harberd suggested that auxin, like the GAs, destabilises DELLA signalling proteins.

However, the internodes of dwarf pea plants respond strongly to GA_1 even when decapitated and, therefore, demonstrably auxin deficient (Ross *et al*., 2002), and furthermore, the GA response is not enhanced by auxin application (Figure 3.12). Therefore, the interesting new auxin–GA interaction discovered by Fu and Harberd (2003) may not be ubiquitous. At this stage, we do not know whether the apparent difference in the auxin–GA signalling interaction between *Arabidopsis* roots and pea stems is attributable to differing species and/or to differing organs.

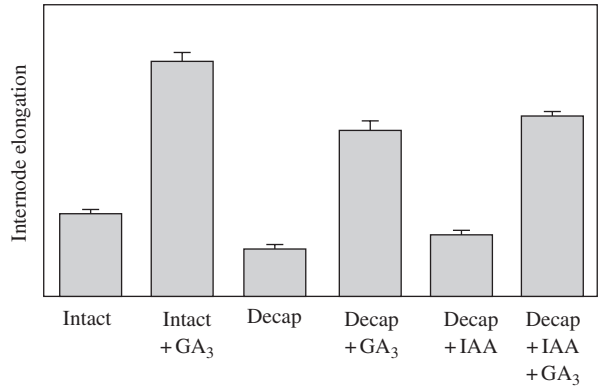

Figure 3.12 Effects of applied GA_3 on internode elongation in dwarf (*le-1*) pea plants. Plants were either left intact or decapitated at the top of the oldest unexpanded internode; the elongation of this internode was subsequently monitored. Some decapitated plants were treated with IAA, applied in lanolin to the cut stump. GA_3 strongly promoted elongation in both intact and decapitated plants, but IAA treatment did not enhance the gibberellin (GA) response. (Data from Naomi Glancy and John Ross.)

How do the BRs fit into the picture? Just as the discovery of the GAs sparked discussion about how GAs and auxin interact, the discovery of the BRs prompted analysis of possible three-way interactions. Bouquin *et al*. (2001) reported that BRs up-regulate the expression of the key GA 20-oxidase gene, *AtGA20ox1*, in *Arabidopsis* – a result implying that BRs, like auxin, might act by modulating GA levels. However, in pea, the BR-deficient mutant *lkb* actually accumulates GA_{20} (the product of 20-oxidation; Figure 3.3), rather than containing less of that GA (Jager *et al*., 2004). Furthermore, Jager *et al*. found no evidence showing that BRs can substitute for auxin in promoting GA 3-oxidation, and concluded that, at least in pea, BRs do not affect stem elongation by altering endogenous GA_1 levels.

With the advent of miocroarrays, it has become possible to gain further insight into hormone 'cross-talk', and, in particular, into how much overlap occurs between the suites of genes regulated by each hormone. Goda *et al*. (2004), for example, showed that while BR induced 409 genes in *Arabidopsis*, and auxin induced 276 genes, only 48 genes were regulated by both hormones. Goda *et al*., therefore concluded that auxin and BR mainly operate by regulating a unique suite of genes, but the group of 48 genes in common does imply some intersection of the signalling pathways. Interestingly, this group of 48 includes some members of the Aux/IAA gene family. Yang *et al*. (2004), working with rice, again showed that for the GAs and BRs, most of the responsive genes were specifically regulated by just one of the two hormones. Furthermore, the observation that GA is largely ineffective at promoting elongation in the BR-deficient *lkb* mutant (Reid and Ross, 1989), and that BR is similarly ineffective on GA-deficient mutants (Nomura *et al*., 2003), indicates that, in pea, BRs and GAs act largely independently to control growth.

3.7 Regulation of stem length by environmental factors

3.7.1 Effects of light on stem growth

Light is one of the most important environmental variables influencing plant growth and development. Light regulates stem elongation in a wide range of higher plants, and its effects are manifested in different ways throughout the plant life cycle. The dramatic effects of light are familiar to anyone who has left potatoes to sprout in the dark, seen weeds growing long and pale under an object left on the ground or watched spinach planted at the wrong time of year bolt rapidly and go to seed.

Seedlings germinating in darkness adopt an extreme light-seeking strategy in which they undergo rapid stem elongation and strongly suppress leaf development. Emergence into light induces a dramatic change to a light-utilising strategy, in which stem elongation is rapidly inhibited and leaf development promoted. This process is called de-etiolation (Figure 3.13). De-etiolation is not an all-or-nothing response, since the extent to which it occurs depends both on the amount and the spectral quality of the incident light. In addition, most plants retain the ability to respond to changes in their light environment even after they undergo de-etiolation as seedlings, and can partially revert to an etiolated habit if the light level drops sufficiently or its spectral quality is altered in certain ways. These vegetative responses to light in older, de-etiolated plants are often collectively referred to as

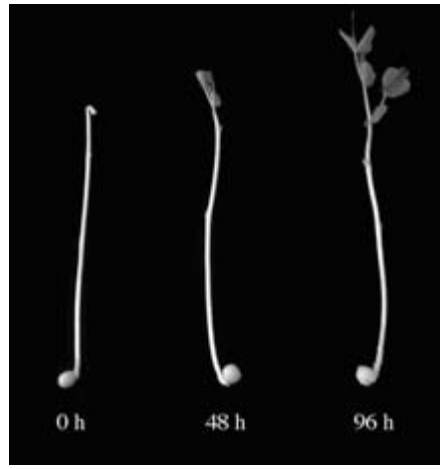

Figure 3.13 Morphological changes in wild-type pea seedlings during de-etiolation. The plant on the left was grown for 7 days at 20°C in continuous darkness. The other plants were grown for 7 days in continuous darkness then transferred to continuous white light (150 μmol m^{-2} s^{-1}) for 48 h (middle) or 96 h (right). Stem elongation reduces dramatically and energies are instead channelled into establishment of photosynthetic structures.

the shade-avoidance syndrome. Finally, the daily duration of light (photoperiod) can also have an important influence on plant growth and development, including stem elongation, in a manner largely independent of photosynthesis and irradiance effects. The most important effect of photoperiod in many species is the regulation of the transition from the vegetative to the reproductive state – a transition which frequently involves complex changes in stem elongation as the vegetative shoot axis is modified to form primary and secondary inflorescences. This kind of photoperiod effect on elongation is seen most dramatically in the rapid stem elongation or bolting of rosette plants that is associated with the transition to flowering. In species with a caulescent growth habit, effects of photoperiod on stem elongation may be less striking, but can also be seen during the vegetative growth phase.

Early physiological experiments showed that light in the blue, red and far-red wavebands were particularly important for the control of seedling de-etiolation, and the associated changes in elongation of the hypocotyl or stem (Went, 1941; Parker *et al*., 1949). It has only been with the advent of a molecular genetic approach that the individual photoreceptors mediating these photomorphogenic effects have been conclusively identified. The isolation of mutants with defective de-etiolation responses under these specific regions of the light spectrum has demonstrated the existence of multiple photoreceptor systems, and the cloning of the corresponding genes has identified a number of new photoreceptors and light signalling components (Briggs and Christie, 2002; Nagy and Schäfer, 2002; Lin and Shalitin, 2003). The molecular characterisation of photoreceptors has also enabled a transgenic approach to understanding photoreceptor function in which photoreceptor genes can be over- or under-expressed in plant species for which photoreceptor-deficient mutants are not available or easily generated (e.g. Olsen *et al*., 1997; Yanovsky *et al*., 2000a; Shlumukov *et al*., 2001). However, these approaches are, in general, most advanced in *Arabidopsis*, and have mainly focused on light effects on early seedling development and on the timing of flowering. As far as stem elongation is concerned, they have dealt mainly with hypocotyl elongation, since, in general, wild-type *Arabidopsis* plants do not undergo any significant internode elongation while vegetative, and the elongation associated with bolting is usually considered as part of the flowering response.

There are now at least three known distinct photoreceptor families that contribute to plant responses to light (Briggs and Christie, 2002; Nagy and Schäfer, 2002; Lin and Shalitin, 2003). These are the phytochrome family (which predominantly absorbs red and far-red light) and the cryptochrome and phototropin families (which predominantly absorb blue light). All of these groups of photoreceptors are reported to affect stem/hypocotyl elongation in some way.

3.7.1.1 De-etiolation

Under normal conditions of full natural daylight or artificial white light, the R and BL wavebands predominate and two photoreceptors in particular, phytochrome B (phyB) and cryptochrome 1 (cry 1), play an important role in allowing the plant to

achieve and maintain the de-etiolated state (Yanovsky *et al*., 1995; Neff and Chory, 1998). In *Arabidopsis*, loss-of-function mutants for either of these photoreceptors have dramatically elongated hypocotyls under white light (Neff and Chory, 1998). Mutants deficient in phyB have been isolated in a number of species, including *Arabidopsis*, tomato, pea, tobacco, cucumber and sorghum, and phyB function has been explored by transgenic approaches in several other species. PhyB controls the classical phytochrome responses to red and far-red light in which developmental responses such as apical hook opening, leaf expansion and inhibition of stem elongation are induced by R and reverted by FR (Reed *et al*., 1994; Shinomura *et al*., 1996). PhyB also has a minor absorbance peak in the BL region and makes a minor contribution to BL sensing. However, the main BL sensor in de-etiolation is cry1, which mediates responses to BL at irradiances above approximately 1 μmol m^{-2} s^{-1} (Lin *et al*., 1998; Weller *et al*., 2001b). Duplication of the phyB gene has been reported in some species including *Arabidopsis* and tomato, each of which has two closely related phyB phytochromes that show a substantial degree of functional redundancy (Aukerman *et al*., 1997; Weller *et al*., 2000).

Although these two photoreceptors may have the most important role in a quantitative sense, other members of the phytochrome and cryptochrome families are also important, with overlapping roles and divergent functions that give additional sensitivity and refinement to the overall light response system. Of these, phytochrome A (phyA) is the best-studied. This photoreceptor has acquired a number of divergent features that confer a number of unique physiological functions. Large amounts are present in dark-grown plants, but it is strongly downregulated by light at both the transcript and protein levels (Nagy and Schäfer, 2002). It is also capable of responding to light at irradiances much lower than those detected by phyB, and in this mode is responsive to wavelengths of light across the visible spectrum (Shinomura *et al*., 1996). The combination of these two features may allow detection of light penetrating the soil surface, and is also important for de-etiolation of seedlings emerging into heavy canopy shade (Yanovsky *et al*., 1995). The other distinct role of phyA, by which it was originally characterised, is in mediating de-etiolation responses to continuous FR (Reed *et al*., 1994; Weller *et al*., 1997). Under natural light or other broad-spectrum sources containing FR, phyA thus acts to oppose the phyB-type phytochromes, which are inactivated by FR. A second member of the cryptochrome family, cryptochrome 2 (cry2), has much the same absorption properties as cry1, but like phyA is photolabile, and this property restricts its influence to lower BL irradiances (Lin *et al*., 1998).

It is thus clear that different photoreceptors have distinct roles in mediating the effects of light quality and quantity on elongation. In addition, studies of short-term kinetics of hypocotyl elongation have revealed significant differences in the timeframe over which various photoreceptors act. On transfer from dark to R, significant inhibition of elongation occurs within 5 min through the action of phyA. The effect of phyA declines at around 3 h and is replaced by a persistent phyB-mediated inhibition (Parks and Spalding, 1999). Following transfer from darkness to BL, cry1- and cry2-mediated inhibition is first detected after approximately

30 min. However, this is preceded by a transient inhibition which can commence as early as 10 s after BL exposure and is mediated by members of the third photoreceptor family – the phototropins (Folta and Spalding, 2001). It is likely that these different kinetics reflect differences in the early response mechanisms of each photoreceptor, which in the case of phototropin involves membrane depolarisation (Briggs and Christie, 2002), and in the case of phytochrome involves nuclear import (Nagy and Schäfer, 2002).

It is also clear that despite their distinct properties, there is also a significant degree of redundancy among the photoreceptors that depends on light quality and quantity. This general redundancy is greatest in blue and white light, where cry1, cry2, phyA, phyB and phototropin can all contribute (Yanovsky *et al*., 2000b; Folta and Spalding, 2001; Weller *et al*., 2001b). There is less functional redundancy under R, where only the phytochromes are active. The situation is simplest for monochromatic FR, where phyA is the only photoreceptor that can act to induce de-etiolation.

Although the roles and interactions of the photoreceptors have been explored in great detail, the signalling pathways leading from photoreceptor activation to whole-plant responses are less well characterised and are still a topic of great interest. These pathways are being explored in *Arabidopsis* by isolation of additional, non-photoreceptor mutants with altered de-etiolation responses, including hypocotyl elongation. The current understanding is that photoreceptor activation may have a variety of effects involving both specific and shared signalling pathways. Phytochromes may act in part through rapid and direct effects on expression of certain key transcription factors (Tepperman *et al*., 2001). Phytochromes and cryptochromes also interact to regulate the activity and subcellular localisation of COP1 – a key negative regulator of photomorphogenesis that targets transcription factors for degradation (Osterlund *et al*., 2000). Loss-of-function *cop1* mutants in both *Arabidopsis* and pea show strong dwarfing at the seedling stage (Deng *et al*., 1991; Sullivan and Gray, 2000). Recent evidence also suggests that photoreceptor activity is itself subject to regulation by the circadian clock (Anderson *et al*., 1997; Dowson-Day and Millar, 1999; Tóth *et al*., 2001), and a number of different genes with primary defects in clock-related processes also have mutant phenotypes that include profound elongation defects (Dowson-Day and Millar, 1999; Huq *et al*., 2000). From another perspective, transcriptional profiling has identified a large number of genes regulated by both phytochrome and cryptochrome (Folta *et al*., 2003). These include transcription factors, hormone biosynthesis genes, hormone response elements and cell-wall-related proteins – not surprising in view of the role of both groups of photoreceptors in the control of elongation.

3.7.1.2 Shade-avoidance

Following successful germination, emergence and de-etiolation, the growing plant may face actual or potential competition for light from its neighbours, or some

other form of shading. These predicaments are signalled to the plant by differential changes in spectral energy and/or changes in total incident light. In contrast, non-foliage shading is almost invariably manifest as a reduction in overall irradiance across the whole light spectrum. This reduces the activation of all photoreceptors, but particularly affects cry1 and phyB, which are active at high irradiances. Competitive or canopy shading also results in a change in spectral distribution, since leaves absorb a high proportion of the incident R but transmit most FR. Thus, in addition to a drop in overall irradiance, the shaded plant also experiences a decrease in the R : FR ratio, which further enhances the inactivation of phyB-type phytochromes (Smith, 2000). Finally, the presence of near but non-shading neighbours may result in a local increase in scattered or reflected FR (Ballaré, 1999), and in responses often described as proximity perception. This response also acts through reduced activity of phyB-type phytochromes, but unlike canopy shade, does not affect cryptochrome activation and has the potential even to increase activity of phyA.

The central role for phyB-like phytochromes in these shade-avoidance responses has led to the suggestion that they may represent an ancestral phytochrome function. The adaptive significance of the phenotypic plasticity enabled by this kind of response may also explain the presence of multiple phyB-like phytochromes in a number of taxa. In addition to the relatively recent duplication within the phyB lineage that was mentioned earlier, many dicot species have an additional more highly divergent phyB-like phytochrome, phyE (Clack *et al*., 1994; Hauser *et al*., 1995). In *Arabidopsis*, phyE has minimal effect on elongation at the hypocotyl stage, but later in development, it plays an increasingly important role in mediating the effects of R (Devlin *et al*., 1998). Tomato seedlings lacking phyB1 and phyB2 still elongate in response to reduced R : FR, suggesting that phyE potentially plays an important role in this species also (Weller *et al*., 2000). In contrast, pea seedlings lacking phyB show no additional elongation in response to FR enrichment, suggesting that only one functional phyB-like phytochrome may be present (Weller *et al*., 2001a). Interestingly, an *Arabidopsis* plant lacking phyA and all three phyB-like phytochromes exhibits a small but unmistakable elongation of vegetative internodes, demonstrating a role for phytochromes in the maintenance of the rosette habit (Franklin *et al*., 2003).

Although early characterisations of phyA-deficient mutants suggested that this photoreceptor had little role in de-etiolated plants, more detailed studies have revealed that it too can make a significant contribution to light responses during later vegetative growth (Devlin *et al*., 1996; Weller *et al*., 1997). However, the extent of its effects vary with species, depending on the degree of redundancy with other photoreceptors, and differences in steady-state level of phyA that may result from species-specific differences in phyA regulation and protein stability. Constitutive overexpression of phyA results in strong dwarfing and a suppression of shade-avoidance responses in white-light-grown plants – a phenotype which may have potential for agronomic application (Robson *et al*., 1996).

3.7.1.3 Photoperiod

The most familiar effects of photoperiod on plant growth are in the control of the transition to flowering. Induction of flowering can occur in response to shorter or longer photoperiod depending on the species, and is often associated with changes in elongation. In *Arabidopsis*, photoperiod responsiveness is determined by interacting effects of light (through phyA, phyB and cry2) and the circadian clock on the level and activity of the CONSTANS protein (Yanovsky and Kay, 2002; Valverde *et al*., 2004). This suggests that the rapid elongation of the inflorescence is controlled by a CONSTANS-dependent signalling pathway, and is thus distinct from the more direct photoreceptor effects on hypocotyl and vegetative stem elongation discussed earlier. In caulescent species, the distinction between direct and photoperiodic mechanisms may not be so clear cut as photoperiod can interact with light quality and irradiance to control elongation of the vegetative plant. However, there is still compelling evidence for the existence of distinct mechanisms. For example, stem internodes in pea are longer during long days than during short days, and based on the lack of such a difference in *phyA* mutants, this response appears to predominantly act through phyA (Weller *et al*., 1997). This phyA-mediated promotion of elongation in response to daylength is clearly distinct from the phyA-mediated inhibition of elongation in response to FR described earlier.

3.7.2 Mediation of light effects by hormones

It is therefore clear that the nature of the light regime can dramatically affect stem length. Next, we review evidence that at least some of these effects are ultimately mediated by changes in the levels of plant hormones. There are several clear examples of the light regime altering GA content sufficiently to account for large changes in elongation rate, and two such examples are discussed in detail. Evidence for the importance of changes in the content of BRs and other hormones is, however, much less convincing.

Photoperiod is an aspect of the light regime that, in certain species, can regulate stem elongation by altering GA_1 content. One of the best examples is provided by *Silene armeria* (Talon and Zeevaart, 1990). Transfer of *Silene* plants from short to long days dramatically increases GA_1 content by over 10-fold in the shoot tip and this increase precedes the substantial stem elongation induced by long days. Furthermore, application of bioactive GA can mimic the effects of long days on stem elongation (Cleland and Zeevaart, 1970).

The effect of long days is not restricted to the shoot tip in *Silene* but was most dramatic in that region (Figure 3.14; Talon and Zeevaart, 1990). Monitoring a range of GAs indicated that the key biosynthetic step up-regulated by long days is the 20-oxidation of GA_{53} (Figure 3.3). GA_{53} was the only GA whose content decreased after transfer to long days, compared with plants kept in short days (Talon *et al*., 1991); like GA_1, levels of GA_{44}, GA_{19} and GA_{20} all increased. Molecular analysis of GA gene expression in *Silene* has not been reported, but in

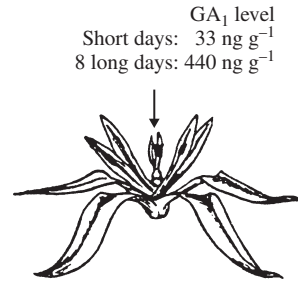

Figure 3.14 Effect of photoperiod on GA_1 content in shoot tips of *Silene* plants. Exposure to 8 long days increased the GA_1 content from 33 to 440 ng g^{-1} dry weight. Subsequently, there was dramatic stem elongation in long days. [From Talon and Zeevaart (1990). Copyright of the American Society of Plant Biologists; reprinted with permission.]

spinach, another rosette species, Lee and Zeevaart (2002) showed that long days strongly up-regulate expression of a key GA 20-oxidation gene (Figure 3.15). *Lolium temulentum* is another species in which photoperiod markedly affects the GA pathway (King *et al*., 2001).

The second example to be considered here also involves a transfer of plants from one environment to another; in this case, pea seedlings from darkness to continuous light. The rapid elongation of dark-grown seedlings is in some ways reminiscent of GA-induced growth in light-grown plants, and early researchers often attributed the spindly habit of dark-grown plants to high endogenous GA levels. However, when the GA content of continuously dark- and light-grown peas was compared by advanced physico-chemical techniques, it was found that the level of GA_1 was not higher in the dark (Weller *et al*., 1994). In fact, by the mid-1990s, it was becoming accepted that in pea, the light regime did not substantially affect the content of bioactive GA.

Then, Ait-Ali *et al*. (1999) reported that transfer of dark-grown pea plants to light caused a rapid drop in GA_1 content. This result was confirmed by subsequent papers (Gil and García-Martinez, 2000; O'Neill *et al*., 2000). Importantly, O'Neill *et al*. showed that the drop in GA_1 level is transient, and that after 3–4 days, the GA_1 content of transferred plants increases to the level found in plants maintained in darkness. This explains why comparison of plants grown in continuous light with those in continuous darkness showed no difference in GA_1 levels (Weller *et al*., 1994). Importantly, Gil and García-Martinez (2000) showed that transferred plants elongate in response to applied GA_1, indicating that the transfer-induced drop in GA_1 contributes to the reduction in elongation, and is not merely passively associated with it. (An inability of transferred plants to respond to applied GA_1 would indicate that the endogenous GA_1 content is irrelevant for the elongation rate.) Furthermore, the drop in GA_1 levels appears to occur sufficiently early to account for at least most of the decrease in elongation rate (Gil and García-Martinez, 2000).

The transfer of plants from dark to light appears to downregulate expression of Mendel's *LE*, and to up-regulate expression of *PsGA2ox2*, which encodes an enzyme that deactivates GA_1 (Figure 3.3; Reid *et al*., 2002). As well as reducing

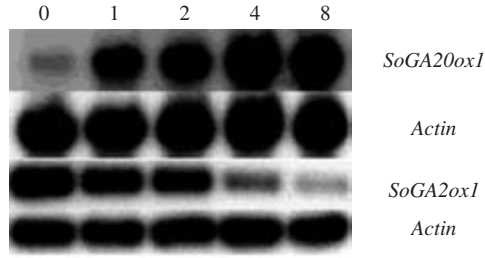

Figure 3.15 Effects of photoperiod on expression of gibberllin (GA) synthesis and deactivation genes of spinach. Exposure to 1, 2, 4 or 8 long days progressively increased mRNA levels of a GA 20-oxidase gene(top), and decreased those corresponding to the GA deactivation gene *SoGA2oxl* (bottom). Thus, long days increase the content of bioactive GA. [From Lee and Zeevaart (2002). Copyright of the American Society of Plant Biologists; reprinted with permission.]

GA levels, transfer from dark to light also reduces the GA responsiveness of seedlings. This is best demonstrated using dark-grown *na* seedlings which respond strongly to GA_1 application. Transferred *na* plants became less responsive, while continuously light-grown *na* plants were least responsive (O'Neill *et al*., 2000). Furthermore, when GA_1 was applied to transferred wild-type plants, it did not restore elongation to that of dark-grown plants (with or without GA_1), although growth was promoted (Gil and García-Martinez, 2000).

It is reduced GA responsiveness that is primarily responsible for the relatively slow growth rate of transferred plants that have been in the light for long enough for their GA_1 content to recover, or of plants that have been continuously light-grown. Of course, the statement that light affects GA responsiveness does not imply that events directly in the GA signal transduction pathway are necessarily directly affected by light. This is an important question for future investigation.

Recent evidence indicates that GAs mediate not only the reduction in elongation that occurs on transfer of seedlings to light, but other aspects of the de-etiolation response as well. Etiolated wild-type plants have a pronounced apical hook, very little leaf expansion and low expression of certain light-regulated genes such as *CAB* and *RbcS*. As these plants de-etiolate on exposure to light, their leaflets expand, their apical hook disappears and the expression of light-regulated genes increases dramatically. Extreme GA-deficient dwarf *na* plants, on the other hand, are considerably de-etiolated even in the dark: they do not have an apical hook and their leaves are quite expanded (Figures 3.16 and 3.17; Reid, 1983); furthermore, *RBsC* appears to be up-regulated, compared with the wild type (Alabadi *et al*., 2004). Similar results have been obtained using the GA-deficient mutant *ga1-3* of *Arabidopsis* (Alabadi *et al*., 2004). On this basis, the GAs have been implicated in the regulation by light of apical hook formation (Achard *et al*., 2003; Alabadi *et al*., 2004; Vriezen *et al*., 2004), and of leaf expansion and light-regulated gene expression (Alabadi *et al*., 2004).

Importantly, Alabadi *et al*. (2004) contended that the enhanced *RBsC* transcript levels of *na* plants in the dark, compared with the wild type, are probably not an

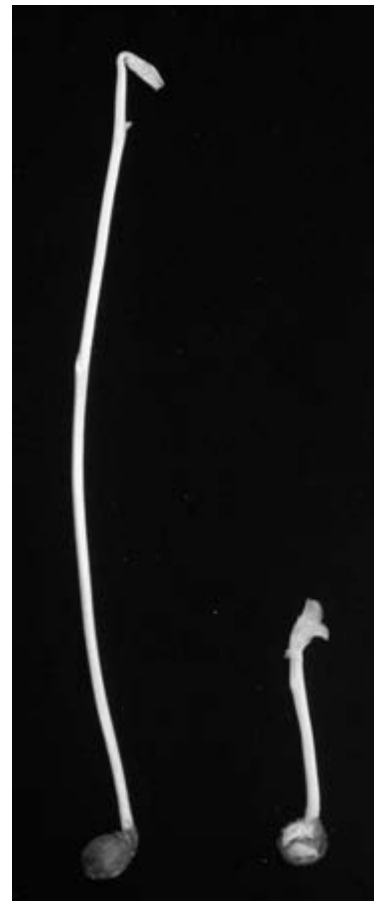

Figure 3.16 Difference between 8-day-old wild type (left) and severely GA-deficient *na* seedlings (right) grown in darkness. Seedlings of genotype *na* do not develop an apical hook.

indirect effect of the short stature of the *na* mutant, because Symons *et al*. (2002b) showed that in other dwarf pea mutants (the BR mutants *lk*, *lka* and *lkb*), *RBCs* are not upregulated. This logic can be extended to other architectural changes during photomorphogenesis, such as apical hook disappearance and leaflet expansion, both of which occur in a wild-type manner in the BR mutants (Symons *et al*., 2002b), but not in *na*. However, it is important to realise that, in the dark, the pea BR mutants are not as short as *na* plants, and therefore the case made by Alabadi *et al*. (2004) is not quite complete. This is especially relevant in view of the fact that moderate GA-deficient mutants, unlike the severe *na* mutant, are not de-etiolated in darkness. Alabadi *et al*'s case can be supported, however, by comparison of the *na* mutant with the double mutant *lka lkb*, which is deficient in both BR levels and reception. These plants, although very short in the dark, do not display leaf

Figure 3.17 Abnormal leaf expansion in dark-grown dwarf plants is not necessarily a consequence of short stature. The *lka lkb* double mutant (left) is short because both BR synthesis and response are impaired, but its leaflets are not expanded. The *na* plant deficient in gibberellin (GA)(right), on the other hand, is taller but its leaflets are relatively expanded.

expansion (Figure 3.17). This supports the suggestion (Alabadi *et al*., 2004) that the enhanced leaf expansion of dark-grown *na* plants, compared with the wild type, is a direct effect of their low GA content.

As mentioned previously, the moderate GA-deficient dwarfs *le-1*, *lh-2* and *ls-1* are phenotypically fully etiolated in the dark, apart from the fact that they are somewhat shorter than corresponding wild-type plants (Reid, 1988). The essentially etiolated phenotype of GA-deficient pea mutants other than the extremely short *na* indicates that the threshold GA_1 level above which the apical hook forms and leaf expansion is inhibited is lower than that above which internode length is impeded.

The lack of an apical hook in severe GA-deficient mutants indicates that an intact GA signalling pathway is required for the development of the hook. The role of GAs in apical hook formation appears to be mediated by DELLA proteins (Achard *et al*., 2003). Ethylene, traditionally thought to be involved in regulating the apical hook, also affects DELLA proteins, which implies a potential link between GAs and ethylene in controlling this phenomenon. A role for GAs is consistent with loss of the apical hook in seedlings transferred from darkness to light (Figure 3.13). The GA_1 content of these seedlings drops rapidly, and this may well contribute to the disappearance of the apical hook on transfer. Possibly, therefore, GAs are not merely a permissive factor with respect to apical hook opening, but actually mediate the regulatory effect of an environmental factor on that process. However, mechanisms of regulation of the differential growth processes that maintain the apical hook remain unclear.

It is ironic that BR-deficient mutants have been used (Alabadi *et al*., 2004) as examples of plants that are not de-etiolated in the dark (apart from being short), because one of the earliest reported characteristics of dark-grown *Arabidopsis* BR mutants was, in fact, a de-etiolated phenotype (Takahashi *et al*., 1995; Li *et al*., 1996). Indeed, an integral part of BR research since the discovery of the first BR-deficient mutants has been the theory that the long internodes of dark-grown plants are attributable to a high level of active BRs. However, measurement of endogenous BRs provides no evidence for this. When Symons *et al*. (2002b) compared BR levels in wild-type pea seedlings grown in continuous light or continuous darkness, the BR levels were actually higher in the light. Importantly, transfer of seedlings from darkness to light also did not reduce BR content (Symons *et al*., 2002b; Symons and Reid, 2003). In summary, therefore, it now appears that BRs are unlikely candidates for the 'etiolation hormone' in pea, and evidence is mounting that this role is, at least in part, actually played by GAs.

3.7.3 Effects of other factors, including flooding and decapitation/grazing

Flooding is another environmental factor that strikingly affects stem elongation. One of the best-studied examples is the promotion of elongation by flooding in deep-water rice. In this case, submersion of the stems causes a rapid increase in elongation rate. The initial stimulus for this dramatic elongation is ethylene, which accumulates because of reduced gas exchange under water (Kende *et al*., 1998). Flooding may also increase the number of ethylene receptors (Watanabe *et al*., 2004). The next step appears to be an ethylene-induced reduction in the level of abscisic acid (ABA), which in turn increases the responsiveness of the tissue to GA (Kende *et al*., 1998; Voesenek *et al*., 2004). Thus, GA is seen as the final hormonal effector of elongation. Interestingly, the role of ethylene as a growth promoter in this system is opposite to its effects on purely terrestrial plants in which it usually inhibits growth (Reid, 1995). Thus, the deep-water rice system again illustrates how hormones can interact to regulate growth, and how the nature of these interactions might differ depending on environmental conditions, species and/or tissue type.

Grazing or pest attack is another environmental factor that can radically affect stem elongation. This factor is not often discussed in this context, even though decapitation caused by grazing is an 'everyday' occurrence for many herbaceous dicotyledonous species (see Chapter 4). As discussed earlier, decapitation reduces the auxin content of the stem, which leads to reduced GA_1 levels and consequently reduced elongation. Assimilates can then be channelled into stimulation of outgrowth of axillary buds; the continued elongation of existing internodes just below the previous apical bud would represent a waste of resources. The converse is that when the apical bud is intact and growing rapidly, the auxin supply to the internodes below that bud is maintained at a relatively high level. Consequently, GA_1 biosynthesis and stem elongation can proceed. Thus, the apical bud exerts an effect on internode expansion, providing an example of hormone 'action at a distance'. Interestingly, BR levels in pea are largely unaffected by decapitation (Symons and Reid, 2004).

3.8 Concluding discussion – are hormones regulators of plant growth or merely permissive factors?

The phenotypes of hormone synthesis mutants demonstrate that normal levels of auxin, GAs and BRs are required for normal stem elongation. Many hormone signalling mutants are also short, confirming the importance of hormone signal transduction. However, these conclusions do not, in themselves, mean that any one of the three hormones is a 'regulator' of stem elongation in the wild type. In order to determine whether or not a hormone actually regulates elongation, in the traditional hormonal sense, we need to consider two critical elements: interactions between the hormones and the effects of environmental and/or developmental factors on hormone levels.

The effect of auxin on GA biosynthesis is a key interaction, because it implies that the promotion of growth by auxin might be mediated to a large extent by GAs. In contrast, auxin does not appear to affect BR levels (Symons and Reid, 2004), and the effects of BRs on auxin or GA levels do not appear to be physiologically significant (Jager *et al*., 2004); nor do GAs appear to operate via changes in auxin content (Ross *et al*., 2002). Thus, of the six possible ways in which auxin, GA and BR can affect each other's level, only the effect of auxin on the GA pathway appears to be important for plant growth. However, it remains likely that each of the three growth-promoting hormones can regulate some of the downstream genes primarily regulated by one of the others; that is, there is 'cross-talk' at the signal transduction level. Goda *et al*. (2004), however, found that a majority of auxin-regulated genes were regulated by auxin only, and not by BRs, and that the majority of BR-regulated genes were regulated by BR only, and not by auxin.

The environment and, in particular, aspects of the light environment, dramatically affect stem elongation. Which hormones actually vary in content in response to environmental factors (i.e. in an hormonal manner), thereby significantly affecting stem length? Certainly, GA content is dramatically affected by the light regime, and this appears to result in substantial changes in elongation. Auxin levels are reduced by decapitation/grazing, and are also thought to be regulated by tropic stimuli. The effects on growth of such changes in auxin content might be mediated by GAs; there is already strong evidence that this is the case for decapitation (Ross *et al*., 2000). Of course, factors that alter GA levels do not necessarily do so by altering auxin content first: there is no evidence that the drop in GA_1 content caused by transferring pea seedlings from dark to light is mediated by changes in auxin level (Symons and Reid, 2003).

It appears, therefore, that GA is a key regulator of stem elongation, in the sense that its level changes in response to environmental and/or ontogenetic factors. Auxin is also critical, not least because without it GA biosynthesis in the stem is dramatically reduced. BRs are required for normal stem elongation, but appear to act largely without affecting the levels of the other hormones. At present, there is little evidence that the BRs mediate environmental effects on stem elongation. However, this should not necessarily raise doubts over the status of BRs as plant hormones.

The BR receptor has been identified (Wang *et al*., 2001), and many genes are regulated by the BRs (Goda *et al*., 2004). Furthermore, like the GAs, BR biosynthesis is subject to feedback regulation, indicating their importance for maintaining plant growth. It is relevant also that the endogenous levels of all the growth-promoting hormones are typically not saturating for elongation, even in wild-type plants, as indicated by the capacity of wild-type peas to respond to treatment with auxin, BR or GA (Yang *et al*., 1996; Nomura *et al*., 1997; Ross *et al*., 2002).

In the above sections we have emphasised factors that alter hormone levels and, consequently, the rate of stem growth. A different perspective is that a major role of hormones is to help the plant maintain a *constant* rate of growth. Consistent with this role, plants have evolved feedback mechanisms for the regulation of GA and BR biosynthesis. Such 'buffering' systems presumably compensate for small changes in hormone synthesis and/or action, and the constant growth rate that results might confer a selective advantage to the plant. However, if feedback systems were too powerful, it would be impossible for an environmental or endogenous stimulus to change hormone biosynthesis. The fact that light and auxin content, to name two such stimuli, can dramatically affect GA biosynthesis means that these factors can override feedback to alter GA content and, consequently, stem elongation.

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4 Shoot architecture II
Control of branching

Colin G.N. Turnbull

4.1 Introduction

Innate human curiosity aside, interest in shoot branching derives largely from two areas. First, shoot branching is a major determinant of plant architecture, governing many aspects of form, function, efficiency and adaptation. Second, many interventions by humans involve, to a greater or lesser degree, modification or manipulation of shoot branching. Deliberate modification of plant shape is a common theme running through several professional and leisure activities. Consider the range of horticultural practices such as orchard pruning, tip removal in pot plants, side shoot pinching in tomatoes, arts of bonsai and topiary, through to productivity of coppicing and functionality of hedging. Every mown patch of turf, every clipped hedge, every pruned fruit tree responds to shoot removal by initiating new branch growth. But, of course, manipulation of branching is not restricted to the activities of the physical cutting of shoots. Many genetic routes have been discovered and then modified for practical purposes. Terms such as upright, spreading, bushy, compact or weeping abound in plant descriptions, usually referring to stable genotypic characters although not exclusively to attributes specific to branching. The environment further modifies shoot branching in dramatic ways. Impact of plant–plant spacing results in many morphogenetic responses, one of which is usually a suppression of branching under crowded conditions due to neighbour perception responses. Similarly, in other competitive situations such as low soil nutrient status, the fundamental and pervasive plant property of developmental plasticity adjusts quantities of shoot growth to match resource availability. Control of branching is a convenient means to achieve this, either through stochastic regulation of branch number or continuous variation in branch length or vigour.

4.1.1 Species differ widely in propensity for branching during normal ontogeny

A major issue in shoot branching is the question of how each species or genotype has a characteristic branching pattern under any given set of conditions; yet, these patterns are enormously diverse from almost never in vegetative nodes of most

palms, to almost always in sympodial systems in the Solanaceae. In contrast, despite this ontogenetic variation among species, almost without exception, every plant will respond to shoot tip damage by initiating additional branch growth. Most likely this has evolved as a universal survival mechanism for plants which have co-existed for millions of years with herbivores, and are also frequently subject to damage from physical extremes of weather – storms, hail, etc.

4.1.2 Responses to decapitation

The ubiquitous initiation of bud outgrowth in response to shoot tip loss suggests that there may be a common mechanism of regulation across higher plant species. Decapitation is a simple manipulation that initiates a cascade of signalling and developmental processes, making it a popular and amenable system for studying branching. Most critical events occur rapidly – within hours to days – not surprising because there are almost certainly selection pressures for rapid recovery and resumption of normal growth and shoot function. What is known about these events? Which are regulatory as opposed to coincidental or consequential?

Some extremely rapid changes occur whenever plant tissues are damaged. Cell disruption alters electrical potentials across membranes, and severing of vascular systems affects hydraulic properties and water relations, probably within seconds (McIntyre and Damson, 1988). In contrast, initiation of new bud growth takes a few hours or more in most species (Hall and Hillman, 1975; Gocal *et al*., 1991, Stafstrom and Sussex, 1992, Turnbull *et al*., 1997). However, much earlier changes have been consistently detected at the molecular level especially in studies of pea, where bud growth has been observed after 8 h but transcriptional changes are readily apparent after only 1 h (Devitt and Stafstrom, 1995; Stafstrom *et al*., 1998).

Stafstrom and Sussex (1988) developed an elegant pea bud system which has provided additional insights into dormancy–growth transitions. By decapitating young seedlings, they caused rapid activation of the multiple existing buds in each axil. However, after a few days, one of these buds maintained growth, becoming the dominant replacement shoot, while the others returned to dormancy. By monitoring transcript and protein changes, a series of dormancy and growth marker genes was discovered. The main findings were rapid transcriptional changes well ahead of detectable growth resumption. Perhaps surprisingly, dormant buds exhibited high metabolic and transcriptional activity indicating that they were far from inactive or 'resting'. Stafstrom (1993) further suggested that there is a definable transitional state through which buds can pass repeatedly in multiple dormancy– growth cycles.

Many genes that are up-regulated are associated with the cell cycle and cell proliferation, including histone H2A and H4 which are markers for S-phase in the mitotic cycle. This is consistent with flow cytometry measurements of dormant bud nuclei which show that the majority of cells are arrested in G1 presumably just prior to the G1–S checkpoint. The most rapid increases on dormancy release were in cycD3-1 and PCNA, suggestive of G1–S transitions. Other early up-regulated

genes include gibberellin-catabolising GA-2-oxidase (Ross *et al*., 2000), cytokinin biosynthesis (IPT), IAA-amino acid conjugate hydrolase, as well as members of the oxylipin/jasmonate pathway (lipoxygenase, allene oxide cyclase) and undefined CYP genes (Shimizu-Sato and Mori, 2001).

In contrast, other pea genes such as *DRM1, DRM2, AD1* and *AD2* (Stafstrom *et al*., 1998; Madoka and Mori, 2000a,b) are significantly downregulated over this period, and, therefore, are considered to represent molecular markers for the dormant state. Because dormant buds clearly maintain high levels of particular transcripts, dormancy should not necessarily be equated with cellular inactivity. *DRM1* may represent a universal marker for non-dividing, non-growing cells throughout the plant, whereas *DRM2* expression is modulated only in buds. Although not confirmed directly, analysis of promoter sequences indicates that some dormancy-associated genes possess putative response elements for different hormones including auxin (*DRM1*) and ABA (*DRM2*, *AD1*) (Stafstrom *et al*., 1998; Madoka and Mori, 2000a,b). However, decline in expression of ABA biosynthesis genes in buds was relatively slow compared with timing of bud growth initiation (Shimizu-Sato and Mori, 2001).

If transcription is altered within 1 h of decapitation, the causative signal(s) must be effective before this time. Most models for regulation of shoot branching have incorporated auxin and cytokinin as two major mobile signals (discussed further in Section 4.4.3). The low velocity of auxin polar transport may present problems for distant buds relying on local auxin depletion, especially in larger plants. In addition, as discussed earlier, auxin moving in the stem polar stream does not significantly enter the bud, and indeed IAA content of buds rises significantly around the time that growth commences (Gocal *et al*., 1991). The source of this IAA is unknown, but given the acknowledged role of auxin in cell cycle control and general growth promotion, it is perhaps not surprising that meristem reactivation is associated with increased auxin content. Rapid elevation of bud cytokinin content is also seen (Turnbull *et al*., 1997) and this appears to be preceded by increased delivery of cytokinins translocated in the xylem (Mader *et al*., 2003). As with auxin, increased cytokinin content is likely to represent a positive signal for cell cycle activation, consistent with one general function of this hormone class.

Alternatively, a more rapid means to change auxin signalling may be achieved via disruption of phloem flow. Although little studied compared with the polar auxin mechanism, phloem sap does contain significant IAA (around 1 μM; Baker, 2000). Phloem translocation appears to be important at least for supply of IAA to root tips, where a post-phloem path exists that uses AUX1 influx and PIN efflux carriers (Swarup *et al*., 2001). Although direct evidence is lacking, it can be surmised that this IAA is largely derived from mature leaves and hence exported in the assimilate flow along with many other metabolites including several classes of hormone that are also detected in the phloem: cytokinins, ABA, gibberellins, jasmonic acid. In some woody species, it has been shown that defoliation rather than decapitation is more effective at inducing lateral bud growth (Champagnat, 1955; Cline and Deppong, 1999), which tends to suggest an inhibitory influence

emanating from the leaves that is not disrupted by the absence of the shoot tip. It is not yet clear whether there is any crossover of IAA between phloem and polar stream during long-distance basipetal transport, nor whether the auxin molecules carried by the two systems have shared or completely discrete targets. Although polar auxin transport, with a velocity of about 10 mm h^{-1}, may be sufficient for signalling over short distances and in smaller plants, effective communication in larger species may require the considerably higher speed (around 50 cm h^{-1}) of phloem mass flow.

4.2 Branch positions and morphologies

Casual observation of different plant species and different growth stages reveals highly divergent and potentially bewildering branching patterns. In this section, some of the major classes and patterns are described. Further coverage of woody plant branching is found in Chapter 8.

4.2.1 Developmental zones

Although the vast majority of leaf axils possess at least one lateral bud, under normal circumstances, not all grow out to form branches. Moreover, several lines of evidence strongly suggest that the outgrowth *potential* varies with position along the main stem axis. Many species seem to have three discrete zones with quite abrupt transitions between them. This is most obvious in caulescent species, such as tomato and pea, but may also exist in rosette plants including Arabidopsis. The three zones are given different terms by different authors, but, in essence, there is a *basal* zone, whose branches will often grow as replicas of the main shoot (e.g. cereal tillers), a *middle* zone where buds are mostly repressed for the lifetime of the plant unless the shoot tip is damaged, and an *upper* zone, often commencing outgrowth shortly before inflorescence emergence and frequently associated with increasing numbers of reproductive growing points (Figure 4.1).

Outgrowth potential can be measured in intact plants under varying conditions, or following shoot decapitation at varying ages and positions along the stem. Intact plants of many species have a tendency to basal branching while vegetative, and then to upper node branching before or after floral initiation. However, environmental factors have a great impact on whether particular buds fulfil their outgrowth potential. Favourable growth conditions such as high light, wide plant spacing, optimum temperature, ample soil moisture and nutrients will give maximum branching whereas resource-poor environments may lead to plants where neither basal nor upper zone buds show significant growth. Impact of environment is discussed further in Section 4.5. Decapitation can induce bud outgrowth at almost any node, with the most common position being the node(s) immediately below the missing shoot tip. Decapitation in the middle zone is often the only circumstance in which buds in this region grow out; so, they can be interpreted as a reserve of meristems for times of urgent need rather than meristems that grow when

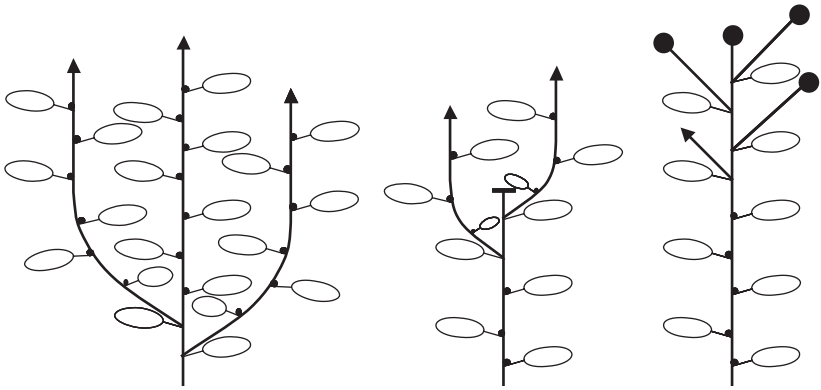

Figure 4.1 Three developmental zones along the shoot axis exhibit different bud outgrowth characteristics. Basal zone buds produce branches that replicate main shoot development pattern (left). Middle zone buds are less likely to grow out in intact plants but after shoot decapitation, can respond by replacing the main stem (middle). Upper zone buds commence outgrowth around the time of floral induction, and some or all of these branches become reproductive (right). Arrowheads are vegetative apices, large circles are reproductive apices and small circles are dormant axillary buds.

resources are plentiful. Basal buds, if they have not already grown, can also respond to decapitation. In pea, there are complex dominance relationships between the multiple buds that coexist within a single axil (see Section 4.1.2), with repeated cycles of onset and cessation of growth.

4.2.2 Shoot dimorphism: orthotropic vs. plagiotropic development

Lateral meristems clearly have many possible fates ranging from permanent dormancy to vegetative outgrowth, indeterminate inflorescence to determinate flower. For vegetative branches, however, within a species, and often within an individual plant, different developmental patterns are possible, varying with age, position and environment. One most obvious divergence in form is seen in many gymnosperms where the main stem axis (trunk) grows vertically (orthotropic growth) whereas lateral branches grow in very different directions, from horizontal to angles above and even below horizontal (plagiotropic growth). Many tropical trees show similar orthotropic/plagiotropic shoot dimorphism (Figure 4.2a). Interestingly, decapitation of the main stem leads to conversion of one or more existing plagiotropic shoots, or dormant lateral buds into replacement orthotropic leader shoots. This indicates multiple potential developmental programmes within such buds and shoots, with realisation of particular patterns depending on internal ontogenetic and external factors. Plagiotropic shoots may also differ from orthotropic shoots in their reproductive potential, with some species such as coffee bearing most or all inflorescences on such laterals and few or none on the main

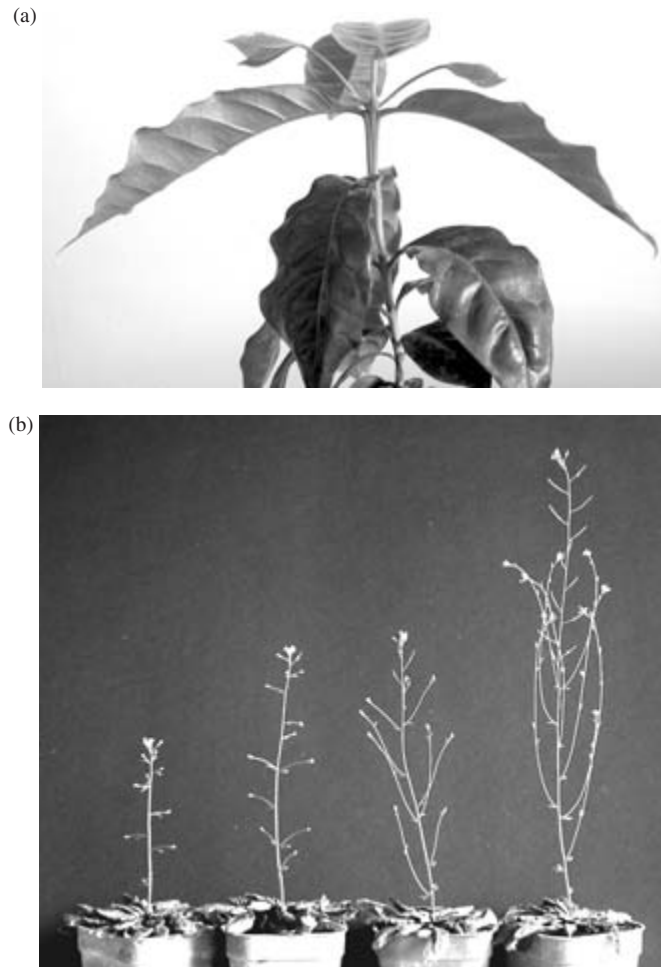

Figure 4.2 Variation in branch angle. Many trees, especially in the tropics, exhibit orthotropic (upright) main stems, but plagiotropic (angled) lateral branches, shown here in a coffee seedling (a). Herbaceous species, including *Arabidopsis*, may also exhibit predictable changes in branch angle. Here, branch angle changes from near horizontal to more upright during development of lateral shoots from cauline nodes of *Arabidopsis* (b).

stem. The opposite is also possible, for example, in cocoa which bears caulifloral branches directly from the trunk. Many other patterns of branch angle and branch fate have been described by Hallé *et al*. (1978) and are discussed in more detail in Chapter 8. The question arises, do herbaceous species exhibit similar branching pattern divergence? Although this issue is not as widely studied as in trees, it is clear that not all branches have equivalent morphologies or potential. Many vigorous basal zone branches adopt an orthotropic habit highly similar to the main stem,

as do almost all shoots that are formed in response to decapitation. However, plagiotropic growth is a clear and stable character of some herbaceous branches. In *Arabidopsis*, branches from nodes on the main inflorescence axis initially grow out horizontally (diagravitropic), but soon convert to a more upward direction (Figure 4.2b). Likewise, simultaneously developing secondary inflorescences generated from rosette leaf axils adopt an increasingly vertical habit during development, nearly replicating the primary inflorescence. Interestingly, in increased-branching mutants of pea and *Arabidopsis*, (Figure 4.3), the branching angle is more strictly vertical than for lateral shoots developed on intact or decapitated wild-type plants.

4.2.3 Relative timing: proleptic vs. sylleptic branching

A further variation on patterns of branching concerns relative timing of growth of different axes. Usually, this is simplified by considering timing of lateral branch growth from particular nodes relative to timing of growth of the main stem. Much of these studies have concerned tropical woody species, possibly because their

Figure 4.3 Fundamental difference between branching architecture induced by decapitation and increased branching due to *max1* mutation in *Arabidopsis*. From left to right: Columbia intact, Columbia 7 days after decapitation and *max1* intact.

multiple phases of vegetative elongation ('flushes') each growth season facilitate time-resolved observation and experiment. Essentially, there are two main options. The first, known as *prolepsis*, is manifest as flushing of the main axis coincident with outgrowth of older pre-existing lateral buds or shoots (Figure 4.4a). The alternative is *syllepsis*, where lateral buds of the currently growing main axis grow out during the flush period in which they were formed (Figure 4.4b). Although little is known of regulatory mechanisms, the fundamental difference is that lateral buds and proleptic shoots enter a period of dormancy before growing, whereas sylleptic buds do not, and therefore can form a shoot much more rapidly. Environmental factors further influence the exact patterns, typically leading to fewer, slower or shorter, lateral shoots under adverse conditions. Even on a sylleptic axis, some buds may not grow, and this may be proportional to resource availability, for example, levels of light and soil nutrients. Further coverage of these patterns is given in Chapter 8, and in a recent review by Wu and Hinckley (2001).

4.2.4 Reiteration: monopodial vs. sympodial systems

The simplest shoot architecture is monopodial where growth is dominated by a single axis, which can subsequently generate flowers or inflorescences in terminal or axillary positions (Figure 4.5). Some more complex species, especially woody types, develop a polypodial form where multiple more or less equivalent axes develop, and ultimately there may be no clear main stem. Both monopodial and polypodial forms may or may not bear higher order lateral branches, which may be plagiotropic (see Section earlier 4.2.2 earlier). In some species, especially well studied Solanaceae

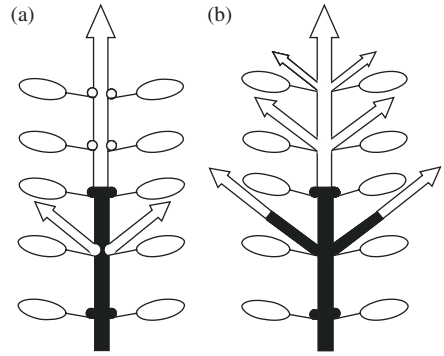

Figure 4.4 Temporal phasing of apical and lateral shoot growth. In proleptic development, (a) laterals grow out from older buds, often at the same time as the main axis is extending. New buds, and often some old buds, remain dormant. In sylleptic development (b), newly formed lateral buds on the main axis grow out immediately to form branch shoots. At the same time, laterals formed from previous growth may extend again. Cyclic vegetative growth is known as flushing, and is a prevalent characteristic of tropical woody species. Previous growth is shaded black, current growth unshaded. Small circles represent dormant buds. For clarity, leaves on laterals have been omitted.

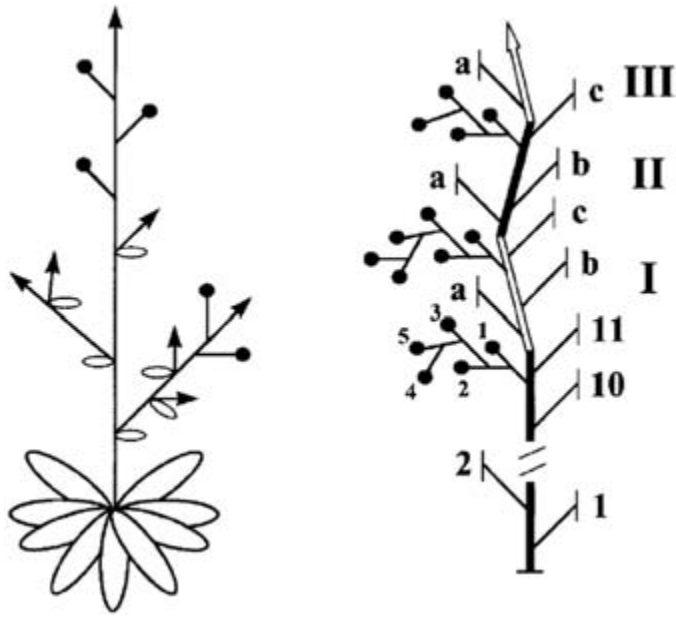

Figure 4.5 Different forms of reiterative shoot architecture. *Arabidopsis* (left) displays monopodial development: branching is normally restricted during the vegetative phase, but after onset of floral development, branches (co-florescences, shown as arrows) grow from the axillary meristems on the primary inflorescence axis. Tomato converts to sympodial development after an initial monopodial vegetative phase (right). The primary vegetative shoot (leaves numbered 1–11) is terminated by a flower. A vegetative shoot develops from the highest axil below the inflorescence and forms a first sympodial unit comprising three nodal leaves (a, b, c in sympodial sections I and II) and a terminal inflorescence, This pattern can then repeat many times. Individual flowers are shown as black circles and leaves/nodes as T-shaped arrows. Adapted, with permission, from Pnueli *et al*. (1998).

members such as tomato, tobacco and petunia, initial monopodial development with the normal branches is later replaced by another form called sympodial branching. This transition normally coincides with the onset of reproductive development and is therefore restricted to the upper zone as defined in Section 4.2.1. In this system, the shoot apical meristem (SAM) completely converts to production of a determinate inflorescence or terminal flower. However, the buds in the axils of one or more of the highest leaves grow out to form sympodial branches. In tomato and petunia, these branches take over the shoot extension function. As they develop, the inflorescence becomes displaced laterally and the sympodial shoot adopts a more vertical orientation, thus giving the appearance of continuity of apical growth. The SAM of the sympodial shoot then becomes floral itself, after producing one (tobacco), two (petunia) or three (tomato) leaves (Figure 4.5). Again upper lateral bud(s) forms new sympodial units, and this modular reiteration can continue almost indefinitely (Reinhardt and Kuhlemeier, 2002). Evidence for the stability of sympodial reiteration comes from

studies of genes such as *SP (SELF-PRUNING)* in tomato (Yeager, 1927). *SP* acts via a mechanism that reserves part of the apical meristem as permanently vegetative and, thus, prevents it from being entirely consumed by formation of the terminal inflorescence. In *sp* mutants, as the name implies, the number of sympodial units is restricted, with a diminished proportion remaining vegetative, thus leading to progressive reduction in numbers of leaves per sympodium from three, then two and finally the shoot terminates in an inflorescence (Pnueli *et al*., 1998). Homologous genes (*TFL1* and *CEN)* are found in *Arabidopsis* and *Antirrhinum*, respectively, but because these species are not sympodial, the genes have slightly different functions, although still associated with maintenance of the vegetative state (Shannon and Meeks-Wagner, 1991; Bradley *et al*., 1996). Further discussion of inflorescence architecture is presented in Chapter 6.

4.3 Bud initiation

Formation of lateral or axillary meristems is an essential first step towards generation of shoot branches. However, there is considerable complexity and variation in the exact timing of bud initiation, and in the extent of bud development. In this section, early events are discussed, up to the point where a recognisable structure containing its own apical meristem and usually a few leaf primordia have developed. This is normally described as a lateral bud, which may or may not enter a period of dormancy (see Section 4.4). Lateral buds superficially resemble the SAM from which they were generated, but physiologically often have some quite different properties.

Pinpointing the exact position and timing of bud initiation is not simple. Visual inspection of longitudinal sections of shoot apices can reveal whether lateral outgrowths exist, but the earliest events of cell determination may occur before this point, and there are complications of leaf primordia being formed from nearby SAM cells, giving a second type of lateral appendage. Ongoing debate on timing and cellular origins of bud formation has concerned two models. The first proposes that a bud arises from cells on the flank of the SAM that never lose their meristematic status. This is often referred to as the *detached* or *reserved* meristem hypothesis. The alternative, described as *de novo* formation, is that buds initiate later, probably from cells at the leaf base, by recommencing meristem activity.

Several means have been employed to investigate the mechanisms and to resolve whether species do, in fact, differ in their fundamental regulation processes. Evidence comes from detailed anatomical observations, clonal analysis and *in situ* gene expression studies. On close examination, it becomes clear that there is probably some confusion in the literature, particularly, where only one experimental approach has been used. Here, an attempt is made to resolve the issue of *detached* vs. *de novo*.

First, species differ in the timing of bud visible formation relative to timing of development of subtending leaves. Environmental factors such as photoperiod are

influential, as is the onset of floral development. In species such as *Arabidopsis*, vegetative rosette nodes exhibit delayed and often minimal axillary bud development especially before flowering under long days. The lack of bud development in rosette nodes has sometimes been interpreted as evidence for the *de novo* hypothesis because the cells that eventually form buds are now very distant from the SAM, often commencing development many plastochrons after leaf initiation (Stirnberg *et al*., 1999). In other species such as tomato (Malayer and Guard, 1964), bud formation is visible virtually from the time of initiation of the subtending leaf primordium. In pea, buds also appear promptly, and basal nodes can carry several visible buds within a single axil (Sarup and Stafstrom, 2000). Subsequent nodes further up the stem, occasionally fail to initiate any bud.

Second, clonal analysis using induced visible mutant sectors has shown that the cells that ultimately form buds are from the same lineage as those forming some or all of the leaf. In particular, bud precursor cells have shared origins with those that form the leaf adaxial surface, and the L2 meristem layer appears significant for bud formation (Furner and Pumfrey, 1992; Irish and Sussex, 1992).

Third, dominant mutations of *PHABULOSA (PHB)* result in conversion of the abaxial domains to adaxial-like development (McConnell *et al*., 2001). In these bi-adaxial leaves, additional axillary bud initiation is associated with this abnormal abaxial side. This suggests that patterning of adaxial leaf features includes specification of a basal packet of cells to commit to bud formation, consistent with results of clonal analysis.

Fourth, patterns of expression of *SHOOTMERISTEMLESS (STM)* indicate that this gene is a good marker for meristem cells. The gene is normally expressed throughout the SAM, but is downregulated in cells about to form leaf primordia (Grbić and Bleecker, 2000). Mutation of *STM* results in a shoot system where cells fail to retain true meristem function, and hence stable reiterative modular shoot development cannot occur (Endrizzi *et al*., 1996). Importantly, *STM* continues to be expressed in a small number of cells in the presumptive leaf axil, suggesting that this is a domain that remains committed to meristem function and can then proceed to generate a bud. However, in *Arabidopsis*, the actual formation of the bud may not be visible for several plastochrons, implying that meristematic activity has, in effect, been suspended.

The question of *detached* vs. *de novo* may therefore be two views of the same process. The cells that ultimately form the bud are determined very early (suggesting detached/reserved model) but the suspension of rapid cell division in those cells in species such as *Arabidopsis* gives the appearance of later formation of a bud from the leaf base (as in the *de novo* model). Of course, cell division arrest in shoot meristems is a commonplace event – this is exactly what happens in every dormant bud or seed. So, it appears that placement of the domain that gives rise to the bud is dependent on patterning processes associated with axis formation during early leaf development, but the cells of this domain may, in fact, never be part of the true leaf, as indicated by suppression of *STM* expression. Perhaps, a better description of the normal sequence of events might be: (i) cell *commitment* occurring around the time

of leaf initiation, and location being dictated by the same positional information processes that include fundamental determination of leaf axes, sometimes followed by (ii) a period of meristem *latency*, then (iii) *evocation* finally leading to visible bud development.

4.3.1 Bud initiation genes

Understanding of bud initiation has been greatly aided by mutants in several species. Most of these mutants fail to form buds, and, therefore, the genes can be considered essential for normal initiation processes. Recent isolation of several of the corresponding genes has expanded the understanding of how meristem formation may be regulated.

4.3.1.1 Lateral suppressor (Ls)

One of the best characterised genes is *Lateral suppressor (Ls)* in tomato (Schumacher *et al*., 1999), which is homologous to *Monoculm1 (Moc1)* in rice and *Lateral suppressor (LAS)* in *Arabidopsis* (Greb *et al*., 2003; Li *et al*., 2003). Heterologous complementation tests indicate these are almost certainly orthologous counterparts, with no apparent close similarities to other members of the GRAS family to which they all belong. In all species studied so far, mutants fail to initiate buds at vegetative nodes, but are largely unaffected in production of lateral structures (inflorescence-bearing branches, flowers or inflorescences) once the plant reaches reproductive maturity. Interestingly, pleiotropic characteristics such as male sterility and other floral abnormalities seen in tomato *ls* mutants are not apparent in *Arabidopsis*. Furthermore, heterologous expression of *Arabidopsis LAS* in *ls* tomato rescues not only bud initiation but also the floral characters, suggesting that LAS/Ls has a broader range of regulatory targets in tomato than in *Arabidopsis*. In *moc1* rice plants, tiller bud formation is suppressed, but, in this case, inflorescence phenotype is also affected by reduced panicle branch numbers (check this!).

4.3.1.2 Blind (Bl)

Blind (Bl), from tomato, is a member of the very large R2R3 class of Myb transcription factors (Schmitz *et al*., 2002). *Blind* shares strong similarity in high conservation of the Myb domain with a total of six of the R2R3 genes in *Arabidopsis* and five in tomato. Typical of this family, very few similarities are found elsewhere in the protein, perhaps reflecting the diversity of specific functions within the gene family. Mutants at the blind locus include several *bl* alleles and other alleles originally identified as *torosa (to)*. Their phenotype is severely, though not completely, affected in formation of vegetative lateral buds, but only moderately affected in sympodial branching patterns than ensue at onset of reproduction. The nodes affected are quite precisely demarcated: buds are formed in axils up to leaf five, and then resume at the second node before inflorescence production (Mapelli and Kinet, 1992).

4.3.1.3 Revoluta (REV)

The *rev* mutant of *Arabidopsis*, similarly, lacks buds at vegetative nodes, but is also affected in initiation of interfascicular fibre strands; hence, its alternative name *ifl1 (interfascicular1)* (Talbert *et al*., 1995; Ratcliffe *et al*., 2000). *REV* is in the class III HD-ZIP gene family which has five members in *Arabidopsis*. Class III HD-ZIPs possess a START domain which has a putative steroid-like ligand binding function. Of the five genes, *REV* is most closely related to the sister gene pair *PHB* and *PHV*, with all three exhibiting similar spatial expression patterns in the apical meristem, the adaxial side of lateral organs and in vascular tissue (Emery *et al*., 2003). The other members are expressed only in vascular regions. Recently, it was discovered that the *REV* transcript possesses a complementary site for two microRNA species, miRNA165 and miRNA166 (Juarez *et al*., 2004). These miRNAs, which appear to represent non-cell-autonomous signalling molecules, are proposed to direct RNA degradation, and may be the means to achieve stable expression domains within the meristem. *PHB* and *PHV* also carry these miRNA target sites.

4.3.1.4 LAX and SPA

A further pair of rice genes, *LAX PANICLE (LAX)* and *SMALL PANICLE (SPA)*, appear to regulate both vegetative and inflorescence branch initiation (Komatsu *et al*., 2003). Single mutants are not affected in tiller initiation, but have substantially reduced number of panicle branches. However, in a *lax spa* double mutant, tiller buds are almost absent, and panicle branching is even more severely restricted. The non-additive nature of the vegetative phenotype suggests a partial redundancy in function between the two genes, but little is known about their functions. To date, *SPA* has not been characterised. The *LAX* gene has been isolated, and has homology with conserved regions of the bHLH (basic helix loop helix) family of transcription factors; but, in other regions, it has minimal homology with sequences in other well-characterised genomes including *Arabidopsis*. It has been suggested that grasses may have evolved genes such as *LAX* subsequent to divergence from other flowering plant groups. *In situ* hybridisation shows that *LAX* is expressed exclusively in cells of axils and presumptive sites of axillary bud formation. No expression is seen in the SAM itself, consistent with normal apical function seen in *lax* mutants. In maize, *bif2* mutants exhibit a similar phenotype with defects in inflorescence branch initiation, but have normal vegetative development (McSteen and Hake 2001).

4.3.1.5 SAX loci

Recently, a number of pea mutants affected in bud initiation were reported, based on a screen of mutagenised populations of increased-branching mutants. Amongst several phenotypes that exhibited reduced branching were three that specifically displayed blind axils in the middle zone of the stem. These have been named *sax1* to *sax3 (suppressed axillaries)* mutants (Rameau *et al*., 2002; Rameau and Parmenter, personal communication). Although none of the genes has yet been isolated, it is clear that these mutants share phenotypic features with some of the

loci described in other species. In particular, all the *sax* mutants have normal initiation of basal buds or branches at the first two nodes, and normal buds and branch outgrowth in the upper zone immediately below the flowering nodes. The genes, therefore, appear only to act on lateral meristem initiation processes during one part of the vegetative phase of the life cycle.

4.3.1.6 Interaction of initiation genes

REV expression in early axillary bud development appears to be dependent on normal *LAS* function, but, curiously, *REV* regulation in relation to vascular and fibre development is not compromised in *las* mutants (Greb *et al*., 2003). This suggests multiple regulation and multiple function of the *REV* gene. *REV*, in turn, appears to be a regulator of *STM* (Otsuga *et al*., 2001) Other genes such as *LOB* and *CUC1* exhibit similar expression patterns to *LAS* (Takada *et al*., 2001; Shuai *et al*., 2002), but no information is available on whether they regulate each other's expression. The finding, mentioned earlier, that miRNAs may regulate *REV* (Juarez *et al*., 2004) provides clues for future studies on links between *LAS* and *REV*.

4.4 Bud dormancy and branch outgrowth

In the simplest terms, all shoot meristems might be put into one of two categories – growing or non-growing. The state of non-growth is normally referred to as dormancy, and indicates that the normal meristem functions of cell division and cell growth (Chapter 1) have been suspended. However, research has revealed a more complex picture, with several classes of dormancy and multiple meristem states, all of which can have consequences for shoot architecture depending on timing, duration and location of dormant meristems. A third option, of course, is that the bud never grows, and ultimately dies or abscises.

The most widely accepted definitions of dormancy for buds (as opposed to seeds) are those of Lang (1987) who proposed three reasons for non-growth of shoot buds. *Endodormancy* is defined as the result of processes internal to the bud itself that restrict growth; *paradormancy* is due to influences on the bud from elsewhere in the plant (classic apical dominance being the most frequently cited relevant example here); and *ecodormancy* is caused by an external influence, such as low temperature, which suppresses growth in a bud that otherwise would be capable of normal growth activity. It is not presently clear whether all three forms of dormancy act on the same cellular pathways. However, the 'engine' of the meristem, namely its cell division function, requires an active cell cycle. Cells can arrest for many reasons, but usually at particular boundaries in the cycle, especially the G1–S and G2–M transitions (Chapter 1). Work by Devitt and Stafstrom (1995) and Shimizu and Mori (1998) has revealed that resumption of the cell cycle after G1 arrest in dormant meristems requires cycD3-1 activation, tied to CDK-activating kinases that operate on CDKs themselves, thus representing a phosphorylation cascade. G2–M activation on the other hand operates with a different series of regulators including cycB, CDKB and tyrosine kinases (Horvath *et al*., 2003).

The inputs into both these control points include several common plant signals including auxin, cytokinin, gibberellin and sugars (Francis and Sorrell, 2001).

4.4.1 Branch outgrowth genes

Whereas bud initiation appears to be governed largely by ontogenetic factors, the 'decision' by a plant to grow many or few branches is influenced by components of the environment, as well as by specific genetic and internal signalling processes.

In essence, intact plants branch more profusely under conditions of plenty (ample light, soil, water and nutrients) but are limited during times of resource scarcity. However, mutant-based studies since the 1990s have revealed suites of genes that regulate bud outgrowth in a specific manner. Most of these mutants were selected on the basis of an increased branching phenotype. The best studied are the *ramosus (rms1* to *rms5)* series from pea, *max1* to *max4 (more axillaries)* from *Arabidopsis* and *dad (decreased apical dominance)* from petunia [see reviews by Napoli *et al*. (1999); Beveridge (2000) and Leyser (2003)]. Unlike many other branching mutants, these lines are relatively non-pleiotropic, with near-normal flowering time and fertility, but sometimes minor alterations to leaf morphology and root development. The mutants are typically shorter than corresponding wild-type plants, but this appears to be a consequence more of the increased numbers of stems competing for available resources rather than a true dwarf character in the sense of shortened internodes (see Chapter 3).

A significant breakthrough was the discovery, first in pea and petunia and more recently confirmed in *Arabidopsis*, that several of the associated genes regulate long-distance signalling (Beveridge *et al*., 1994, 1997b; Napoli 1996; Morris *et al*., 2001; Turnbull *et al*., 2002). As discussed below, physiological studies including grafting, decapitation, hormone measurement and hormone application have collectively revealed that auxin and cytokinin are almost certainly not the only signals involved in regulation of bud outgrowth. Grafting of other mutants to wild-type rootstocks did not rescue branching back to the wild-type (inhibited) state, suggesting that these genes acted largely in the shoot.

The precise functions of these branching genes and the relationships among them are not yet entirely clear. However, several of the genes have now been cloned from *Arabidopsis* (Stirnberg *et al*., 2002; Sorefan *et al*., 2003), and some of them are known to have orthologues in pea (Sorefan *et al*., 2003; C. Rameau, personal communication). *MAX4* is a member of the carotenoid cleavage dioxygenase (CCD) family which has nine members in *Arabidopsis* (Tan *et al*., 2003), and is represented in mammals and certain bacteria. Homologues of *MAX4* (also known as *CCD8*) are present in pea (*RMS1)* and rice, and are closely related to *CCD7/MAX3* (Booker *et al*., 2004). More distant members of the CCD family in *Arabidopsis* comprise mainly a group of five genes *(CCD2, CCD3, CCD5, CCD6, CCD9)* which appear to function in one step of the ABA biosynthesis pathway (Schwartz *et al*., 2003).

It is conceivable that CCD7/MAX3 and CCD8/MAX4 proteins might also generate ABA precursors, but there is no evidence for this: *max3* and *max4* mutants,

in common with all the currently known branching mutants, do not exhibit any phenotypes characteristic of ABA deficiency, such as a tendency to wilt or reduced seed dormancy. Based on biochemical evidence, *CCD1* also appears not to have a role in ABA synthesis because it cleaves carotenoids at the 9, 10 position to yield C_{13} apocarotenoids rather than the C_{15} precursors of ABA (Schwartz *et al*., 2001). A recent breakthrough is the discovery that recombinant MAX3 and MAX4 proteins expressed in *E. coli* are able to act on a range of carotenoid substrates (Booker *et al*., 2004; Schwartz *et al*. 2004). The MAX3 protein cleaves a range of carotenoids into C_{13} and C_{27} fragments. In contrast, MAX4 protein did not cleave intact carotenoids but was able to cleave the C_{27} product, generating two further products (C_{18} and C_9). It is therefore possible that MAX3 and MAX4 act sequentially on a carotenoid cleavage pathway. However, this awaits direct demonstration in plants, as does identification of the endogenous cleavage products and evidence for their ability to influence bud outgrowth.

MAX1 encodes a putative cytochrome P450-like (CYP) protein (O. Leyser, personal communication), again suggestive of an enzymatic function. However, as with *MAX3* and *MAX4*, there are presently few clues as to potential substrates or products. *MAX2*, also isolated as *ORE9*, encodes an F-box protein (Woo *et al*., 2001; Stirnberg *et al*., 2002), one of over 600 in the *Arabidopsis* genome. Recent findings strongly suggest that pea *RMS4* is orthologous to *MAX2* (C. Rameau, personal communication). F-Box proteins have functions in ubiquitin-mediated targeted protein degradation pathways. Although we do not yet know the pathway in which MAX2 operates, this ubiquitination role is consistent with the current understanding of mechanisms within other plant hormone signalling pathways including auxin, ethylene and jasmonate (Gray *et al*., 2001; Xu *et al*., 2002; Potuschak *et al*., 2003).

Teosinte branched1 (*Tb1*) from maize is another gene with functions in regulating branch outgrowth, but also results in conversion of axillary inflorescences from female to male (Hubbard *et al*., 2002). Mutated forms of the gene exist in ancestral teosinte genotypes which display increased branch numbers compared with modern cultivated maize (Doebley *et al*., 1997). Likewise, homozygous *tb1* mutants in modern maize have many more branches than their wild-type progenitors. *Tb1* is a member of the TCP gene family which act as transcriptional regulators. Other known plant members include *Cycloidea* from *Antirrhinum*, and *TCP1* in *Arabidopsis* (Cubas *et al*., 1999). All three genes appear to participate in floral development, especially male features, but it is not clear whether dicot versions have a direct role in shoot branching. More recently, a rice homologue, *OsTB1*, was reported; this corresponds to the increased branching *fineculm1 (fc1)* mutation (Takeda *et al*., 2003). It is, therefore, possible that branching functions of these genes evolved subsequent to divergence of monocots.

4.4.2 Physiology of branching mutants

All the original studies on increased-branching mutants were physiological, and were followed only recently by the molecular experiments described above.

Encouragingly, in terms of advancing our understanding of the whole regulatory system, both routes have led to essentially the same conclusions. Here, a brief coverage is given of some of the key physiological experiments, which then leads into a section discussing models for branching control, and changes in these models in the light of genetic studies.

Several mutagenesis screens have uncovered mutants with altered branching. Because the default phenotype in wild-types of the species studied (mainly pea and *Arabidopsis*) is unbranched, most of the original mutants have increased branching. Specifically, one or more axillary meristems grow out to generate a plant with significantly greater branch numbers. The genes, therefore, appear to act to suppress branching. Much less is known about mutants with reduced branching, but, logically, the genes involved are likely to regulate pathways that promote branching.

In characterising these mutants, initial work focused on relationships with auxin and cytokinin, at the time thought to be the primary, if not the only, regulatory signals. It soon became clear that none of the *rms* were deficient in auxin in the shoot (Beveridge *et al*., 1997b; Morris *et al*., 2001); if anything, some, especially *rms2*, had increased IAA levels (Beveridge *et al*., 1994). Similarly, polar auxin transport in *rms* shoots was not impaired, and may be slightly faster than in wild types (Beveridge *et al*., 2000). Analysis of xylem sap revealed that cytokinin content was significantly lower in all *rms* mutants, by as much as 40-fold for major components such as *trans*-zeatin riboside in *rms4* (Beveridge *et al*., 1997a). In superficial terms, these findings run counter to the original Sachs–Thimann model which might predict auxin deficiency or cytokinin overproduction as a cause of the branching phenotype. A more plausible explanation is that downregulation of xylem cytokinin export is a consequence of a branching phenotype (e.g. Beveridge *et al*., 1997a), perhaps operating as a feedback mechanism. *RMS2* may have a role in this feedback process, as *rms2* is the only *rms* mutant that does not have reduced xylem cytokinin content (Beveridge, 2000). The fact that the mutants have alterations in physiology of both auxin and cytokinin suggests that they may play central roles, but in more complex ways than originally envisaged. A link between auxin and *RMS* genes was uncovered in experiments with decapitated plants which could not be prevented from branching by applying auxins to the cut stump (Beveridge *et al*., 2000).

Further advances were made by the use of simple grafting techniques which revealed that mutant shoots of *rms1, rms2* and *rms5* could be restored to normal (suppressed) branching phenotypes if grafted to wild-type rootstocks (Figure 4.6; Beveridge *et al*., 1994, 1997b; Morris *et al*., 2001). This strongly implicated the *RMS1, RMS2* and *RMS5* genes in regulation of long-distance signals that could be transmitted from root to shoot. In *Arabidopsis, max1, max3* and *max4* can be similarly restored by grafting (Turnbull *et al*., 2002; Sorefan *et al*., 2003), as can *dad1* in petunia (Napoli, 1996). In contrast, *rms3, rms4* and *max2* shoots were not rescued by grafting to wild-type rootstocks (Figure 4.6), suggesting that these genes act largely in the shoot (Beveridge *et al*., 1996; Beveridge, 2000; J. Booker, C. Turnbull, O. Leyser, unpublished). Reciprocal grafts of wild-type shoots to mutant rootstocks showed no increase in branching for any of the known mutants.

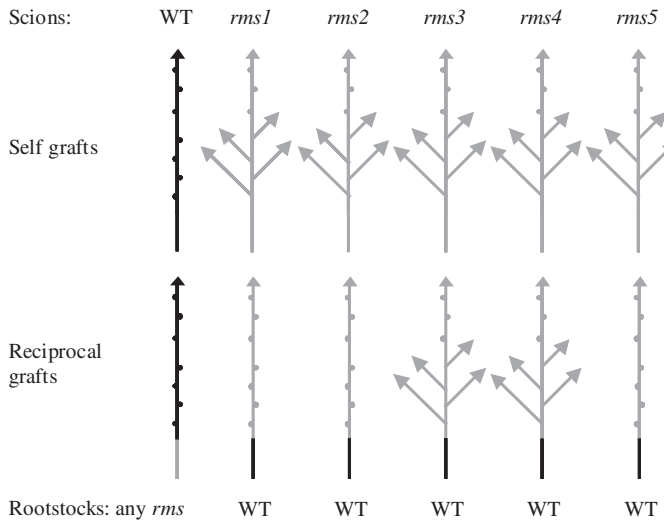

Figure 4.6 Graft-transmissible effects of *RMS* genes in pea. Lateral branches are represented as arrows, and dormant buds as small circles. Wild-type tissue is shown in black and mutant tissue in grey. The normal phenotypes of wild types (unbranched) and mutants (branched) are exhibited by self-grafted controls (top row), and effect of reciprocal grafting (WT/mutant, or mutant/WT) is shown on bottom row. For clarity, leaves are not shown, and all branched phenotypes are displayed as identical. Data are collated from Beveridge *et al*. (1994, 1996, 1997a,b) and Morris *et al*. (2001). Very similar responses occur in *max* mutants of *Arabidopsis.*

This suggests that all the genes with graft-transmissible effects can act in the shoot as well as in the root.

Work by Foo *et al*. (2001) revealed more of the characteristics of the mobile signal regulated by *RMS1*. Using more complex grafts, it was shown that a very small wild-type interstock inserted between mutant scion and rootstock was sufficient to almost completely suppress branching (Figure 4.7), similar to previous results for *dad1* in petunia (Napoli, 1996). Construction of grafts with two shoots – one wild-type and one *rms1* – resulted in very different phenotypes in each shoot despite being supported by a common *rms1* rootstock. The wild-type shoot remained unbranched, but was not able to suppress branching of the mutant shoot (Figure 4.7b). This most likely means that although wild-type tissue is able to downregulated its own branch outgrowth, the signals involved are not transmitted down that shoot and up into the mutant shoot. Therefore, there appears to be a polarity of signal movement, from root to shoot. Finally, grafting single *rms1* shoots to two rootstocks led to downregulation of branching if at least one of the rootstocks was wild-type (Figure 4.7c). From this it can be concluded that *RMS1* is involved in generating a branching inhibitor rather than the mutant rootstock producing increased amounts of a branching promoter. Putting this evidence together, there appears to be a mobile inhibitor that can move up but not down the plant.

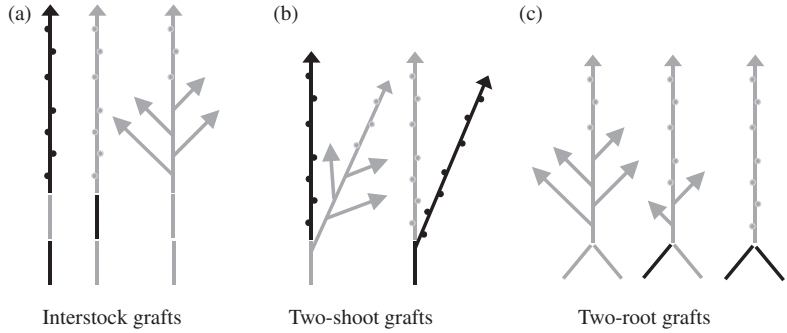

Figure 4.7 Complex grafts reveal the site of action of the *RMS1* gene in pea. Wild-type tissue is shown black and *rms1* tissue shown grey. All grafts were done at the epicotyl: interstocks are 5–10 mm epicotyl segments inserted between scion and rootstock (a), two-shoot grafts are achieved by allowing one cotyledonary axil bud to grow out from the rootstock of a single-grafted plant (b), two-root grafts are approach grafts of two complete rootstocks under a single scion. Adapted from Foo *et al*. (2001).

Auxin is a most unlikely candidate for this signal because its direction of transport is downwards, and cytokinin can probably be ruled out because it generally promotes branching when fed into the xylem stream or applied direct to lateral buds. It has, therefore, been suggested that there is a novel hormone-like substance regulated by *RMS1*, and probably by *RMS5* and *RMS2* (Beveridge, 2000; Foo *et al*., 2001; Morris *et al*., 2001).

Combining grafting with auxin and decapitation treatments showed that auxin response in *rms1* shoots could be completely restored if the rootstock was wild-type (Beveridge *et al*., 2000), and this led to the proposal that the novel inhibitor regulated by *RMS1* is essential for auxin response. In terms of the model of Sachs and Thimann (1967), this signal could represent the hypothetical second messenger for auxin action. Consistent with this, levels of *RMS1* transcript decline in pea stems following decapitation, perhaps because the auxin level also drops rapidly (Sorefan *et al*., 2003). If auxin is applied to the stem stump at concentrations sufficient to inhibit branching, it prevents this loss and even increases the transcript level. *RMS1*, therefore, appears to be an auxin-inducible gene, at least in the context of exogenous IAA application following shoot decapitation. Interestingly, auxin response of *MAX4*, the orthologue in *Arabidopsis*, is restricted largely to root tips (Sorefan *et al*., 2003), but it is not clear if there is a fundamental difference in regulatory mechanisms between pea and *Arabidopsis*.

4.4.3 Shoot branching and apical dominance models

The classic auxin–cytokinin ratio model has pervaded texts and research on control of shoot branching for decades. Indeed, auxin–cytokinin ratios are attributed much wider powers including organogenesis, xylogenesis and overall balancing of shoot

and root growth. The elegant simplicity of this concept is supported by weighty evidence, only a small proportion of which can be recounted here. It is worth mentioning in passing that very few of these studies made use of genetic tools – in particular, mutants affected in branching phenotype or in auxin and cytokinin signalling. The term 'apical dominance' is used widely to refer to the inhibitory influence exerted by the shoot tip over some or all the lateral buds below it. However, as discussed later, apical dominance is not synonymous with shoot branching control, because it is now clear that factors that restrict branching do not emanate exclusively from the shoot apex (Napoli *et al*., 1999). The almost universal plant response to shoot decapitation reveals release of apical dominance, where one or more dormant lateral buds initiate growth to replace the lost shoot. Auxin is widely considered the best candidate for this activity because (i) it is transported in a polar basipetal pattern down the stem from the shoot tip; (ii) this transport stream rapidly disappears on shoot decapitation, coinciding with onset of lateral bud outgrowth; (iii) chemical blocking of auxin polar transport with substances such as TIBA and NPA mimics the effect of decapitation; (iv) replacement of the shoot tip with a dose of auxin prevents lateral bud outgrowth.

Cytokinin, arriving from the root tip or synthesised elsewhere in the plant, is proposed to act opposite to auxin, as a stimulus for bud outgrowth. How auxin and cytokinin interact is not entirely clear. Recent evidence indicates that transcript abundance of certain members of the cytokinin biosynthesis gene family in *Arabidopsis (IPT5* and *IPT7)* is actually enhanced, rather than repressed, by auxin (Miyawaki *et al*., 2003). These genes and others *(IPT1, IPT3)* are also repressed by cytokinin itself, suggesting a feedback control system. As yet, it has not been established whether altered expression of these *IPT* genes has an impact on cytokinin content. The availability of knockout mutants may facilitate such studies. However, physiological evidence suggests that the transient increase in xylem cytokinin content in decapitated plants is suppressed if the shoot stump has been treated with auxin (Bangerth, 1994; Li *et al*., 1995). One hypothesis is that auxin enhances cytokinin degradation. This does appear to happen with a cytokinin O-xylosyl transferase (Martin *et al*., 1997) and direct studies of auxin effects on cytokinin degradation strongly point to up-regulation of cytokinin oxidase (CKX) activity (Palni *et al*., 1988), both probably operating via post-transcriptional regulation. So far, regulation by auxin has not been demonstrated at the transcriptional level for the *CKX* family (Brugière *et al*., 2003). Overexpression of *Arabidopsis CKX* genes in tobacco led to increased branching, mainly after onset of flowering. Although superficially surprising, this result may be an indirect consequence of low cytokinin on shoot apex vigour, with consequently reduced auxin export (Werner *et al*., 2001). Possibly, the apical meristem becomes limiting for cytokinin in activation of the cell cycle, and the direct consequences of cytokinin on lateral bud growth are relatively minor. Interestingly, in *Arabidopsis*, an *AtCKX5::GUS* reporter showed enhanced expression in axillary meristems, but only after shoot decapitation or commencement of lateral growth due to onset of flowering (Werner *et al*., 2003).

The complementary situation, namely cytokinin regulation of auxin levels, may also occur: plants overexpressing *AtCKX1* had modest but significant reductions in

free IAA in the whole shoot (Werner *et al*., 2003). There are, however, lines of evidence which lead to the conclusion that cytokinin, at least that supplied from the root in the xylem stream, may have little impact on shoot branching at least in intact plants. In particular, grafts of tobacco plants conditionally expressing a bacterial *IPT* cytokinin biosynthesis gene exhibited no enhancement of branching and indeed no increase in shoot cytokinin content (Faiss *et al*., 1997), whereas expression of the same gene in the shoot did increase branching and cytokinin content. The interpretation is that local, but not distant (root), cytokinin synthesis has a role in regulation of branching. A recent report suggests that auxin can downregulate endogenous *IPT* gene expression in pea stems (Tanaka *et al*., 2003), and some *Arabidopsis IPT* genes show highly specific expression patterns in shoot tissues based on analysis of *IPT::GUS* promoter–reporter fusion studies (Miyawaki *et al*., 2003). In pea, as mentioned earlier, many of the *rms* branching mutants have greatly reduced xylem cytokinin content (e.g., Beveridge *et al*., 1997a). Calculations that include sap flow estimations show that delivery (pmol per hour per gram of tissue) to the shoot is six-fold lower in the *rms4* mutant than in wild-type plants; yet, mutant shoots have virtually unaltered cytokinin content (Dodd *et al*., 2004). The capacity of shoots to synthesise cytokinins is well-known, but it is not clear whether this is normally sufficient to supply the entire cytokinin requirement within the shoot. Clearly, at least in the *rms* mutants, the shoot is able to cope with reduced cytokinin delivery, which suggests homoeostatic mechanisms operating either to up-regulate local biosynthesis, or to downregulate catabolism, or to alter cytokinin export in the phloem. Alterations in cytokinin delivery and distribution following decapitation may lead to the rapid increases in axillary buds (Mader *et al*., 2003), so there are possibly different regulatory mechanisms operating in intact and damaged plants.

4.4.4 Branching control: more than auxin and cytokinin

It is apparent that there are several gaps and inconsistencies in the auxin–cytokinin model. In particular, auxin transport studies show that auxin from the polar stream does not actually enter the buds, and that auxin levels in buds released from apical dominance often show rapidly increased rather than decreased auxin levels (Gocal *et al*., 1991). Ever since the original model proposed by Sachs and Thimann (1964, 1967), it has been suggested that some ‘second messenger’ inhibitory substance(s) must mediate between auxin and the bud tissues. As discussed earlier, cytokinins in this context are unlikely candidates because they activate buds. Other known growth inhibitors such as ABA have been proposed, but seem unlikely universal candidates. In particular, genetic evidence shows that ABA-deficient ‘wilty’ mutants do not have increased-branching phenotypes, and neither do any of the increased branching mutants discovered to date have reduced ABA levels (Morris, Turnbull and Beveridge, unpublished). From the studies of branching mutants, especially the *rms* and *max* series, it appears that at least one novel mobile inhibitory substance awaits identification. As discussed in Section 4.4.1, carotenoid cleavage products are

probably the best candidates, and compounds such as 3-hydroxy-β-ionone are known endogenous growth inhibitors in plants (Kato-Noguchi, 1992).

4.5 Environmental influences

In addition to intrinsic diversity in branching architecture due to genetic differences between and within species, several factors in the external environment have major modifying influences on timing, position and extent of shoot branching. Some of these effects are quantitative, for example, continuous variation in branch length, whereas others are qualitative, such as whether branches form at the base or top of the plant (or both or neither). What follows is a brief coverage of major factors, with discussion of interactions with specific genes and interactions with other components of development.

4.5.1 Light effects

4.5.1.1 Photoperiod

Photoperiod responses in plants are not restricted to control of flowering time. Many other developmental processes have a direct relationship with changing daylength, including tuberisation in potato, onset of bud dormancy in many temperate trees and petiole elongation in strawberry (Thomas, 1998). Extent of shoot branching, too, appears to be under photoperiod control in many species, although coincident influences on flowering often make it difficult to see branching responses in isolation. Pea is probably the best studied model in this regard, with a quantitative increase in branching, largely from basal nodes with decreasing photoperiod (Floyd and Murfet 1986; Murfet and Reid, 1993; Napoli *et al*., 1999; Beveridge *et al*., 2003). Increased basal branching is associated with delayed flowering in this long-day plant. The two processes can be interpreted as opposite strategies for resource investment – namely increased vegetative shoot structure via multiple stems with later enhanced capacity for reproductive nodes, *vs*. rapid onset of flowering at the expense of restricted vegetative investment. Each strategy requires a different principal direction of assimilate flow – essentially upwards or downwards from source leaves – but the control of this directionality remains obscure. In addition to effects on basal branching, photoperiod regulates position of branching at upper nodes of pea, usually just prior to emergence of lateral inflorescences at the next nodes. The number and length of the upper branches varies, and was originally thought to indicate a release of bud dormancy which was a prerequisite indicator of onset of flowering. However, late flowering mutants such as *gigas* and *veg* have shown that branching and flower initiation can be uncoupled. In these genotypes, outgrowth of upper branches occurs at the same nodes as in wild-type plants, but flowering does not necessarily ensue (Reid and Murfet, 1984; Beveridge and Murfet, 1996). The perhaps surprising conclusion is that photoperiod primarily controls bud release but not flowering, which instead appears to be under autonomous regulation by genes such as *GIGAS* and *VEG*.

4.5.1.2 Light intensity and spectrum: shade and neighbour responses

Under low light conditions, photoassimilate availability is inevitably less than under high light. The question here is how varying light levels impact specifically on branching, and the answer is that branching is almost always restricted under relatively lower light intensities. In addition, plants usually respond in several ways to moderate competition from neighbours of equivalent stature, and to strong competition from over-topping neighbours. These are widely known as proximity responses and shade responses, respectively. The perception of neighbours and shade is largely via phytochrome photoreceptors, but may also involve blue light sensing through cryptochromes and phototropins. However, experiments on grasses have shown that reductions in light intensity *without* changes in spectral composition ('neutral shade') cause substantial reductions in basal branching (tillering), suggesting that quantitative effects of assimilate availability may be sufficient to dictate developmental outcomes (Casal *et al*., 1986; Gautier *et al*., 1999). However, in these studies, an additional response to increased or decreased R : FR ratio was noted, giving higher or lower numbers of tillers, respectively, which indicates a significant role of phytochromes possibly independent of carbon availability. In contrast, depletion of blue light had no significant effects, which seems to rule out a major role for the blue light receptor systems.

Because neighbours and shade cause multiple coordinated developmental changes, it is not easy to describe the branching component in isolation. Other elements include alteration in leaf area, leaf thickness, leaf attitude, petiole length and stem elongation (Smith and Whitelam, 1997). How phytochrome signalling is tied to suppression or acceleration of branching is not yet clear: generally branching will be suppressed at all nodes under complete shade, but reduced only on the sides nearest the closest neighbour(s) under proximity responses. Some of the complexities of tree development are discussed in Chapter 8, including specific alterations in branch angles and branch lengths, and divergent development when a tree crown is emergent from a forest canopy but the lower branches are in deep shade. What is currently known about tree branching comes largely from observation together with some informative manipulative experiments. It is clear that highly regulated response systems controlling branching architecture exist across wide ranges of species and environments, but we do not yet understand the molecular genetic and physiological bases of the internal processes. It seems reasonable to speculate that woody plants may have evolved additional or even completely different branching control mechanisms to herbaceous species. Detailed comparative studies between plant types are presently lacking but this should be an attractive research area which can generate enormously informative data in the near future.

4.5.2 Nutrition

Not surprisingly, plants grow more slowly under nutrient-deficient conditions. The exact nature of changes in shoot biomass and architecture appears to depend on species and on which nutrients are limiting. For example, in potato, combinations

of N, P and K deficiency resulted in up to five times fewer branches, and the total leaf number on those branches was reduced by as much as eight-fold (Jenkins and Mahmood, 2003). In this study, and in a similar one on peach trees (Mediene *et al*., 2002), N deficiency had greater effects on basal than on apical branching, but with much less influence on main stem growth and first flowering node. It is not clear whether this targeted effect on branching is purely nutritional or whether some direct or indirect signalling response is involved. In roots, nitrate supply has major effects on root development, especially influencing the number and extension of lateral roots (see Chapter 7).

4.6 Conclusions and prospects

A theme introduced at the start of this chapter concerned the diversity of branching habits exhibited by different plants depending both on genotype and on environment. There is the intrinsic plasticity of branching phenotype attributable to every individual, and the extent of branching is governed by multiple input factors that lead to ontogenetic patterns and final architecture that to the observer appears patently well-suited to environmental circumstances and ecological niches. Shoot branching, in keeping with other aspects of shoot development is almost always suppressed when one or more resources, particularly light, water or mineral nutrients, are limiting.

A central challenge in attempting to devise means to manipulate branching concerns how much we know about genetic mechanisms, and whether we can predict the effects of altering genes that directly or indirectly impact on branch or lateral meristem development. At present, our ability to answer either of these issues is limited by incomplete knowledge of the genetic and regulatory networks that govern branching architecture. However, the past decade, in keeping with advances in most aspects of developmental biology, has seen extraordinary progress in this area. Major discoveries via mutational or gene isolation strategies include suites of genes implicated in lateral bud initiation and branch outgrowth. Many of the former also have roles in governing broader aspects of meristem function. In addition, we know of many genes involved in hormone signalling. There is much potential for further exploration of influences of altered regulation of cytokinin action, for example, via *IPT, CKX, AHK* and *ARR* genes, which are central to biosynthesis, degradation, perception and response, respectively. For auxin, the other hormone classically implicated in regulation of branching, there are still surprising gaps in knowledge of biosynthesis, but huge progress has been made on mechanisms of auxin polar transport and auxin signalling. The recent findings that *MAX* and *RMS* genes probably regulate one or more novel branching signals have led to the first revision of models for regulation of branching for about 30 years. Although currently far from definitive, it is clear that such models need to embrace auxin, cytokinin and these novel compounds, and must, in the future, explain their mutual interactions. Discovering new hormone-like compounds presents technical and conceptual challenges, but the rewards are potentially great if new means to regulate branching can ensue.

The broader vision might be to have a repertoire of genes driven by specific promoters that would act reliably across a wide range of species to modify branching in precisely defined ways. This may well be realised in coming years, but one cautionary comment is worth making: the benefits of altered branching, for example simple increases or decreases in branch numbers, are not easy to predict. Especially, when considering closed canopies in agricultural contexts, there is a high degree of buffering and compensation inherent at the population level, such that the net growth or yield of plants can be very stable across a wide range of planting densities and branching intensities. In other words, altering stem numbers through genetic alterations may have minimal effect on yield, despite substantial alterations in shoot architecture of individual plants.

So, in conclusion, although traditional practices such as pruning will continue to represent a cornerstone of our means to manipulate shoot architecture, it can be predicted with reasonable certainty that knowledge of genetics and signalling systems will open up exciting alternatives for stable alterations to branching patterns.

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5 Floral architecture

Regulation and diversity of floral shape and pattern

Elena M. Kramer

5.1 Introduction

'Darwin's abominable mystery' has, at this point, become a somewhat clichéd way of referring to the sudden appearance of angiosperms in the fossil record approximately 150 mya. In fact, the speed with which flowering plants diversified is as much a mystery as their origins. Over a similar timescale, mammals evolved species numbering fewer than 10 000, while angiosperms radiated into hundreds of thousands of species. Although the fundamental characteristics of angiosperm flowers are generally conserved, the enormous morphological diversity suggests a high degree of plasticity in the genetic control of floral development. Variation is observed in every aspect of floral architecture, including phyllotaxy, merosity, floral symmetry and floral organ identity. In-depth analyses of model species such as *Arabidopsis thaliana* and *Antirrhinum majus* have contributed significantly to our understanding of the genetic pathways that control these morphological components. By using this work as a foundation for comparative studies, a picture is gradually coming into focus of how alterations in floral genetic programs have contributed to the evolution of floral architecture.

The goal of this chapter is to review the current state of the field in the broad area of floral architecture. While some of the relevant genetic pathways, such as the program directing floral organ identity, are well understood, others, like the control of merosity, remain more elusive. Overall, these genetic programs appear to be strikingly conserved, but when diversification has occurred, it has been due to a number of different phenomena. These include gene duplication, shifts in gene expression pattern, independent instances of genetic co-option and functionally significant changes in protein sequence. In addition, genetic dissociability between pathways is an important factor in the evolution of complex floral morphologies. The reader is encouraged to consult the following recent reviews for additional information and alternative viewpoints: Hudson (2000), Running and Hake (2001), Zik and Irish (2003a), Ferrario *et al*. (2004), Jack (2004), Kellogg (2004) and for a complete discussion of morphological issues related to floral architecture, Endress (1994).

5.2 Phyllotaxy and merosity

The organizational pattern of primordia on the floral meristem is referred to as phyllotaxy. Two main types of phyllotaxy are observed in flowers – whorled and

spiral – although chaotic patterns are found in some taxa (Endress, 1994). The term merosity can be broadly defined as the number of organs of each type present in the flower, but it is typically used to refer to the fact that when the parts of the flower are whorled, members of the different whorls often have the same basic number. This type of organization, described as 'fixed' merosity, is particularly observed in the calyx and corolla (collectively termed the perianth), but somewhat less frequently in the androecium and gynoecium. In flowers of the basal ANITA grade, magnoliid dicots and lower eudicots (Figure 5.1), both phyllotaxy and organ number tend to be highly variable (Zanis *et al*., 2003). In contrast, among the monocots and core eudicots, whorled phyllotaxy and fixed merosity predominate (Endress, 1987). All of the most commonly studied model species exhibit this latter type of floral plan, including *Arabidopsis*, which is tetramerous (also called 4-merous), and *Antirrhinum*, which is pentamerous (Figure 5.2).

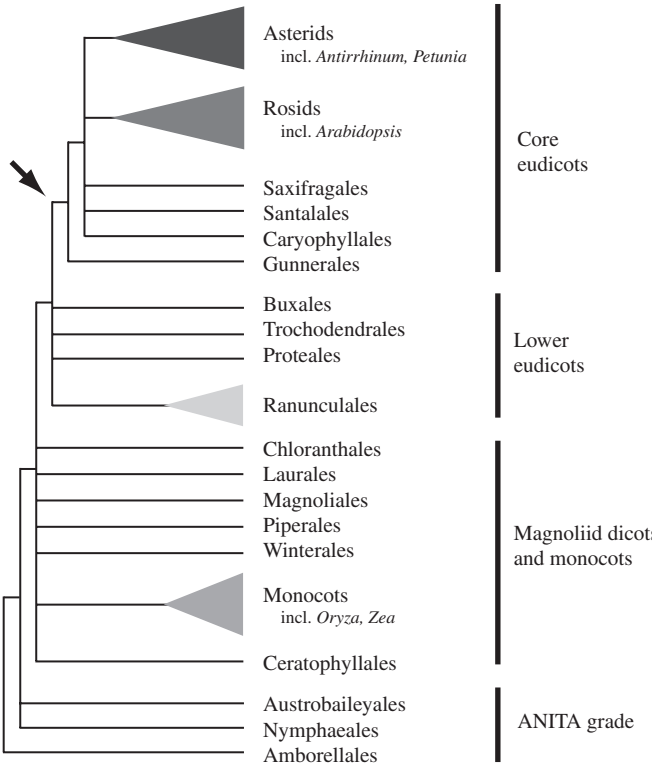

Figure 5.1 Simplified phylogeny of the angiosperms based on recent molecular analyses (Zanis *et al*., 2003). The acronym 'ANITA' is used to refer to the representatives of the basal angiosperm lineages. Arrow indicates the relative position of gene duplications in the *AP3*, *AP1* and *AG* gene lineages (see Figure 5.5 and Section 5.4.2).

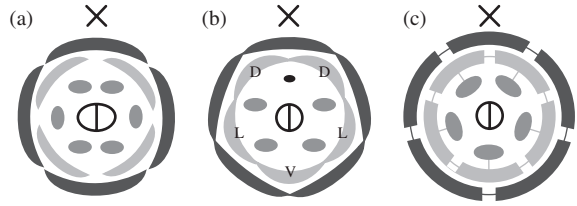

Figure 5.2 Floral diagrams indicating the arrangement and number of organs in the flowers of *Arabidopsis* (a), *Antirrhinum* (b) and *Petunia* (c). The X indicates the position of the inflorescence axis. In the case of *Antirrhinum*, morphologically distinct petal types are indicated: V = ventral; L = lateral; D = dorsal.

5.2.1 Genetic control of floral phyllotaxy

In both *Arabidopsis* and *Antirrhinum*, whorled phyllotaxy serves as a distinguishing characteristic of floral meristems relative to vegetative meristems, which typically exhibit spiral phyllotaxy. This shift in meristematic behavior is a major component of the floral meristem identity program and appears to be controlled by the combined activity of several genes. Primary among these are *LEAFY (LFY)* from *Arabidopsis* and its *Antirrhinum* ortholog *FLORICAULA (FLO)* (Table 5.1; Coen *et al*., 1990; Schultz and Haughn, 1991), representatives of an ancient lineage of unique transcription factors (Frohlich and Parker, 2000). Mutations in either gene transform meristem identity from floral to inflorescence, resulting in development of spiral rather than whorled phyllotaxy, as well as disruption of proper floral organ identity. Similar spiral phenotypes are observed in mutants of *LFY* homologs from diverse angiosperms (Hofer *et al*., 1997; Molinero-Rosales *et al*., 1999; Bomblies *et al*., 2003), suggesting a general conservation of this aspect of gene function. The other *Arabidopsis* genes known to contribute to floral meristem identity, *APETALA1 (AP1)* and *APETALA2 (AP2)*, exhibit whorled phyllotaxy as single mutants (Kunst *et al*., 1989; Irish and Sussex, 1990), but enhance the development of spiral phyllotaxy when combined with alleles of *lfy* (Huala and Sussex, 1992). This indicates that *AP1* and *AP2* also promote the establishment of whorled floral phyllotaxy, although *LFY* is the major player. In *Antirrhinum*, mutants of the *AP1* ortholog *SQUAMOSA (SQUA)* show a stronger floral to inflorescence transformation than that observed in *ap1*, including a breakdown in normal whorled phyllotaxy (Huijser *et al*., 1992), but a role in floral phyllotaxy has not yet been uncovered for the characterized *Antirrhinum AP2*-like genes, *LIP1* and *LIP2* (Keck *et al*., 2003).

The question of how the floral meristem identity program mediates the control of phyllotaxy remains unanswered. In *Arabidopsis*, the best characterized direct targets of *LFY* are the floral organ identity genes (see Table 5.1 and Section 5.4; Parcy *et al*., 1998). Of these, only *AP1* appears to contribute to phyllotaxy, reflecting its dual role in floral meristem identity (Irish and Sussex, 1990). For instance, ectopic expression of *APETALA3 (AP3)* and *PISTILLATA (PI)* can rescue aspects of the organ identity defects in *lfy*, but these genes cannot restore the whorled

Table 5.1 Important loci in genetic pathways controlling floral architecture in *Arabidopsis* and *Antirrhinum*

Genetic pathway	Arabidopsis loci	Antirrhinum loci	Gene function
Phyllotaxy and merosity	*LEAFY*	*FLORICAULA*	Establishment of floral meristem identity
	PERIANTHIA	nk	Control of primoridium number
	ETTIN	nk	
Symmetry	*TCP1**	*CYCLOIDEA*	Dorsal/ventral symmetry of axillary meristems and floral organs*
		DICHOTOMA	
	nk	*DIVARICATA*	
Floral organ identity			
A class genes	*APETALA1*	*SQUAMOSA*	Floral meristem identity, sepal and petal identity
	APETALA2	*LIP1, LIP2*	
B class genes	*APETALA3*	*DEFICIENS*	Petal and stamen identity
	PISTILLATA	*GLOBOSA*	
C class genes	*AGAMOUS*	*PLENA*	Stamen and carpel identity, floral meristem determinacy
E class genes	*SEPALATTA1*	*DEFH49*	Facilitation of ABC gene function
	SEPALATTA2		
	SEPALATTA3	*DEFH72*	

*The genetic orthology and specific function of *TCP1* are yet to be established.

organization of the floral meristem (Krizek and Meyerowitz, 1996a). Furthermore, several studies have demonstrated that phyllotaxy is dissociable from organ identity, suggesting that each is controlled by independent pathways (Hill and Lord, 1989; Bossinger and Smyth, 1996; Meyerowitz, 1997). In vegetative meristems, auxin trafficking and signaling pathways are known to play critical roles in the control of primordium position (Reinhardt *et al*., 2000, 2003; Vernoux *et al*., 2000), and there seems to be every reason to believe that similar mechanisms function in flowers. This would suggest that the floral meristem identity program controlled by *LFY/AP1/AP2* must influence aspects of the auxin signaling pathway at some level. Unfortunately, forward mutagenesis screens and identification of *LFY* targets using microarray analyses have not yet identified clear candidates for this mechanism (but see also Section 5.2.2) (Schimid *et al*., 2003; William *et al*., 2004).

5.2.2 Genetic control of merosity

Although many known mutations alter the merosity of *Arabidopsis* flowers, the majority of these affect more profound aspects of meristem size or organization

(reviewed in Running and Hake, 2001). One of the most notable exceptions to this trend is the gene *PERIANTHIA (PAN)*, which encodes a member of the bZIP family of transcription factors (Chuang *et al*., 1999). In *pan* mutant flowers, the wild type tetramerous structure is replaced by a fairly stable pentamerous pattern, with five sepals, five petals, five stamens and two carpels (Running and Meyerowitz, 1996). The floral meristem and organs otherwise appear quite normal, with the organs of consecutive whorls arising in the expected alternate positions (similar to Figure 5.2b). Unfortunately, the exact nature of *PAN* function is unclear due its broad expression domain, which seems to suggest the existence of other important cofactors, or perhaps post-transcriptional regulation (Chuang *et al*., 1999). The most likely model is that *PAN* is involved in modulating the inhibitory fields that appear to be associated with developing primordia.

Analysis of another gene, *ETTIN (ETT)*, has provided some insight into *PAN* function and the general control of merosity (Sessions *et al*., 1997). *ETT* encodes a member of the auxin response factor (ARF) family of transcription factors and is expressed in early floral meristems, petals, stamens and carpels. The floral meristems of *ett* mutants are of normal size but display alterations in merosity, with increased numbers of sepals and petals but decreased stamen number, as well as disruptions in stamen and carpel development. Interestingly, double *pan*; *ett* mutants exhibit a synergistic phenotype in which sepal and stamen numbers are severely reduced while petal primordia proliferate in the second whorl. These findings indicate that *PAN* and *ETT* function in a partially redundant manner to determine the correct number and sites of primordium initiation. Although *lfy*, *ap1* and *ap2* are largely epistatic to the *pan* and *ett* phenotypes (Running and Meyerowitz, 1996), both of the merosity genes are properly expressed in the floral meristem identity mutants (Sessions *et al*., 1997; Chuang *et al*., 1999). This suggests that proper establishment of floral meristem identity is required for *PAN* and *ETT* to function, but neither gene is a direct target of *LFY*, *AP1* or *AP2*. The finding that *ETT* mediates aspects of the auxin signaling pathway (Nemhauser *et al*., 2000) fits nicely with the known role for the hormone in controlling organ position (Reinhardt *et al*., 2003), and may represent a link between meristem identity and auxin-based regulation of primordium position. Interestingly, no single or double mutant combinations of *pan* or *ett* have altered phyllotaxy, perhaps indicating that merosity and floral phyllotaxy are controlled by separate genetic pathways which are both downstream of *LFY* and ultimately converge on aspects of auxin trafficking.

5.2.3 Evolutionary aspects of phyllotaxy and merosity

Overall, very little is known concerning the genetic basis for naturally occurring changes in phyllotaxy or merosity. Within the close phylogenetic vicinity of *Arabidopsis* and *Antirrhinum*, phyllotaxy is always whorled but considerable variation is seen in organ number, particularly in the stamens (Endress, 1992). In the Capparaceae and Cleomaceae (*sensu* Hall *et al*., 2002), which are closely related to the Brassicaceae, stamen number ranges from 1 fertile stamen to 200, with

massive ring primordia often giving rise to the highly proliferated stamens. Relatives of *Antirrhinum* also exhibit alterations in stamen number, but this is typically due to differing patterns of organ abortion as a component of floral symmetry (see Section 5.3). Of course, at deeper phylogenetic levels, great diversity is observed in both phyllotaxy and organ number. In the basal angiosperms, alternation between spiral and whorled phyllotaxy is common, even among closely related lineages (Zanis *et al*., 2003). Given that the role of *LFY* in floral meristem identity is thought to be very highly conserved (Frohlich and Parker, 2000), this suggests that there is plasticity in the genetic pathways controlling phyllotaxy that are downstream of *LFY*.

Merosity also varies radically, with organ numbers in basal lineages ranging from three, four or five, to indeterminate (Zanis *et al*., 2003). Although the previously discussed genes *PAN* and *ETT* could play roles in the generation of this variability, many other loci also present themselves as candidates. In cases of highly proliferated organ numbers, genes involved in the maintenance of meristematic activity, such as *SHOOT MERISTEMLESS (STM)* (Long *et al*., 1996), could promote the development of this condition in a manner analogous to their known function in the elaboration of certain types of compound leaves (see Chapter 2; Bharathan *et al*., 2002). Less dramatic shifts in organ number may reflect changes in the expression patterns or functions of any number of loci, including *AGAMOUS (AG), UNUSUAL FLORAL ORGANS (UFO), PETAL LOSS (PTL)* and *JAGGED (JAG)*. *AG*, an organ identity gene (Bowman *et al*., 1989), and *UFO*, an F-box containing protein (Ingram *et al*., 1995), appear to act in an antagonistic manner to control the initiation of second whorl organs (Durfee *et al*., 2003). Similarly, *PTL* promotes the proper orientation and development of second whorl primordia regardless of their identity (Griffith *et al*., 1999). Perhaps most intriguing is *JAG* – a zinc-finger containing protein that is critical to the proper outgrowth of organ primordia (Dinneny *et al*., 2004; Ohno *et al*., 2004). In *Arabidopsis*, subtending bracts do not develop in association with floral meristems, despite the fact that many molecular markers for organ initiation are expressed in the position of the presumptive bract (Dinneny *et al*., 2004). The wild type bractless condition is 'rescued' by *JAG* over-expression, suggesting that the presence of JAG can release suppression of the organs. It is possible that similar omissions of critical growth factors could be responsible for the selective abortion of single floral organs or even whole whorls. While all of the loci discussed above represent avenues for comparative study, it will be necessary to obtain a much better understanding of the genetics of phyllotaxy and merosity in model species before significant progress can be made in evolutionary studies of the phenomena.

5.3 Floral symmetry

The symmetry of a flower is fundamentally determined by the number and morphology of the floral organs. The most common types of floral symmetry are radial symmetry (actinomorphy, having many planes of symmetry) and bilateral symmetry (zygomorphy, having one plane of symmetry). The former is exemplified

by model species such as *Petunia* (Figure 5.2c), while the latter is present in *Antirrhinum* (Figure 5.2b). Although *Arabidopsis* is often treated as actinomorphic, it is more properly considered disymmetric (having two planes of symmetry) due to the positioning of its two lateral and four medial stamens (Figure 5.2a) (see Endress 1992 for a very thorough discussion of this phenomenon). Asymmetry and enantiomorphy, which are comparatively rare, are generally associated with odd patterns of orientation in the stamens or carpels. Enantiomorphy is an intriguing form of dimorphism in which individual asymmetric flowers are present in 'right-handed' and 'left-handed' forms that are mirror images of one another. All available evidence suggests that actinomorphy represents the primitive state for angiosperms, with zygomorphy and other forms of symmetry having evolved independently many times (Crane *et al*., 1995; Endress, 2001).

5.3.1 Genetic control of floral symmetry

The genetic pathway controlling zygomorphic development has been extensively studied in *Antirrhinum*, whose bilateral symmetry is primarily due to differing growth patterns within the petals and stamens (Luo *et al*., 1996). The petals can be separated into three types based on their size, shape and epidermal patterning: two dorsal, two lateral and one ventral (Figure 5.2b). While the ventral petal is internally symmetric, the dorsal and lateral petals are individually asymmetric but have mirror symmetry within each pair. This morphology imposes a bilateral symmetry on the corolla that is echoed in the stamen whorl. Here, the single dorsal stamen aborts during development to leave the lateral and ventral pairs, which further undergo torsion so that their anthers face the ventral side of the flower. The genes *CYCLOIDEA (CYC), DICHOTOMA (DICH)* and *DIVARICATA (DIV)* have been identified as major players in the generation of this morphology (Table 5.1; Luo *et al*., 1996; Almeida *et al*., 1997). *CYC* and *DICH* encode closely related paralogs of the TCP family of transcription factors (Luo *et al*., 1996, 1999; Cubas *et al*., 1999a). In *cyc; dich* double mutants, the flower becomes actinomorphic (referred to as a peloric phenotype) with all petals assuming the ventral identity and a loss of both dorsal stamen abortion and torsion in the fertile stamens (Luo *et al*., 1996). In addition, merosity is affected such that six organs arise in each of the first three whorls of the flower. Single mutants of *cyc* have a fairly strong semipeloric phenotype while *dich* plants show only a weak disruption of dorsal petal development. This indicates that although there is a degree of redundancy, *CYC* is the major contributor to dorsal meristem identity. Both genes are expressed on the dorsal side of the early floral meristem, and in the dorsal petals and staminode through later stages of development (Luo *et al*., 1996, 1999). This may reflect some degree of non-cell autonomy in *CYC/DICH* function since the phenotype of the lateral petals is also affected in the double mutant background (Luo *et al*., 1996). Overall, the genes are thought to function by regulating growth rates, in some cases positively but in others, negatively (Cubas *et al*., 1999a; Gaudin *et al*., 2000).

One particularly interesting aspect of the *cyc; dich* phenotype is that it exhibits a combinatorial interaction with organ identity. In *Antirrhinum ovulata* mutants,

where the petals are transformed into stamens, the two dorsal stamens arising in the second whorl exhibit an aborted phenotype similar to the single dorsal stamen normally present in the third whorl (Carpenter and Coen, 1990). Consistent with this, when the stamens are transformed into carpels in *deficiens* mutants of *Antirrhinum*, the dorsal carpeloid organ in the third whorl does not abort. These findings suggest that the *CYC/DICH*-mediated abortion of the dorsal stamen is dependent on the identity of the primordium rather than its position. Another curious phenomenon is the alteration of merosity in *cyc; dich*. One possible interpretation is that the genes play a direct role in controlling the meristic pattern of the dorsal side of the meristem and that in their absence this region reiterates the primordium arrangement of the ventral side. Alternatively, the phenotype may be an indirect result of an apparent overall increase in meristem size, which in turn results in the production of more primordia (Cubas, 2002).

In contrast to *cyc* and *dich, div* mutants have a semipeloric phenotype in which the ventral petal adopts the morphology of the lateral petals (Almeida *et al*., 1997). *DIV* encodes a member of the MYB family of transcription factors and shows fairly broad expression throughout the flower (Galego and Almeida, 2002). Given that the triple *cyc; dich; div* mutant exhibits a fully lateralized corolla phenotype, it appears that 'lateral' identity represents the default state for *Antirrhinum* petals (Almeida *et al*., 1997). Genetic studies support a model whereby *DIV* function establishes the identity of the ventral petal while *CYC/DICH* promote dorsal identity in the upper petals and stamen. In addition, *CYC/DICH* functions non-cell autonomously to prevent *DIV* from affecting the lateral petals, although this repressive interaction is post-transcriptional (Almeida *et al*., 1997; Galego and Almeida, 2002).

5.3.2 Evolutionary aspects of floral symmetry

Due to the evolutionary plasticity of floral symmetry, patterns of functional conservation and modification of *CYC/DICH* homologs have become a major focus of comparative genetic studies (Cubas, 2002). Through analysis of a classic peloric mutant of *Linaria* (toadflax), an ortholog of *CYC* has been shown to be critical to the production of zygomorphic flowers in this relative of *Antirrhinum* (Cubas *et al*., 1999b). The *Linaria cyc* allele is the result of heavy methylation in the promoter region of the gene, which produces a ventralized phenotype (albeit with no change in merosity). Another interesting example from a very close relative of *Antirrhinum* is the desert ghost flower *Mohavea confertiflora*. *Mohavea* flowers have a superficially radial corolla and exhibit abortion of the lateral stamens as well as the dorsal (Endress, 1998). This morphology is correlated with a restriction of *DICH* homolog expression in the petals, in addition to an expansion of the expression domain of the *CYC* and *DICH* homologs into the lateral stamen primordia (Figure 5.3) (Hileman *et al*., 2003). These findings suggest that shifts in *CYC/DICH* gene expression may have been important components in the evolution of *Mohavea*'s altered floral form. Broader studies across the predominantly zygomorphic order Lamiales (of which *Antirrhinum* is a member) indicate that *CYC* homologs are present in all taxa,

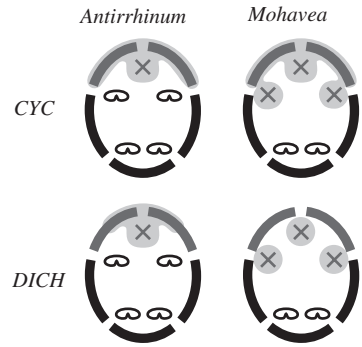

Figure 5.3 Floral diagrams of *Antirrhinum* (left) and *Mohavea* (right) indicating the extent of the *CYC* (top) and *DICH* (bottom) homolog expression domains (Hileman *et al*., 2003). Expansion of *CYC*/*DICH* expression into lateral stamens in *Mohavea* is correlated with the abortion of these organs, while the absence of *DICH* in dorsal petals correlates with a greater degree of internal symmetry in these organs.

although it is clear that several duplication events have occurred in the gene lineage (Citerne *et al*., 2000; Hileman and Baum, 2003). Interestingly, a possible mutant allele of *CYC* was found in a peloric horticultural hybrid of *Sinningia*; however, two naturally actinomorphic genera, *Ramonda* and *Conandron* of the Gesneriaceae, seem to have functional forms of the gene (Citerne *et al*., 2000). While these results generally support a conserved role for *CYC*-like genes in the production of zygomorphic flowers across the Lamiales, they also suggest that *CYC* loss-of-function models may be too simplistic to explain evolutionary reversals to actinomorphy.

Broader questions related to the evolution of *CYC* homolog function include whether the genes have been independently recruited to promote zygomorphy in distant angiosperm lineages, and how the loci might be functioning in taxa with actinomorphic flowers. The former issue is being addressed in the Asterales, which are thought to have separately evolved zygomorphic flowers (Donoghue *et al*., 1998). Normally, the capitulum inflorescence of *Senecio* produces actinomorphic disc flowers and zygomorphic ray flowers but, in some mutant forms, ray flowers are transformed into the disc form (Gillies *et al*., 2002). A possible role for *CYC*-like genes in this phenotype is currently under investigation (Gillies *et al*., 2002). Another well-known case of independently derived zygomorphy is the Fabales (legumes), which are in the Rosids (Figure 5.1). Members of the TCP gene family that appear to be closely related to *CYC* have been identified in many members of the legumes but, as yet, it is not clear whether they are involved in development of bilateral symmetry (Citerne *et al*., 2003; Fukuda *et al*., 2003). *Arabidopsis*, another member of the Rosids, is known to possess 24 TCP genes (Cubas, 2002), including the *CYC*-like gene *TCP1*, which is expressed on the dorsal side of all lateral meristems, including the disymmetric flowers (Cubas *et al*., 2001). One main difference between the expression of *TCP1* and *CYC* is that the former is only expressed at

early stages and is not maintained in the floral organs (Cubas *et al*., 2001). It is possible that this reflects a common function in establishing inherent polarity within axillary meristems, regardless of floral symmetry. Consistent with this hypothesis, *TEOSINTE BRANCHED 1 (TB1)*, a *CYC*-like gene from *Zea mays*, also regulates the growth of axillary meristems (see Chapter 4; Doebley *et al*., 1997). If *CYC*-like genes do, in fact, play deeply conserved roles in marking the dorsal side of axillary meristems and/or in regulating meristematic growth, it is quite possible that these loci have been recruited numerous times to promote zygomorphy; but, this remains to be demonstrated.

5.4 Floral organ identity

The majority of angiosperm flowers possess four types of floral organs: two outer whorls of sterile organs, the sepals and petals; and two inner whorls of fertile organs, the male stamens and female carpels, with the carpels positioned centrally. Although this organization is strictly adhered to in the major core eudicot model species (Figure 5.2), great variation is observed in other taxa, affecting both organ position and type. In terms of alteration in organ position, the most famous example is certainly *Lacandonia*, with its centrally located stamens (Vegara-Silva *et al*., 2003), but another notable case is *Eupomatia*, where the fertile stamens are external to the petaloid organs (Endress, 1993). It is also not uncommon, particularly among basal lineages, to observe morphological transformation series within a single flower (Weberling, 1989). In such instances, organ identities are not discrete but exist on a gradient from one type to another. Perhaps most striking are the examples of what appear to be novel organ identities. While these are often thought to represent modifications of preexisting organs (and hence, preexisting identity programs), they should not be underestimated in terms of their significance as evolutionary innovations. Based on our current understanding of angiosperm evolution (Figure 5.1), it appears that the ancestor of extant angiosperms possessed petaloid organs, stamens and carpels, but, subsequently, differentiation of the perianth into outer sepals and inner petals has independently evolved several times (Zanis *et al*., 2003).

5.4.1 Genetic control of floral organ identity

The elucidation of the genetic program controlling floral organ identity has been largely based on genetic and molecular studies of floral homeotic mutants in *Arabidopsis* and *Antirrhinum* (Bowman *et al*., 1989; Carpenter and Coen, 1990). This groundbreaking work led to the now classic ABC model (Figure 5.4), which holds that the overlapping domains of three classes of gene activity, referred to as A, B and C, produce a combinatorial code that determines floral organ identity in successive whorls of the developing flower (Coen and Meyerowitz, 1991). Genes in each class were identified based on their homeotic mutant phenotypes, which affect two adjacent whorls of organs. For instance, loss of B gene function results

in the transformation of petals into sepals and stamens in carpels (Bowman *et al*., 1989; Carpenter and Coen, 1990). Analyses of both mutants and over-expressing lines has demonstrated the completely homeotic nature of this developmental program (Bowman *et al*., 1991; Mizukami and Ma, 1992; Krizek and Meyerowitz, 1996a), indicating that floral primordia are initially equivalent in potential. Another critical component of the ABC program is that A and C functions are mutually exclusive (Bowman *et al*., 1991), such that elimination of C gene activity causes the A domain to expand and vice versa (Drews *et al*., 1991; Gustafson-Brown *et al*., 1994). An additional class of organ identity genes, the E class, has recently been identified as critical facilitators of ABC gene activity in the floral meristem (Figure 5.4) (Pelaz *et al*., 2000, 2001). These loci were not recovered in initial mutant screens due to their triple redundancy, but complete loss of E class function results in the transformation of all floral organs into sepals (Pelaz *et al*., 2000). The 'D' class genes, which function in ovule development (reviewed Kramer *et al*., 2004), will not be considered here.

Molecular identification of the genes corresponding to these homeotic mutants revealed that genetically homologous loci control similar aspects of floral identity in *Arabidopsis* and *Antirrhinum* (Table 5.1; reviewed in Weigel and Meyerowitz, 1994; Lohmann and Weigel, 2002). In *Arabidopsis*, the A class genes are represented by *APETALA1 (AP1)* and *APETALA2 (AP2)*, which have early roles in determination of floral meristem identity (see Section 5.2.1) but are thought to be required for sepal and petal identity as well. The B class genes, *APETALA3 (AP3)* and *PISTILLATA (PI)*, are responsible for the establishment of petal and stamen identity in the second and third whorl, respectively. *AGAMOUS (AG)* – the C class gene – is necessary for stamen and carpel identity, but is also required to specify the determinacy of the floral meristem. Finally, the three E class genes known as the *SEPALLATA*s *(SEP1–SEP3)* are necessary for proper functioning of all the previously described loci, with the exception of *AP2* (Pelaz *et al*., 2000). In *Antirrhinum*, the functions of the C class gene, *PLENA (PLE)*, and the B class genes, *DEFICIENS (DEF)* and *GLOBOSA (GLO)*, are very similar to those of their *Arabidopsis* homologs (reviewed in Irish and Kramer, 1998). Although mutant forms of *SEP*-like genes have not yet been isolated in *Antirrhinum*, their homologs appear to have similar patterns of gene

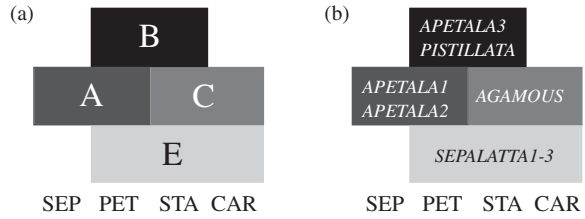

Figure 5.4 (a) Schematic representing the ABC program of floral organ identity, with addition of the E class genes as suggested by Theissen (2001). (b) *Arabidopsis* representatives for each of the four gene classes.

expression and protein interaction (see below; Davies *et al*., 1996). The *Antirrhinum* A class genes, *LIP1* and *LIP2*, do not exhibit direct functional equivalence with their *Arabidopsis* homolog, *AP2*. Although they both contribute to organ identity, they have not yet been ascribed a role in floral meristem identity (Keck *et al*., 2003). Conversely, the *Antirrhinum AP1*-like gene *SQUAMOSA (SQUA)* clearly functions as a determinant of floral meristem identity but appears to be dispensable for organ identity (Huijser *et al*., 1992).

With the exception of *AP2*, all of the organ identity genes identified to date are members of the pan-eukaryotic MADS transcription factor family (reviewed in Becker and Theissen, 2003; Messenguy and Dubois, 2003). More specifically, they are type II MADS proteins, which are characterized by a distinct 'MIKC' domain structure (Alvarez-Buylla *et al*., 2000). Our understanding of the biochemical nature of ABCE gene function is based on the distinct roles of each of these domains. DNA binding at sequence elements known as CArG boxes is controlled by the highly conserved N-terminal MADS domain (Riechmann *et al*., 1996b). This only occurs, however, when the proteins are dimerized, which is primarily mediated by the adjacent I and K domains (Riechmann *et al*., 1996a). Different dimerization preferences are observed between proteins and this appears to be important for determining functional specificity (Krizek and Meyerowitz, 1996b; Riechmann *et al*., 1996a). The AP3 and PI gene products are known to function as obligate heterodimers while AP1, AG and SEP1–3 have broader interaction potentials (Davies *et al*., 1996; Riechmann *et al*., 1996a; Honma and Goto, 2001). The current model is that AP1/SEP and AG/SEP are the critical heterodimers functioning in floral organ identity (Honma and Goto, 2001; Theissen and Saedler, 2001). These various dimer combinations are now thought to associate in larger complexes – an interaction mediated by the C terminal domain of the proteins (Egea-Cortines *et al*., 1999; Honma and Goto, 2001). This region exhibits much lower levels of overall sequence conservation (Purugganan *et al*., 1995), but has previously been shown to be essential for proper gene function (Krizek and Meyerowitz, 1996b). The C domain contains short, highly conserved motifs that are lineage specific and are implicated in transcriptional activation in some cases (Moon *et al*., 1999; Honma and Goto, 2001) and aspects of functional specificity in others (Lamb and Irish, 2003). The so-called 'quartet' model holds that tetramers, consisting of two MADS protein dimers, are responsible for the specification of organ identity in each whorl (Theissen and Saedler, 2001). For instance, in the second whorl, AP3/PI dimers would associate with AP1/SEP dimers to control petal identity. Presumably, differentiation of organ identities would result from the distinct DNA-binding specificities of each complex (Egea-Cortines *et al*., 1999). It is important to note that although this 'quartet' model is very attractive, it is currently supported by limited direct data (Jack, 2004).

In contrast to the detailed understanding of MIKC-type gene activity, we know relatively little about the specific functions of *AP2*, which is a member of the AP2/EREBP family of transcription factors (Okamuro *et al*., 1997; Riechmann and Meyerowitz, 1998). This is due in part to the complexity of the gene family and the functional redundancies that exist among its members in both *Arabidopsis* and

Antirrhinum (Elliott *et al*., 1996; Krizek *et al*., 2000; Keck *et al*., 2003). In addition, although *AP2* is known to be necessary for the repression of *AG* in the first two whorls of the flower (Drews *et al*., 1991), its expression pattern is much broader (Jofuku *et al*., 1994), suggesting post-transcriptional gene regulation. It now appears that *AP2* function is restricted to the first two whorls via translation repression by a microRNA that is expressed in whorls 3 and 4 (Chen, 2004).

Along with the difficulties in deciphering the activity of *AP2*, the general concept of A function has remained somewhat elusive. This is a result of several factors, including the dual role of these genes in separate stages of floral meristem development, and the lack of one-to-one correspondence between A class gene functions in *Arabidopsis* and *Antirrhinum* (Gutierrez-Cortines and Davies, 2000). These issues are particularly problematic for *AP1* and *SQUA*, but recent work in *Arabidopsis* may be starting to resolve the problem. Analysis of *AGL24* – a MIKC-type MADS gene involved in flowering time control (Yu *et al*., 2002; Michaels *et al*., 2003) – indicates that many aspects of the *ap1* phenotype are due to over-expression of *AGL24* (Yu *et al*., 2004). *AP1* is required in the early floral meristem to limit expression of *AGL24*, which normally promotes inflorescence meristem identity. Double *agl24; ap1* mutants show a considerably improved phenotype relative to *ap1*, including significant rescue of organ identity defects. These findings indicate that *AP1* itself may not be absolutely required for sepal or petal identity, similar to what is observed with *SQUA* in *Antirrhinum* (Huijser *et al*., 1992). One caveat to this suggestion is that any role for *AP1* in organ identity might be complemented by the presence of the *SEP* genes, which appear to have some functional equivalency with *AP1* (Honma and Goto, 2001).

5.4.2 Evolutionary aspects of floral organ identity

The detailed functional characterization of the MIKC-type organ identity genes in *Arabidopsis* and *Antirrhinum*, together with the genes' high degree of sequence conservation, have made these loci prime targets for comparative studies of floral morphology across the angiosperms (reviewed in Theissen *et al*., 2000). Putting aside the noted difficulties regarding the A class genes, most aspects of *AP3, PI, AG* and *SEP* homolog function seem to be well conserved across the core eudicots (reviewed in Becker and Theissen, 2003). In the two major grass model species, rice and maize, there also appears to be considerable conservation (Kang *et al*., 1998; Kyozuka *et al*., 2000; Kyozuka and Shimamoto, 2002), leading to the suggestion that the ABC model is applicable to all angiosperms (Ma and dePamphilis, 2000). Such analyses have tended to highlight conserved aspects of the program, but many complicating factors have also been uncovered. These include the very common occurrence of gene duplications, independent patterns of functional evolution, changes in aspects of protein biochemistry and gene regulation, and shifts in gene expression patterns. While these considerations may be dismissed as trivial in the big picture, they have actually provided rich insight into the complexities of the evolution of floral developmental programs.

5.4.2.1 Patterns of gene duplication and their functional significance

Numerous gene duplications are known to have occurred at every phylogenetic level in the various lineages of MIKC-type MADS genes (Kramer *et al*., 1998, 2003, 2004; Munster *et al*., 2002; Theissen *et al*., 2002; Becker and Theissen, 2003; Litt and Irish, 2003; Malcomber and Kellogg, 2004; Stellari *et al*., 2004). Each of these lineages is commonly referred to by the name of the best characterized homolog from *Arabidopsis* or *Antirrhinum*, such as the *AG* lineage. Given that some of the observed gene duplications are quite ancient, even within the angiosperms, it seems likely that the retained paralogs are being selectively maintained. One cluster of duplication events that has received considerable attention is a group that occurred in the *AG*, *AP1* and *AP3* lineages close to the base of the core eudicots (Figure 5.1) (Kramer *et al*., 1998, 2004; Litt and Irish, 2003). These are of particular interest because the core eudicot radiation was a critical event in flowering plant evolution, giving rise to approximately 75% of all angiosperms species (Magallon *et al*., 1999). Furthermore, following the duplications in the *AP3* and *AP1* lineage, the resultant paralogs underwent unusual patterns of sequence evolution (Figure 5.5) (Kramer and Hu, unpublished data; Litt and Irish, 2003; Vandenbussche *et al*., 2003). This process involved frameshift mutations in the *C*-terminal domain that remodeled otherwise highly conserved sequence motifs. In the *AP3* lineage, the ancestral paleoAP3 motif was retained in one of the paralogous core eudicot lineages, known as the *TM6* lineage, while in the other gene lineage a new conserved sequence was formed, the euAP3 motif (Kramer *et al*., 1998). As it turns out, *Arabidopsis* has lost its representative of the *TM6* lineage and only retains the euAP3 containing gene, which is the B class gene *AP3*. Motif-swapping experiments in *Arabidopsis* have demonstrated that the paleoAP3 and euAP3 motifs are not functionally equivalent, indicating that the sequence change does reflect a shift in biochemical function (Lamb and Irish, 2003). The model species *Petunia* has retained both *TM6* and

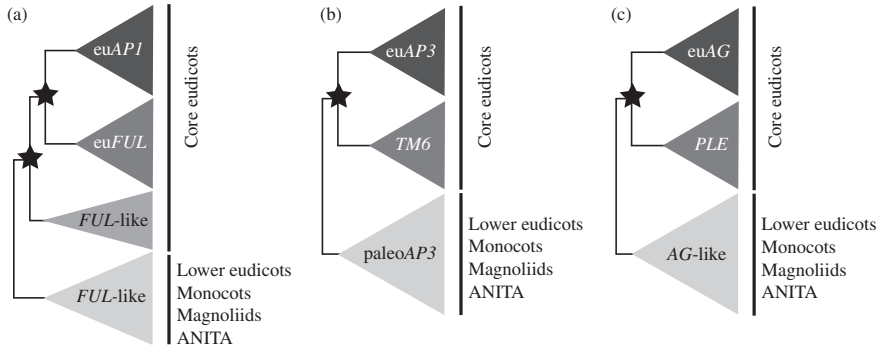

Figure 5.5 Simplified phylogenies of the *AP1* (a), *AP3* (b) and *AG* (c) gene lineages (Kramer *et al*., 1998, 2004; Litt and Irish 2003). Stars indicate gene duplication events. The angiosperm groups that have representatives of each lineage are indicated to the right of each phylogeny.

eu*AP3* orthologs, however, and genetic analysis has shown that while the eu*AP3* functions in both petal and stamen identity, the *TM6* ortholog has been restricted to a function in stamen identity (Vandenbussche *et al*., 2004).

The functional significance of the *AP1* duplication, which produced the eu*AP1* and eu*FUL* paralogs from the ancestral *FUL*-like lineage (Figure 5.5), is equally unclear (Litt and Irish, 2003). The *Arabidopsis* representatives of these lineages, *AP1* and *FRUITFULL (FUL)*, exhibit partially overlapping functional repertoires: both genes contribute to floral meristem identity (Ferrandiz *et al*., 2000), while *AP1* plays a specific role in sepal and petal identity (Bowman *et al*., 1993) and *FUL* is required for proper fruit formation (Gu *et al*., 1998). As mentioned above, simple functional conservation is not observed between *AP1* and its *Antirrhinum* ortholog *SQUA*, and a function has not yet been assigned to the *Antirrhinum AmFUL* locus (Litt and Irish, 2003). The *AP1* lineage has an even more complex evolutionary history than that of *AP3*, however, and many eudicots actually possess a third paralogous *FUL*-like lineage (Litt and Irish, 2003) (Figure 5.5). An *Antirrhinum* ortholog of this lineage, *DEFH28*, has been functionally characterized and this gene plays a very similar role as *FUL* in *Arabidopsis*, controlling floral meristem identity and fruit development (Müller *et al*., 2001). This may suggest that the ancestral function of *FUL*-like genes is more similar to this set of roles, and that aspects of eu*AP1* function are more recent acquisitions (Litt and Irish, 2003). One tantalizing possibility is that the changes in eu*AP3* and eu*AP1* represent coevolutionary processes (Litt and Irish, 2003; Vandenbussche *et al*., 2003). In contrast to the rather dramatic changes observed in eu*AP3* and eu*AP1*, the *AG* duplication is not associated with major modification of gene sequence, and the paralogs, known as eu*AG* and *PLE*, seem to have been primarily retained due to subdivision of the ancestral functional repertoire (Kramer *et al*., 2004).

Independent gene duplications affecting aspects of gene function are also known in the Solanaceae (core eudicots) and Ranunculaceae (lower eudicots, Ranunculids) (Figure 5.1). In the former case, a relatively recent duplication has given rise to two *PI* paralogs in *Petunia* (van der Krol *et al*., 1993). Protein interaction studies indicate these two genes have become specialized to favor dimerization interactions with either the eu*AP3* or *TM6* ortholog (Vandenbussche *et al*., 2004). Since the *PI* duplication occurred much later that the eu*AP3*/*TM6* event (Kramer *et al*., 1998), this represents a recent coevolutionary process. Similarly, in the Ranunculaceae, two ancient *AP3* duplications that predate the diversification of the family have been followed by a spate of later independent events in the *PI* lineage (Kramer *et al*., 2003). Given that the *AP3* paralogs appear to have undergone both sub- and neofunctionalization (Kramer and Jaramillo, unpublished data; Kramer *et al*., 2003), the more recent *PI* duplications may reflect repeated biochemical specialization to promote specific paralog interactions.

On the whole, these complex patterns of gene duplication have raised numerous caveats to the generally conserved functional repertoires that are observed across the various ABCE gene lineages. Independent instances of subfunctionalization have produced situations where functional homologs are not direct genetic

orthologs (Kramer *et al*., 2004). In addition, duplication events may provide new genetic material, in turn allowing the elaboration of existing identity programs or the evolution of unique gene combinations to yield novel organ identity programs (Kramer and Jaramillo, unpublished data; Kramer *et al*., 2003; Malcomber and Kellogg, 2004). There even appears to be a case where major aspects of gene function have shifted between nonhomologous genes. This relates to the relative roles of *AG* and *CRABS CLAW (CRC)* in establishing carpel identity. In *Arabidopsis, AG* is the major determinant of carpel identity but *CRC* – a member of the YABBY gene family – also contributes (Alvarez and Smyth, 1999; Bowman and Smyth, 1999). However, in rice, a *CRC* homolog known as *DROOPING LEAF (DL)* appears to be the more critical player (Nagasawa *et al*., 2003; Yamaguchi *et al*., 2004). Consistent with this, mutations in *AG*-like genes from both *Zea* and *Oryza* do not exhibit transformations of carpel identity, although this may also be due to redundancy among *AG* paralogs (Mena *et al*., 1996; Kang *et al*., 1998).

5.4.2.2 Patterns of gene expression and their morphological significance

Another aspect of evolution of organ identity that has received considerable attention is the correlation between shifts in gene expression pattern and changes in floral morphology. Largely through comparative gene expression studies, many researchers have sought to establish the degree of conservation of the ABC program while also testing the 'sliding boundary' hypothesis (*sensu* Kramer *et al*., 2003). This hypothesis suggests that significant changes in floral morphology may, in fact, be due to very simple shifts in the expression domains of the homeotic organ identity programs (Bowman, 1997; Albert *et al*., 1998). A key aspect of this idea is that the ABC program is very deeply conserved, which seems likely to be true for the stamen and carpel identity programs, but is somewhat more complicated for the sepal and petal pathways. In addition to the complications presented by the eu*AP3/TM6* and eu*AP1*/eu*FUL* duplications, there also seem to be conflicting data from studies outside the core eudicots (Kramer and Irish, 2000; Kramer and Jaramillo, 2004).

Due in part to the inherent difficulties of analyzing A class function (see above), the majority of these investigations have focused on the B gene lineages represented by *AP3* and *PI*. Determination of gene expression patterns across a diversity of angiosperms has found that homologs of *AP3* and *PI* are almost always expressed in petaloid organs (Kramer and Irish, 1999, 2000; Tzeng and Yang, 2001; Kanno *et al*., 2003; Kramer *et al*., 2003; Park *et al*., 2003). Furthermore, functional studies in *Oryza* of *SUPERWOMAN1 (SPW1)* – a paleo*AP3* ortholog – and the *PI* homologs *OsMADS2* and *OsMADS4* indicate that the genes are necessary for lodicule and stamen identity (Kang *et al*., 1998; Nagasawa *et al*., 2003). Similar results have been obtained from genetic analysis of the *Zea* paleo*AP3* gene *SILKY* (Ambrose *et al*., 2000). Since one interpretation of lodicule evolution is that the novel organs represent modified petals (Dahlgren and Clifford, 1982; Clifford, 1987) and given the ancient divergence of core eudicots and grasses (Figure 5.1),

these findings are taken as evidence that the role of *AP3* and *PI* homologs in petal identity is deeply conserved (Ma and dePamphilis, 2000; Ng and Yanofsky, 2001).

This idea of a conserved petal identity program is at odds, however, with traditional botanical theories regarding the evolution of the petal, which have long held that the perianth organs were derived independently many times (reviewed in Endress, 1994). Potentially in support of this notion, the expression patterns of paleo*AP3* and *PI* homologs exhibit a surprising degree of temporal and spatial variability, despite the fact that they are generally expressed in petaloid organs (Kramer and Irish, 1999, 2000). This is significant because in the core eudicots, constant expression of eu*AP3* and *PI* is necessary throughout the developing petal in order to maintain petal identity (Bowman *et al*., 1989; Zachgo *et al*., 1995; Jenik and Irish, 2001). Biochemical interactions of the B gene homologs are also known to have changed over time because PI-like proteins can bind DNA as homodimers in the monocots *Lilium* and *Tulipa*, which is never observed in the core eudicots (Winter *et al*., 2002; Kanno *et al*., 2003). Additionally, in some taxa that seem to be good candidates for the sliding boundary hypothesis, a simple shift in the organ identity program is not observed (Kramer and Jaramillo, 2004). For example, in the monocots *Lilium* and *Asparagus*, gene and/or protein expression differs between the first and second whorl organs, which are both petaloid (Tzeng and Yang, 2001; Park *et al*., 2003). Most notably, in *Asparagus*, no paleo*AP3* or *PI* transcripts are detected in the petaloid first whorl organs at all (Park *et al*., 2003, 2004). Similarly, paleo*AP3* and *PI* homologs of the magnoliid *Aristolochia* are not expressed in the petaloid regions of the modified calyx (Jaramillo and Kramer, 2004). In the Ranunculid *Aquilegia*, it appears that paleo*AP3* and *PI* loci may contribute to the petaloidy of the first whorl organs, but they are not expressed at early stages of sepal development (Kramer and Jaramillo, unpublished data; Kramer *et al*., 2003).

One interpretation of these complex findings is that B gene homologs have been repeatedly recruited to promote the development of independently derived petaloid organs (Kramer and Irish, 1999). Alternatively, processes related to developmental system drift (True and Haag, 2001), particularly gene duplication, may have caused diversification in a commonly inherited genetic program (Kramer and Jaramillo, 2004). In this regard, it has been suggested that the eu*AP3/TM6* duplication was correlated with fairly significant changes in both biochemical and developmental aspects of *AP3* homolog function (Lamb and Irish, 2003; Vandenbussche *et al*., 2004). Furthermore, it appears that the functions of paleo*AP3* and *PI* homologs outside the core eudicots are often dissociable into distinct early and late phases (Kramer and Irish, 1999, 2000; Jaramillo and Kramer, 2004). While the former stage is consistent with a role in organ identity, the latter seems to be correlated with late aspects of organ/tissue differentiation. Extreme cases of this phenomenon are observed in taxa with particularly complex petaloid organs, such as *Aquilegia, Aristolochia* and *Asimina* (Kramer, Stellari, and Jaramillo, unpublished data; Jaramillo and Kramer, 2004). Another issue is the general simplicity of the sliding boundary hypothesis. Although botanists often consider the perianth of species such as *Lilium* to be 'undifferentiated', each whorl, in fact, exhibits distinct developmental patterns and even

final morphology. It is perhaps not surprising then to find that while B gene homologs may contribute to the petaloidy of the first whorl organs, a simple translocation of the second whorl identity program is not indicated. Similarly, the many instances of novel forms of petaloidy in a diverse array of organ types (floral and non-floral) may involve the redeployment of B gene homologs, but they are also likely to include other modifications ranging from subfunctionalization, to temporal and spatial restriction of expression, to new interactions with other genetic pathways.

In summary, comparative studies of the organ identity program across diverse angiosperms have revealed a complex pattern of conservation and divergence. It seems likely that a program relatively similar to that first described in *Arabidopsis* and *Antirrhinum* was functioning in the ancestor of extant flowering plants. However, considerable developmental system drift has occurred over time, giving rise to new gene combinations and novel forms of floral organs. Teasing apart the complex interactions among gene duplication, functional evolution and shifts in gene expression will require considerably more research, but promises to be very illuminating.

5.5 Elaboration of organ identity

Given the great detail in which organ identity genes have been studied, surprisingly little is known about the downstream genetic pathways responsible for the elaboration of organ development. Evidence from microarray-based studies in *Arabidopsis* indicates that AP3/PI act relatively directly on the genes involved with petal and stamen morphogenesis (Zik and Irish, 2003b). Since the morphology of these organs is highly variable, even within the core eudicots where AP3/PI function is thought to be conserved, this would seem to suggest a high degree of plasticity in the *cis*-regulatory regions of the downstream loci. One possible target for the B class genes that has been identified in *Antirrhinum* is the MYB family gene *MIXTA*, which is necessary for the development of the characteristic conical epidermal cells found in petals (Glover *et al*., 1998; Martin *et al*., 2002). Other genes known to control aspects of later organ differentiation include *RABBIT EARS (RBE)* (Takeda *et al*., 2004) and *FRILL 1 (FRL1)* (Hase *et al*., 2000), but how all these loci interact to produce a given organ morphology remains unknown. Another important factor is likely to be parallel genetic pathways, such as the determinants of abaxial/adaxial polarity within lateral organs (see Chapter 2), which undoubtedly also contribute to floral organ development.

The one organ that has received a considerable amount of attention in terms of morphogenesis is the *Arabidopsis* carpel (reviewed Ferrandiz *et al*., 1999). This is largely due to the complexity of the carpel, which is composed of numerous distinct cell types in addition to being of great economic importance. The carpels of *Arabidopsis* develop into a dry, dehiscent form of fruit known as a silique. The genes *CRC* and *SPATULA (SPT)* were identified as important to the establishment of carpel identity in combination with *AG* (Alvarez and Smyth, 1999). Loci that are

required for aspects of apical–basal differentiation in the gynoecium include *ETT* (Sessions and Zambryski, 1995), *PINOID (PID)* (Bennett *et al*., 1995) and *TOUSLED (TSL)* (Roe *et al*., 1997). A diverse array of genes has also been characterized as necessary for the specification of carpel tissue types, particularly the ovary wall, or valve, and the dehiscence zone. These include the *SHATTERPROOF* loci *(SHP1* and *2)* (Liljegren *et al*., 2000), *FUL* (Gu *et al*., 1998), *ALCATRAZ (ALC)* (Rajani and Sundaresan, 2001), *INDEHISCENT (IND)* (Liljegren *et al*., 2004) and *REPLUMLESS (RPL)* (Roeder *et al*., 2003). Due to the fact that carpel/fruit morphology is among the most plastic aspects of floral architecture (Endress, 1994), future comparative studies of any of the above-mentioned loci are likely to be very informative.

Other aspects of organ morphology that remain largely unexplored include diverse phenomena such as organ fusion, petal spurs and nectaries. Congenital and postgenital fusion between organs of the same whorl or separate whorls is one of the most important sources of variation in floral architecture, particularly in the large core eudicot group (Endress, 1990). Our current knowledge of the mediation of floral organ fusion is primarily drawn from studies of organ identity mutants, where it has been shown that attenuation of identity often results in loss of fusion or improper fusion patterns (van der Krol and Chua, 1993; Alvarez and Smyth, 1999; Vandenbussche *et al*., 2004). This suggests that the genetic control of organ fusion is downstream of organ identity, perhaps an integral component of the organogenesis program. Other loci that could be involved with fusion include the *CUP-SHAPED COTYLEDON (CUC)* genes, which are required for separation of organ primordia (Aida *et al*., 1997), and the gene *FIDDLEHEAD (FDH)*, which appears to control epidermal adhesion between organs (Lolle *et al*., 1992). The genetic control of spur formation is almost completely unexplored. Some evidence from *Antirrhinum* suggests that expression of KNOX homeodomain genes in the petal may give rise to spur formation (Golz *et al*., 2002), but this remains to be tested in a taxon with naturally occurring spurs. Nectaries are typically associated with spurs, but they also can be borne on other organs or develop as independent structures. The nectaries of *Arabidopsis* are dependent on the activity of *CRC* for their proper development (Baum *et al*., 2001). Evolutionary, all of these architectural elements are thought to have evolved many times independently (Brown, 1938; Endress, 1990; Hodges, 1997). Moreover, they are quite labile in terms of position, suggesting that their genetic control can be dissociated from specific organ identity programs. It remains to be determined whether similar genetic mechanisms are functioning in these structures across the angiosperms or if distinct pathways have been recruited in conjunction with separate evolutionary events.

5.6 Sex determination as a modification of floral architecture

Although the primitive condition in angiosperms is thought to be hermaphroditism, diverse forms of sex determination have evolved independently many times

(Westergaard, 1958). Most dioecious or monoecious plants go through a hermaphroditic stage early in flower development, followed by differential abortion or arrest of sex organs (Ainsworth, 2000). Examples of this type of unisexual flower development include the most commonly studied dioecious plants, such as *Silene* and *Rumex*, as well as monoecious species such as maize and cucumber. On the other hand, some dioecious plants like *Spinacia oleracea* (spinach), *Mercurialis annua* (mercury) and *Cannabis sativa* (hemp) have unisexual flowers with no vestiges of organs of the opposite sex. The genetic pathways underlying sex determination have been a subject of study since the time of Darwin, but still remain somewhat elusive. From the standpoint of floral architecture, analyses of various model species have revealed divergent systems controlling organ abortion. In cucumber, for instance, developmental arrest appears to be largely position dependent (Kater *et al*., 2001), while in maize, abortion relies on both identity and positional cues (Ambrose *et al*., 2000). A possible role for the organ identity genes has been investigated in several taxa (reviewed in Ainsworth, 2000), but has not been found to be of great significance. This is not especially surprising, however, since in all of these cases, sex determination occurs after organ identity is established. It remains to be seen whether developmental programs that produce flowers which are entirely male or female from inception act upstream of organ identity (Di Stilio *et al*., in preparation).

5.7 Future perspectives

The emerging picture of the genetic control of floral architecture presents many complex, interacting pathways. It is striking, however, to see the degree of dissociability that exists among these genetic programs. Organ identity appears to be superimposed on an independently controlled pattern of floral primordia, whose development is then influenced by the additive effects of the identity program as well as other pathways such as meristem and organ symmetry. The end product of floral development, therefore, represents the compounding effect of these many genetic pathways: a whole which is greater than the sum of its parts. Genetic dissociability of this type has been suggested to allow for greater 'evolvability' (Wagner and Altenberg, 1996) and may have played a critical role in allowing the diversification of floral morphology.

These genetic findings have significant relevance to the study of floral evolution because they suggest that many aspects of floral morphology that have previously been treated as unified syndromes should perhaps be considered as independent characters. The evolution of petaloidy is the most notable example, where conclusions regarding the homology of petaloid organs have often been made by combining issues such as phyllotaxy, developmental kinetics, vascular patterning and phenotype (Albert *et al*., 1998; Baum and Whitlock, 1999). We have now come to understand that positional homology is often dissociable from the expression of a homologous organ identity program. It has also become clear that our elegant models for morphological evolution, such as the 'sliding boundary' hypothesis or the

concept that *CYC* loss-of-function would be responsible for reversals to actinomorphy, may be overly simplified.

Even after 15 years of highly productive research, many fundamental questions regarding the genetic control of floral architecture remain to be answered. These include: how are phyllotaxy and merosity controlled by the floral meristem identity program? How do the organ identity genes manage morphogenesis? How are interactions among the diverse array of floral genetic pathways mediated? Beyond these issues, the field of floral developmental evolution remains largely unexplored. The enormous potential of genomic approaches, as well as the development of new model species, is likely to greatly advance this field of research in the near future. Through this combination of techniques, we will hopefully come to eventually resolve the great mystery of angiosperm diversification.

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6 Inflorescence architecture

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Inflorescence architecture plays an important role in the reproductive success of flowering plants. First, through its influence on pollination, and subsequently as the axis bearing fruits and seeds, it is important for seed set and dispersal. The term 'inflorescence' was introduced by Linnaeus [in Parkin (1914)], and essentially the inflorescence represents a modified shoot system which functions to bear flowers (Troll, 1964). The inflorescence, like other structures on the plant shoot, develops after germination. Shoot development in a seedling is centred on the activities of the shoot apical meristem (SAM), which produces leaves, or shoots/inflorescences, or flowers on its periphery. The SAM and its activities are central to the development and organisation of flowers on the inflorescence axis, and influence the variation in inflorescence architecture seen amongst plants. Kellogg (2000) has proposed a generalised model for inflorescence development which states that meristems proceed to bear either meristems or determinate flowers on their flanks, and that each subsequent meristem reiterates the same decision to either develop a meristem or a determinate flower on its flanks. In this manner, the meristem and its activities are likely to play a central role in the generation of varied inflorescence architecture of plants.

The different morphologies of inflorescences found in plants have been used to classify them into different types, for example, raceme, spike, etc; most of these describe the mature inflorescence structure of a plant, which is the end result of a specific developmental programme and growth. In *Arabidopsis* and *Antirrhinum*, the inflorescence has a single main axis, whose apex remains meristematic and is indeterminate, but which generates numerous floral meristems (FMs) on its flanks. While plants like tulip have a determinate inflorescence and their entire inflorescence apex is transformed into a single terminal flower, solanaceous plants like petunia and tomato have a cymose type of branching inflorescence whose apex is transformed into a terminal flower, and on which additional inflorescences grow from a new meristem that arises from the axil of the flower. The principal classes of inflorescence structures and the molecular and genetic basis of inflorescence architecture in model and crop plants are discussed here; for a comprehensive analysis of the morphology and the types of inflorescences see Weberling (1989).

6.1 Determinate and indeterminate inflorescence types

Inflorescences can be divided into two primary forms: determinate and indeterminate. In determinate inflorescences, the main shoot stops producing additional lateral

branches with flowers or bracts, and the entire inflorescence apex ends in a terminal flower, whereas, in inflorescences of the indeterminate type, the inflorescence apex continues to grow and produces either flowers or inflorescences on its flanks, until it eventually declines in activity. It is believed that the indeterminate form of inflorescence architecture seen in many flowering plants was independently derived from determinate inflorescence structures several times during evolution (Stebbins, 1974). *CENTRORADIALIS (CEN)* of *Antirrhinum* (Bradley *et al*., 1996) and *TERMINAL FLOWER1 (TFL1)* in *Arabidopsis* (Shannon and Meeks-Wagner, 1993) encode orthologous genes that are important for the maintenance of indeterminate inflorescence growth (Bradley *et al*., 1997). Mutations in *cen/tfl1* convert the indeterminate inflorescences of these plants to determinate inflorescences bearing a terminal flower (Shannon and Meeks-Wagner, 1991; Bradley *et al*., 1996). *CEN* and *TFL1* shows homology to a class of phosphatidyl-ethanolamine-binding proteins also referred to as Raf1-Kinase-inhibitor proteins (RKIP). In order to understand the mechanisms used by indeterminate species to avoid the development of a terminal flower, *CEN/TFL1* homologues have been isolated from different plants like pea, tobacco, tomato, ryegrass and petunia (Kato *et al*., 1998; Pnueli *et al*., 1998; Amaya *et al*., 1999; Jensen *et al*., 2001; Nakagawa *et al*., 2002; Foucher *et al*., 2003). Subsequently, these *CEN/TFL1* homologues have been used as tools to test if they too control indeterminate inflorescence architecture in different plants. This has been the case in pea, where the *CEN/TFL1* homologue, *PsTFL1a*, corresponds to *DETERMINATE (DET)*, required for maintaining the indeterminate state of the primary inflorescence (Foucher *et al*., 2003). In contrast, the tomato *CEN/TFL1* homologue, *SELF PRUNING (SP)*, has a function distinct to that of *CEN/TFL1* as it controls the determinacy of sympodial meristems and has no effect on inflorescence architecture in tomato (Pnueli *et al*., 1998).

6.2 Simple and compound inflorescences

In addition to the indeterminate or determinate nature of growth, inflorescences can be further classified into simple or compound types depending on the extent of branching of the flowering shoot. The definitions outlined here are useful to categorise inflorescences into different types, but, occasionally, a rigid definition may not accommodate the varied inflorescence architecture found in nature.

6.2.1 Simple inflorescences

Troll defined a simple inflorescence as one in which branching does not extend beyond the first order (Troll, 1964). Simple inflorescences can be further divided into several different types as raceme, spike, umbel, spadix or capitulum, depending on whether individual flowers are borne on stalks or are sessile, and if the main axis of the inflorescence is extended or compact (Figure 6.1). These main classes

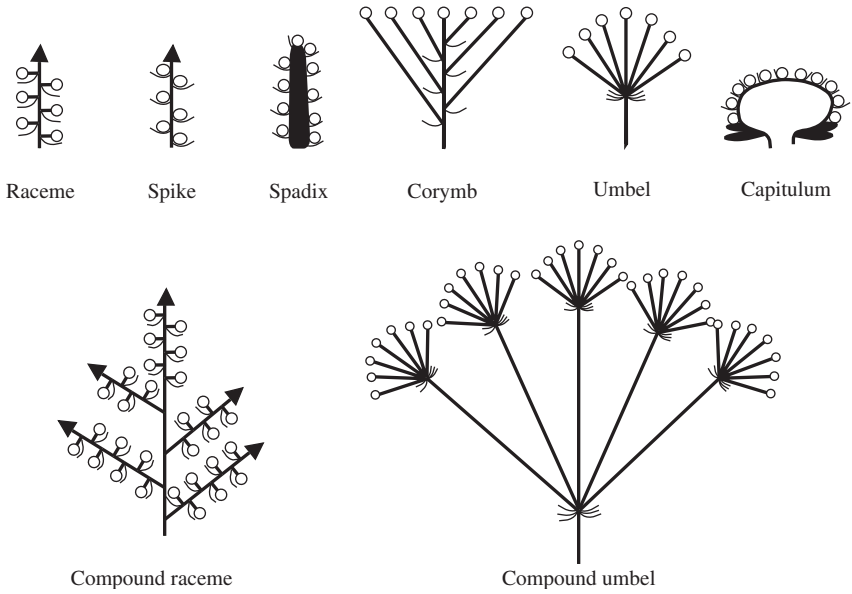

Figure 6.1 Schematic representation of different inflorescence types. A raceme with an indeterminate apex and bearing flowers with pedicels and bracts is shown, along with a spike with sessile flowers, a spadix with a thick rachis, a corymb, an umbel and a capitulum. A compound raceme and a compound umbel (shown as a double umbel with five partial umbels) are shown as representations of compound inflorescence forms. Flowers are depicted as circles, and bracts as curved lines; the apical meristem is shown as a triangle.

are summarised here and are based on a comprehensive analysis of inflorescence types discussed by Weberling (1989) and Rickett (1955).

- Raceme is derived from the Latin term *racemus*, meaning a bunch of grapes or similar fruits. A raceme has distinct developed internodes on its main axis – the rachis, and all the flowers borne on the rachis have stalks, that is, they are pedicellate flowers. The rachis has an indeterminate apex, and, consequently, lacks a terminal flower. The flowers borne on a raceme open in the order in which they are initiated, that is, those from the base first, and proceeding upwards along the main axis; the *Arabidopsis* inflorescence is an example of a raceme.
- Spike is derived from the Latin *spica*, which means a point; the main axis of a spike has distinct internodes, is indeterminate and can be distinguished from a raceme by the sessile flowers it bears in the axils of bracts; wheat has an inflorescence that is classified as a spike.
- Umbel has its roots in the Latin word *umbella*, which means a little shade, hence a little parasol or umbrella; an umbel is typified by its extensively compressed rachis, which distinguishes it from a raceme. In an umbel, the

pedicels of flowers are long and radiate from a central point, subtended by a cluster of bracts; the *Daucus carota* inflorescence is a good example of an umbel structure.

- Corymb is originally derived from the Greek κορυμβοζ through the Latin corymbus – a cluster of ivy berries or of other berries. A corymb is essentially a raceme in which all flowers are more or less at the same level due to the proportional elongation of pedicels.
- Spadix is another Greek term derived from two words suggesting a torn-off branch, especially a palm frond. A spadix is essentialy similar to a spike, but it has a thickened fleshy axis, stalkless flowers and often there is a large bract subtending the entire structure – the spathe. Members of the Araceae display this inflorescence type.
- Capitulum is a distinctive inflorescence type, from the Latin *caput*, meaning head. A capitulum or a head type of inflorescence is a highly condensed inflorescence, which bears sessile flowers or florets on a receptacle; the receptacle can be wide flattened or elongated, with a convex or concave curvature. In the daisy type of capitula, the rim of the receptacle bears two rows of outer ray florets which encircle numerous tubular disc florets. This type of inflorescence is most widespread in the Asteraceae, represented by sunflower, but variants of the type can also also be found in several other families of the Asteridae and Monocotyledonae (Harris, 1999).

The simple inflorescence types outlined here need not be exclusively indeterminate, but can also be as determinate forms; they provide a general outline and further details of these and more elaborate inflorescence forms are found elsewhere (Weberling, 1989; Harris, 1999; Singer *et al*., 1999; Tucker and Grimes, 1999).

6.2.2 Compound inflorescences

The structure of compound inflorescences is more complex, and this type of inflorescence arises when individual flowers are replaced by an inflorescence with the same branching pattern, for example, if a raceme or umbel is repeated in the branch, they give rise to double racemes and double umbels, respectively; the panicle of rice or sorghum is an example of a compound raceme. Reiteration of this branching pattern on the secondary branches can generate even more complex compound inflorescence structures with even more extensive triple branching character.

6.3 Growth and branching patterns of shoots

In addition to these distinctions in inflorescence types, shoot growth can be further divided into a monopodial or sympodial pattern (Figure 6.2). Plants exhibiting monopodial growth have a main axis that develops through the activities of a single apical meristem; *Arabidopsis*, maize and *Antirrhinum* shoots show

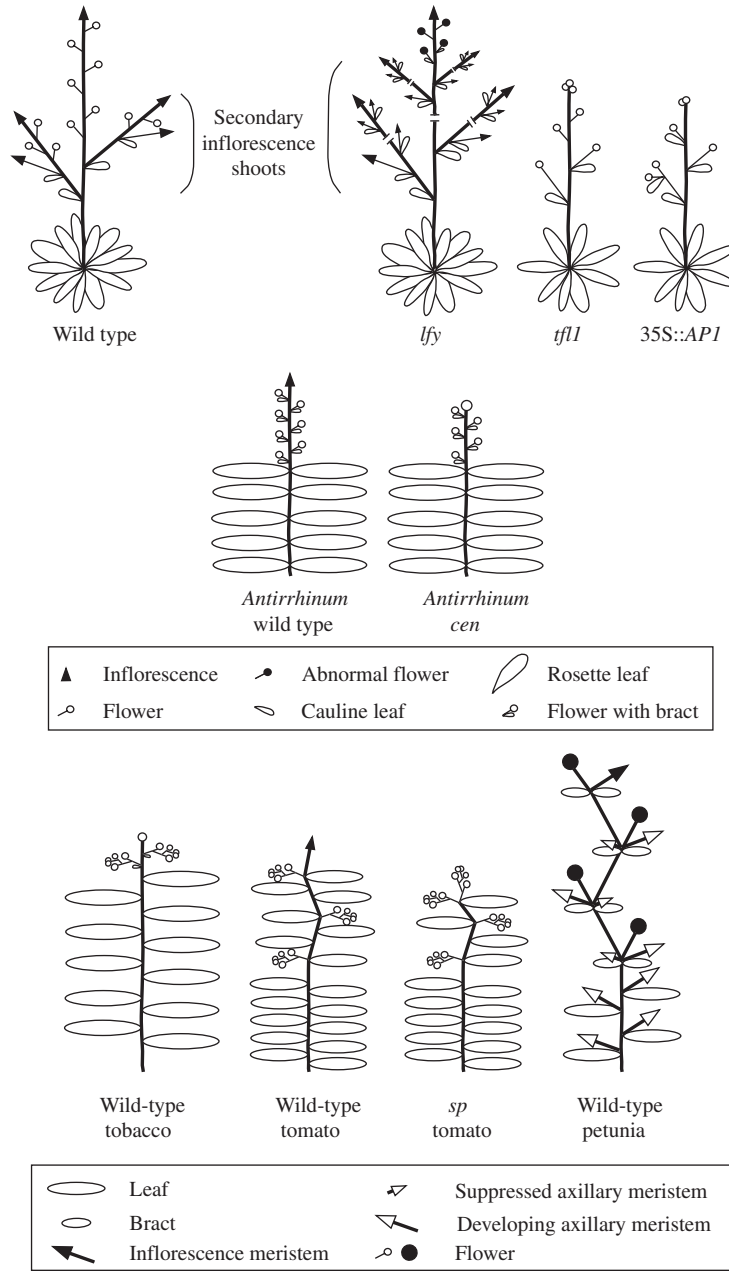

Figure 6.2 A schematic representation of plants with indeterminate and determinate inflorescences. A wild-type *Arabidopsis* inflorescence initially bears secondary inflorescences subtended by cauline leaves, before it initiates the development of flowers on its

monopodial growth. In plants that show sympodial growth, the main axis develops through the activity of a succession of apical meristems, like petunia and tomato. Each sympodial shoot unit goes through a vegetative phase before it undergoes the transition to making flowers, and gives rise to the next sympodial unit. A key difference between these two types of shoot growth is that the monopodial axis may be indeterminate, whereas each sympodial unit has a determinate inflorescence.

6.4 Vegetative to reproductive transition

Prior to the elaboration of inflorescence structures, plants must make the transition from the vegetative to the reproductive phase. Our current knowledge of the genes that regulate this developmental transition is based largely on studies with *Arabidopsis thaliana*, and these are described in this section. During the vegetative phase of growth, the SAM has vegetative identity, and cells on the periphery of the SAM give rise to leaf primordia that bear secondary (axillary) meristems, which may be dormant or develop into side shoots. In response to appropriate environmental stimuli and developmental signals, the plant then switches from its vegetative phase to the reproductive phase of development, and the meristem acquires an inflorescence identity. At this stage, the SAM of *Arabidopsis* produces two or three cauline leaf primordia with axillary inflorescence meristems (secondary inflorescence shoots) before it produces numerous primordia with a floral identity.

Figure 6.2 (*cont.*) flanks. The inflorescence meristem remains indeterminate and continues to produce flowers until its activity ceases. Mutations in the floral meristem (FM) identity gene *lfy* results in a plant whose inflorescence continues to produce secondary inflorescences instead of flowers until it eventually produces aberrant flowers, some with subtending bracts. In *tfl1* mutants, the inflorescence apex is converted to a flower and stops growth. The secondary inflorescences of *tfl1* also terminate in a flower and *tfl1* has a shorter vegetative and reproductive phase than wild type. Ectopic expression of the (FM) identity gene *AP1* in *Arabidopsis* results in main and secondary inflorescences that have a terminal flower. Wild-type *Antirrhinum* also has an indeterminate inflorescence, and mutations in *cen* make it determinate; *cen* plants have an inflorescence with a terminal flower. Wild-type tobacco shoot meristems are determinate and terminate in a flower; terminal flowers are also found on the inflorescences that develop from axillary meristems below the apex. After the vegetative phase in wild-type tomato, the apex produces a terminal inflorescence. Further growth of the shoot occurs from an axillary meristem, which generates three leaves before it too terminates in an inflorescence. Shoot growth continues through reiteration of this process. In the *self pruning (sp)* mutant of tomato, each sympodial unit has fewer leaves, and eventually the shoot terminates with two inflorescences. The sympodial growth of a wild-type petunia shoot is shown. The black triangle represents the apical inflorescence meristem, while flowers are shown as circles. Vegetative axillary meristems are represented by open triangles; the small triangles represent axillary meristems that are dormant and the large triangles represent axillary meristems that are growing.

The main inflorescence apex is indeterminate and keeps on producing FMs on its flanks until this activity also fades due to senescence. The switch to reproductive development is mediated by the activities of numerous flowering time genes (Blazquez, 1997; Simpson *et al*., 1999; Blazquez and Weigel, 2000; Hempel *et al*., 2000; Araki, 2001); the signals generated by the flowering time genes are integrated and in turn regulate the activity of a limited number of meristem identity genes, namely *TFL1, LEAFY (LFY)* and *APETALA1 (AP1)*. Of these, the shoot meristem identity gene, *TFL1*, is required to make the inflorescence meristem indeterminate, and for it to have a non-floral character. In contrast, the FM identity genes, *LFY* and *AP1*, are the principal regulators of floral identity and are required to make lateral organs develop as flowers.

6.5 Meristem identity

6.5.1 Shoot/inflorescence meristem identity

TERMINAL FLOWER1 function is required for the SAM to retain shoot identity, and in *tfl1* mutants, the shoot inflorescence meristem takes on a floral identity and develops into a flower. *TFL1* is proposed to operate by the slowing down of progression through the different growth phases of *Arabidopsis. TFL1* expression can be detected very early on in plant development; 2–3-day-old *Arabidopsis* seedlings grown in a long-day environment accumulate low levels of *TFL1* mRNA in a group of subapical cells in the shoot apex. At this stage, *TFL1* expression in the SAM is proposed to prevent the plant from flowering prematurely; at a later stage, *TFL1* expression at the shoot apex is increased. *TFL1* plays a role in antagonising the activity of the FM identity genes, *LFY* and *AP1*, in two ways – first, by delaying their upregulation in the meristem, and second, by preventing the meristem from responding to *LFY* and *AP1* activity (Ratcliffe *et al*., 1998, 1999). In *Arabidopsis, TFL1* is expressed below the apical cells in the shoot apex before the transition from the vegetative to the reproductive phase, where it is proposed to play a role in preventing premature flowering (Bradley *et al*., 1997). Subsequently during development, its expression increases and *TFL1* is involved in maintaining the indeterminacy of the inflorescence meristem. *TFL1* also controls the rate of progression through the different phases in *Arabidopsis*; increasing levels of *TFL1* in *Arabidopsis* has the opposite effect to the loss of *TFL1* function, as both the reproductive and vegetative phases get extended and the plants have increased numbers of rosette leaves and bear highly branched inflorescences with flowers (Ratcliffe *et al*., 1999). The consequences of the over-expression of *CEN* in tobacco (Amaya *et al*., 1999), and the *TFL1/CEN* homologues (*RCN1* and *RCN2*) in rice are also similar (Nakagawa *et al*., 2002); they cause an extension of the vegetative phase and have no effect on the determinacy of the inflorescence meristem. It, therefore, appears that most *CEN/TFL1* homologues are involved in a conserved mechanism that regulates transition between developmental phases.

LFY and *AP1* in turn exclude *TFL1* expression from FMs on the periphery of the apex, thus conferring floral identity rather than shoot identity to lateral organs. It has been proposed that the separation of shoot and FM identity relies on the mutual inhibition of *TFL1* by *LFY* and *AP1*. Analysis of both loss and gain of function phenotypes of *TFL1, LFY* and *AP1* supports this antagonistic regulation of these meristem identity genes. An exception to this mutual exclusion of expression is seen in tomato, where *SP*, the *CEN/TFL1* orthologue is expressed in all meristems, and overlaps with expression of the FM identity gene *FALSIFLORA (FA)*, the tomato *LFY* orthologue. It is unclear how antagonistic activities of FA and SP are separated in tomato.

6.5.2 Flower meristem identity genes

Floral meristem identity genes are also important in determining inflorescence architecture as their expression domain will mark where a flower is formed. *LFY* and *AP1* are the two principal meristem identity genes of *Arabidopsis*, and both encode transcriptional regulators; LFY is a novel plant specific transcription factor and AP1 is a transcription factor of MADS box class. Mutants of either *lfy* or *ap1* show a partial conversion of flowers to shoot, and in plants mutant for both *lfy* and *ap1*, the lateral meristems do not specify floral primorida; instead, the lateral organs show a strong conversion to shoots (Mandel *et al*., 1992; Weigel *et al*., 1992; Bowman *et al*., 1993). In contrast, ectopic expression of either *LFY* or *AP1* by the CaMV35S promoter shortens the vegetative phase, converts shoots to flowers and plants eventually produce a terminal flower, similar to that of *tfl1* plants (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995). Even though either *AP1* or *LFY* can specify FM identity, they do not function independently in promoting flowering in *Arabidopsis*. There is a hierarchy operating between these regulators and *AP1* has been shown to function downstream of *LFY*, as *AP1* expression increases rapidly in response to *LFY* induction and as floral promotion by ectopic *LFY* is blocked in *ap1* mutants; the converse is not true as ectopic *AP1* in *lfy* mutants did not promote floral identity.

In addition to *LFY* and *AP1*, genes like *CAULIFLOWER (CAL), FRUITFULL (FUL)* – a MADS box proteins related to *AP1, APETALA2 (AP2)* and *UNUSUAL FLORAL ORGANS (UFO)* also play a secondary role in conferring FM identity (Bowman, 1992; Jofuku *et al*., 1994; Lee *et al*., 1997; Ferrandiz *et al*., 2000). Mutations in *FUL* in combination with the related genes *CAL* and *AP1* is sufficient to prevent the formation of FMs, partly because *LFY* is not upregulated and the shoot meristem identity gene *TFL1* is expressed ectopically (Ferrandiz *et al*., 2000). However, once FM identity is established by the activity of FM identity genes, it must be maintained to prevent reversion of the FM to an inflorescence meristem. The continued expression of *LFY* and *AP1* in FMs represses expression of the MADS box transcription factor *AGAMOUS-LIKE24 (AGL24)* and ensures that floral reversion does not happen (Yu *et al*., 2004).

As *FLO/LFY* is an important regulator of floral identity, its homologues have been cloned from several other plants; also, some of these mutant alleles have also been identified and these reveal that *FLO/LFY* function is conserved. For some, like maize, duplicate genes, *ZFL1* and *ZFL2*, provide redundancy (Bomblies *et al*., 2003). Plants mutants for both *zfl1* and *zfl2* have several inflorescence defects; they lose determinacy and have defects in ear and tassel architecture, floral organ identity and patterning. In addition to these, *zfl1* and *zfl2* plants also have defects in the vegetative to reproductive phase transition. The function of maize *LFY* homologues is thus conserved with the role of the dicot counterparts. The rice *FLO/LFY* homologue is predominantly expressed in very young panicles, but is absent in mature florets, suggesting a role in panicle branching; its loss of function phenotype should demonstrate if this is so (Kyozuka *et al*., 1998). The pea *LFY* homologue – *UNIFOLIATA (UNI)* regulates indeterminacy in inflorescence development, thus affecting its branching pattern and it also regulates floral development (Hofer *et al*., 1997); uniquely, amongst all known *FLO/LFY* homologues, *UNI* is the only one with a role in leaf development.

6.6 Genetic regulation of inflorescence architecture

Inflorescence architecture is being studied in several model species for which mutants with defective inflorescences are known. The application of insertion mutagenesis, with transposons or T-DNAs, available for some of the plant models has facilitated the isolation of mutants for known target genes and also the identification of novel genes influencing inflorescence architecture. A candidate gene approach focusing on key regulators of inflorescence form has been successfully applied to pea (Hofer *et al*., 1997; Taylor *et al*., 2001; Foucher *et al*., 2003) which has a rich collection of inflorescence architecture mutants but for which tools of reverse genetics are limiting. Such analyses have facilitated the comparative analysis of a gene's function in inflorescence development in diverse plants. The subsequent sections describe the progression through inflorescence development, and highlight the function of different genes in sculpting inflorescence form in different plants.

6.6.1 Maize inflorescence development

Two types of inflorescences develop on monoecious maize plants – the tassel, bearing male flowers, and the ear, bearing female flowers. The tassel arises directly from the SAM after it has ceased producing leaves, whereas the ear develops from the tip of an axillary branch. Both of these distinct inflorescence types develop in a strikingly similar manner after each meristem undergoes a series of branching, and transitions in their identity [Figure 6.3; Irish (1997)]. The developmental analysis of wild-type inflorescence meristems, and mutants defective in specific stages of inflorescence development have been instrumental in revealing the transitions that occur during this

process (McSteen *et al*., 2000). Two models have been proposed to explain these transitions. The conversion model (Irish, 1997) states that the maize inflorescence meristem progresses through a sequence of states/identities, and that a meristem with a particular identity gives rise to a specific derivative meristem with another identity (Figure 6.3). In contrast, the proposition of the second model is the retention and persistence of the residual meristem after formation of FMs (Chuck *et al*., 1998); a recent analysis (Kaplinsky and Freeling, 2003) favours the conversion model. The transitions that occur during the elaboration of the maize inflorescence are described later.

The first event is a change in the identity of the meristem to an inflorescence meristem, and this occurs after the plant switches from the vegetative to the reproductive phase in response to intrinsic and extrinsic factors. Once an inflorescence meristem is initiated, it produces a second type of meristem – the spikelet pair meristem (SPM); these arise in multiple rows (polystichous) of SPM and in an acropetal manner, that is, the meristems are initiated from the base towards the tip. In tassels, the SPMs that arise first give rise to branch meristems that initiate tassel branches bearing more SPMs, each of the remaining SPMs produces a third type of meristem – the spikelet meristem (SM), each SPM produces one SM before it too gets transformed to an SM. In the tassel, each SM produces a pair of bract like organs – the glumes – and initiates the lower FMs before becoming the upper floret meristem. Each FM then gives rise to the terminal floral organs; in tassels, the pistil aborts, while in ears, the lower pistil and the anthers abort.

There are several maize mutants that affect different stages of tassel or ear development (see Figure 6.3 and Table 6.1); some of these affect the number of flowers in a spikelet, while others block the switch in identity of branch meristems to spikelets, or affect inflorescence meristem size. Often additional functions for these genes are revealed when mutants are introgressed into different genetic backgrounds. For example, the *barren inflorescence2 (bif2)* mutant has a rachis that lacks branches and florets; however, the *bif2* phenotype is much weaker in an A188 background, revealing a role for *BIF2* in the maintenance of branch, spikelet and floral meristems (McSteen and Hake, 2001).

fasciated ear2 (fea2) and *knotted-1 (kn-1)* mutants show defects at an early stage of inflorescence development that suggest a role in meristem maintenance; *FEA2* encodes leucine-rich repeat receptor-like protein similar to the *Arabidopsis* CLV2 protein, and regulates meristem size specifically in the ear inflorescence meristems (Taguchi-Shiobara *et al*., 2001). *fea2* ear inflorescence meristems undergo massive overproliferation and eventually produce short and wide ears. In contrast, recessive loss-of-function mutations of the homeodomain protein *Kn-1*, reduce the number of branches and spikelet pairs (Vollbrecht *et al*., 2000); *kn-1* mutants often lack ears, and the ears that grow have few spikelets. Maize mutants with defects in branch meristems either increase branching of the inflorescence or reduce it. The *ramosa* class of mutants have extensively branched inflorescences as meristems proliferate or continue to produce branches. In *ra1* ears, the meristem fails to switch from making long branches to SPM. Other mutants that also increase inflorescence branching are the *tassel seed* class II mutants (Phipps, 1928; Irish,

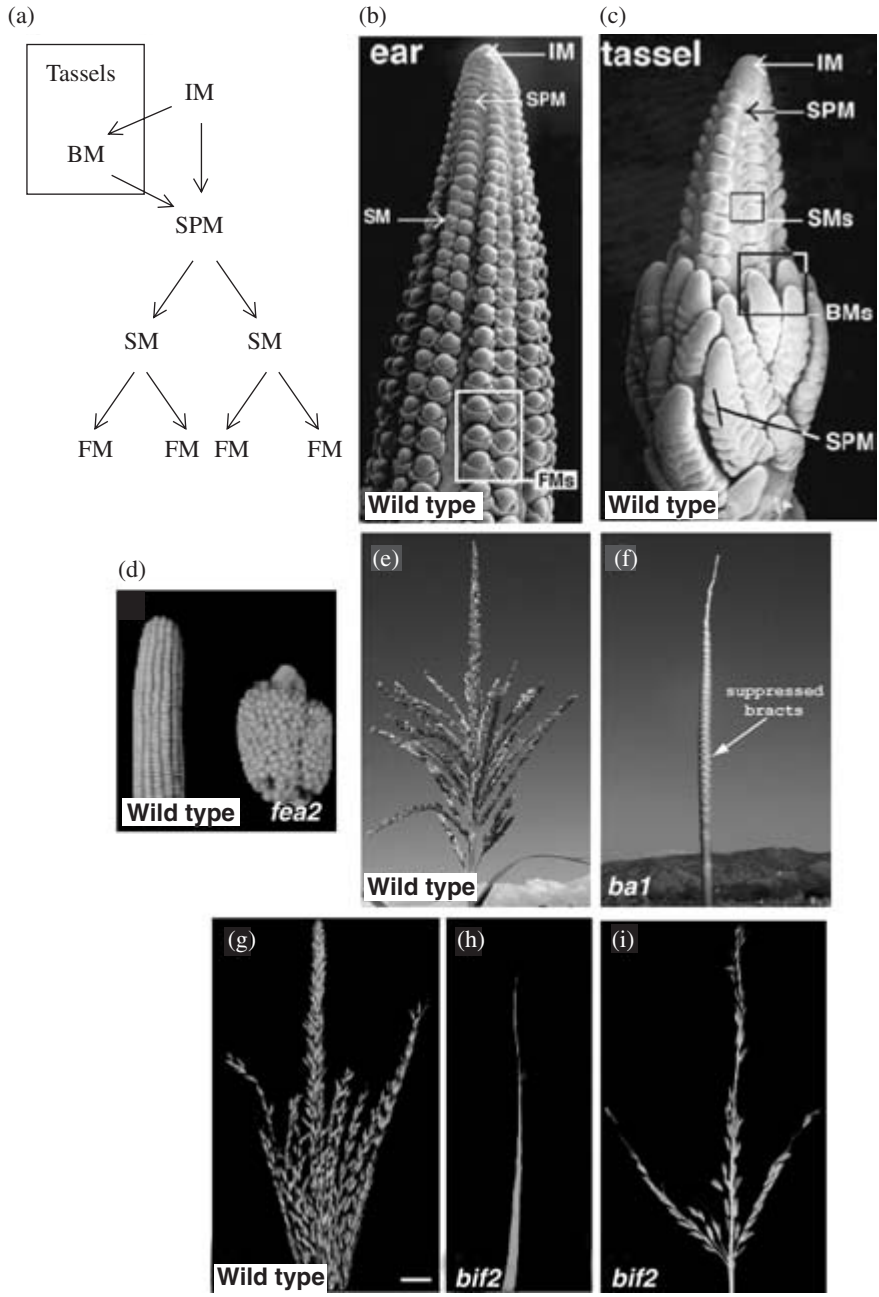

Figure 6.3 Inflorescence development in maize. (a). Schematic of meristem transitions during inflorescence development. The inflorescence meristem (IM) gives rise to spikelet pair meristems (SPM), which in turn form two spikelet meristems (SMs). Individual

1997), *tasselseed4 (ts4)* and *Tassel seed6 (Ts6)*, which are required for floral organ abortion, but also affect branching in the inflorescence meristem. *ts4* plants have tassels that are highly branched as SPM fail to form SM, but instead they bear branches which make more SPM. The entire *ts4* inflorescence is eventually covered by meristems by the reiterative development of SPM as, both, the switch from SPM to SM identity and SPM determinacy are affected.

Several other mutants like *barren inflorescence2 (bif2), Suppressor of sessile spikelets1 (Sos1)* and *barren stalk1 (ba1)* reduce the number of branches and spikelets (Doebley *et al*., 1995; McSteen and Hake, 2001; Ritter *et al*., 2002). The phenotype of *ba1* mutant is severe and resembles that of *pin1* and *pinoid* mutants of *Arabidopsis; ba1* plants have defects in axillary meristem development and lack tassel branches, spikelets, tillers or ears. It has, therefore, been speculated that *BA1*, like *PIN1* and *PINOID*, could also play a role in polar auxin transport or auxin signalling (Ritter *et al*., 2002).

Mutants that alter meristem determinacy can also affect the branching of the inflorescence. The *branched silkless1 (bd1)* mutant fails to make the transition of SM to FM; instead, SM give rise to indeterminate branches bearing glumes and more SMs. *BD1* encodes an ethylene-responsive element-binding (ERF) class of transcription factor proposed to repress indeterminate branch fate within the lateral domain of the SM (Colombo *et al*., 1998; Chuck *et al*., 2002). The *indeterminate floral apex1 (ifa1)* mutant affects the determinacy of several distinct meristems of the maize inflorescence, as SPM, SM and FM, all become less determinate in *ifa1* plants (Laudencia-Chingcuanco and Hake, 2002). Unlike *ifa1*, mutants like *indeterminate spikelet1 (ids1)* and *reverse germ orientation1 (rgo1)* only affect determinacy of the SM; *IDS1* encodes an *AP2*-like transcription factor required for SM determinacy (Chuck *et al*., 1998), and *ids1* mutants bear more florets per spikelet than is normal.

Figure 6.3 (*cont.*) SM give rise to two floral meristems (FMs). In tassels, the IM also develops indeterminate branch meristems (BMs) on its base, which in turn develop SPM. SEM image of a (b) wild-type maize ear and (c) tassel. Female inflorescence development in maize also proceeds similar to that of the male inflorescence, except for the supression of branch meristems in the female inflorescence. In the boxed area, six spikelets are shown; the upper FM and outer glume can be seen but the lower FM and glume are not visible. Figures shown in (b)–(c) are reproduced with permission from Laudencia-Chingcuanco and Hake (2002). (d) Mature ear phenotype of wild-type inbred B73 line and *fea2* mutant. The wild-type ear has straight vertical rows of kernels while the *fea2* mutant ear is shorter, wider and flattened with irregular rows of kernels. Images reproduced from Taguchi-Shiobara *et al*. (2001) with permission. (e)–(f) Wild-type and *ba1* tassels; *ba1* tassel lacks tassel branches and spikelets and has ridges of suppressed bracts. Images (e)–(f) are reproduced from Ritter *et al*. (2002) with permission. (g)–(i) The branching phenotype of *bif2* assels in different backgrounds. (g) Wild-type inbred line B73, and (h) almost total lack of branching seen in *bif2* tassels in a B73 background. (i) *bif2* in a A188 genetic background produces tassels that have a few branches and spikelets on both the main spike and the branches. Images (g–i) are reproduced from McSteen and Hake (2001) with permission.

Table 6.1 Maize inflorescence development mutants

Gene	Mutant phenotype	Identity/sequence	Reference
FASICLED EAR1 (FAS1)	*Fas1*-dominant allele; inflorescence meristem is fasciated and its branching pattern is affected	Not known	Orr *et al*. (1997)
FASCIATED EAR2 (FEA2)	*fea2*-ear inflorescence meristems proliferate excessively	Leucine-rich repeat receptor-like protein; has homology to CLAVATA2 of *Arabidopsis*	Taguchi-Shiobara *et al*. (2001)
KNOTTED1 (Kn1)	*kn1*-has reduced number of branches and spikelet pairs	Homeodomain transcription factor; similar to *SHOOT-MERISTEMLESS* of *Arabidopsis*	Vollbrecht *et al*. (2000)
ZFL1 and *ZFL2*	Duplicate genes; *zfl1 zfl2* double mutants have defects in ear and tassel architecture, floral organ identity and patterning	*FLO/LFY* homologues; transcription factor, plant specific	(Bomblies *et al*. (2003)
RAMOSA1, *RAMOSA3*	Increased branching of tassels	Not known	Neuffer *et al*. (1997)
TASSEL SEED6 (TS6)	*Ts6* semi-dominant mutant; indeterminacy of pedicillate SM is extended; initiates extra FM	Not known	Nickerson and Dale (1955), Irish (1997)
TASSEL SEED4 (ts4)	*ts4* is blocked in the transition of SPM to SM, additional SPM are formed reiteratively; consequently, ears and tassels are highly branched	Not known	Phipps (1928); Irish (1997)
INDETERMINATE FLORAL APEX1 (ifa-1)	*ifa1*-makes extra spikelets as it reducesdeterminacy of SPM, SM and FM. *IFA1* also has a role in meristem identity	Not known	Laudencia-Chingcuanco and Hake (2002)

(*Continues*)

Table 6.1 (*Continued*)

Gene	Mutant phenotype	Identity/sequence	Reference
UNBRANCHED1 (UB1)	Tassels are unbranched as all SPM become determinate	Not known	Neuffer *et al*. (1997)
BARREN STALK1 (BA1)	*ba1* mutant lacks axillary meristems Possibly involved in polar auxin transport, as its phenotype resembles that of pin-1 or *pinoid* mutants of *Arabidopsis*	Not known	Hofmeyer (1930); Ritter *et al*. (2002)
BARREN INFLORESCENCE2 (BIF2)	*bif2* axillary meristem development is defective, mutants have fewer branches	Not known	McSteen and Hake (2001)
REVERSED GERM ORIENTATION1 (RGO1)	SM generates an extra FM before terminating as a FM. Lower spikelet develops instead of aborting. Suppresses SM determinacy	Not known	Kaplinsky and Freeling (2003)
SUPPRESSOR OF SESSILE SPIKELET1 (SOS1)	*Sos1*-dominant allele; SPM are unbranched; consequently, single spikelets are produced, plants have reduced number of tassel branches	Not known	Doebley *et al*. (1995)
BRANCHED SILKLESS1 (BD1)	*bd1* florets are not formed in the ear; affects determinacy in the ear. It alters SM identity, and is required for the transition from a spikelet meristem to a floral meristem	ERF class transcription factor	Kempton (1934); Colombo *et al*. (1998); Chuck *et al*. (2002)

Table 6.1 (*Continued*)

Gene	Mutant phenotype	Identity/sequence	Reference
INDETERMINATE SPIKELET1 (IDS1)	*ids1* spikelets become indeterminate and produce more florets. *IDS1* suppresses SM determinacy	*APETALA2*-like gene	Chuck *et al.* (1998)

The development of numerous florets per spikelet is proposed to be an ancient trait in grasses (Stebbins, 1987), with most derived species having a reduced number of florets per spikelet. It has been suggested that genes like *IDS1*, which act to suppress SM meristem indeterminacy, could play a role in regulating number of florets per grass spikelet (Chuck *et al*., 1998). Mutating *RGO1* has a similar effect on SM determinacy and affects floret number (Kaplinsky and Freeling, 2003). Intriguingly, plants heterozygous for *ids1* and *rgo1* mutations show non-allelic non-complementation, despite bearing mutations in distinct genes. In addition, plants homozygous for both *ids1* and *rgo1* have a novel phenotype – SPM with increased branching, not seen in the single mutants. The identity and expression pattern for *RGO1* are not known, but *IDS1* is expressed in SPM and floral primordia; this and the phenotype of *ids1* and *rgo1* double mutants support a wider role for *IDS1*. Based on the analysis of *ids1* and *rgo1*, Kaplinsky and Freeling (Kaplinsky and Freeling, 2003) have proposed that the transition through the distinct meristem identities in a maize inflorescence may depend on the dosage and levels of *IDS1* and genes like *RGO1*.

6.6.2 Pea mutants

The pea inflorescence also has a complex branching structure and is classified as a compound raceme. Like maize, pea inflorescence architecture develops from inflorescence meristems of distinct orders that bear branches on which flowers develop. The development of this complex inflorescence architecture and the molecular identity of key regulators are outlined here [Table 6.2; for additional details see Singer *et al*. (1999)]. Prior to its switch to reproductive growth, the vegetative meristem (V_1) gives rise to leaves and axillary V_2 meristems; subsequent to the change to a reproductive phase, the SAM converts to an inflorescence meristem – the indeterminate inflorescence meristem (I_1) – which produces nodes that give rise to second order inflorescence meristems (I_2) in leaf axils. Each I_2 meristem is determinate and produces one or more FM (F) laterally, before it stops growing and terminates in a stub with epidermal hair. Pea mutants which arrest at different stages of inflorescence development are also available, identifying genes important for these transitions. The candidate gene approach has been very successful in linking specific inflorescence development mutants

Table 6.2 Inflorescence mutants of pea

Gene	Mutant phenotype	Identity/sequence	Reference
BROCCOLI (BROC)	*broc* mutants have no phenotype; *broc pin* double mutants have a more severe phenotype	Not known; mutant phenotype suggests it could be the equivalent of the *Arabidopsis* meristem identity gene *CAULIFLOWER*	Singer *et al*. (1999)
DETERMINATE (DET)	Converts I_1 meristem to I_2 meristem	*PsTFL1a – CEN/TFL1* homologue; RAF-kinase- like-inhibitor protein	Foucher *et al*. (2003)
NEPTUNE (NEP)	Acts on I_2 meristems; *nep* plants have multiple pods per branch	Not known	Singer *et al*. (1999)
PROLIFERATING INFLORESCENCE MERISTEM (PIM)	Delayed floral meristem specification and floral defects	*SQUAMOSA* orthologue – MADS box transcription factor	Taylor *et al*. (2002)
STAMINA PISTILLOIDA (STP)	Specifies floral meristem identity	*FIM/UFO* orthologue; F-box protein	Taylor *et al*. (2001); Monti and Devereux (1969)
VEGETATIVE (VEG)	Plants do not make I_2 stubs or flowers	Not known	Singer *et al*. (1999)
UNIFOLIATA (UNI)	I_1 meristem becomes determinate and flowers are defective	*FLO/LFY* homologue; plant specific transcription factor	Hofer *et al*. (1997); Yaxley *et al*. (2001)

to their corresponding genes in pea (Hofer *et al*., 1997; Taylor *et al*., 2001; Foucher *et al*., 2003). Some of these, like *DET* and *UNI* encode conserved regulators of meristem determinacy or identity.

DET encodes one of the pea *CEN/TFL* homologues, *PsTFL1a*, required for maintaining the indeterminate state of the primary inflorescence. The phenotype of *det* mutants, like that of *tfl1* and *cen* mutants, also alters indeterminate growth to determinate growth during reproductive phase; however, *det* mutants do not produce a terminal flower, instead the I_1 meristem terminates in a stub; nor do *det* plants flower early like *Arabidopsis tfl1* mutants. Another *CEN/TFL1* homologue of pea, *PsTFL1c*, corresponds to the *LF* gene, and has a role in repressing flowering. In pea, at least, the function for indeterminacy of the inflorescence meristem and regulation of the floral transition is separate (Foucher *et al*., 2003). Only plant mutant for both *det* and *veg* produce a terminal flower.

The phenotype of several different mutants like *vegetative (veg)*, *stamina pistilloida (stp), proliferating inflorescence meristem (pim)*, *unifoliata (uni)* suggests a role for these genes in regulating FM identity. Defects of *pim* mutant inflorescences are similar to those seen in *squamosa* and *apeatala1* mutants, which encode FM identity genes of *Antirrhinum* and *Arabidopsis*, respectively. *PIM* is a homologue of *SQUA* and *AP1* and is also required for both FM identity and floral development. FMs on *pim* plants convert to inflorescence meristems before they form defective flowers, increasing branching on the inflorescence (Taylor *et al*., 2002). *STP* is also a homologue of another FM identity gene, *UFO/FIM*, and *stp* mutants are affected in several developmental processes (Taylor *et al*., 2001). The presence of ectopic secondary flowers on *stp* inflorescences is indicative of a role for *STP* in FM specification. *UNI* is a *FLO/LFY* homologue (Hofer *et al*., 1997) and regulates indeterminacy in inflorescence development, but it also has a function in vegetative development. In *uni* plants, second order inflorescence growth (I_2) is enhanced as I_1 meristems become determinate and initiate several I_2 meristems before terminating in stubs. Loss of *UNI* also affects flower development as mutant flowers mainly contain leaf-like sepals and carpels.

veg mutants make the transition to develop I_1 meristems but are unable to produce any floral organs or I_2 stubs. Some of these mutants, like *pim* and *stp*, are homologues of conserved FM identity genes. Other mutations, like *neptune (nep)* appear to affect determinacy of I_2 meristems as they increase the number of FMs produced by each I_2 meristem; consequently, *nep* plants bear multiple pods per branch (Singer *et al*., 1999). *BROCCOLI* is likely to encode a redundant FM identity gene, as *broc* mutants have no phenotype on their own (Singer *et al*., 1999); however, when combined with *pim*, with which it is partly redundant, *broc pim* double mutants have a more severe phenotype with the inflorescence resembling a head of broccoli. This is similar to the interaction between *ap1/cal* mutants of *Arabidopsis*, which also result in a cauliflower phenotype (Bowman, 1992).

6.6.3 Tomato inflorescence development

In tomato, shoot growth is sympodial, that is, the inflorescences are not borne on a single main shoot. Instead, the vegetative and reproductive phases alternate on a tomato plant. The primary shoot produces about 8–12 leaves and terminates in an inflorescence, and further growth is from an axillary bud which develops below the inflorescence axis. The shoot from this axil generates three additional leaves and itself terminates in an inflorescence, and growth continues in this pattern from an axillary bud below each inflorescence. The shoot of a tomato plant, thus, has repeated sympodial units made up of three vegetative nodes and a terminal inflorescence, and each unit arises from the proximal vegetative node of the earlier sympodial entity. The wild-type habit of tomato produces sympodial shoot units continuously and is thus indeterminate, but the inflorescence meristems of tomato plants are determinate (Figure 6.2).

SELF PRUNING is a homologue of *CEN/TFL1* and it functions in tomato to control the determinacy of sympodial meristems (Pnueli *et al*., 1998); mutations in *SP* result in termination of the sympodial units of the shoot, which produces a bushy, compact plant, but it has no effect on the inflorescence [Table 6.3]. The termination of growth is gradual as the number of vegetative nodes made by each sympodial unit diminishes from three to two to one, until the vegetative phase is bypassed and the apex terminates with two successive inflorescences. So, although *SP* does not affect inflorescence determinacy, its function in preventing premature flowering in the tomato sympodial shoot parallels the role of *TFL1* in *Arabidopsis. FALSIFLORA (FA)* is the tomato *FLO/LFY* homologue (Molinero-Rosales *et al*., 1999), and like its conserved counterparts in other plants, the loss of *FA* also prevents the shoots from making the transition from an inflorescence meristem to an FM. Mutants like *single flower truss (sft)* and *jointless (j)*, and lines with reduced *TM29* transcript all show reversion of the inflorescence meristem to vegetative growth (Molinero-Rosales *et al*., 2004; Ampomah-Dwamena *et al*., 2002; Mao *et al*., 2000). At present, the identity of *SFT* is not known, but *TM29* and *JOINTLESS* both encode MADS box transcription factors required for maintenance of FM identity. *UNIFLORA (UF)*, on the other hand, is required for the maintenance of inflorescence meristem identity as *uf* mutants bear solitary flowers instead of producing inflorescences (Dielen *et al*., 2004). In contrast, the inflorescences of *anantha (an)* plants show excessive proliferation and resemble the cauliflower inflorescence (Allen and Sussex, 1996), as inflorescence meristems fail to switch to floral identity in *an* plants.

6.6.4 Petunia inflorescence development

In petunia inflorescences, shoot growth continues through a sympodial meristem in the axil of a flower (Figure 6.2); each inflorescence has two leaf-like bracts at each node, a single flower develops from the axil of one bract, while an inflorescence shoot grows from the axil of the other bract and reiterates this branching pattern. Once the flower and inflorescence have grown out, axillary meristems arise in the axils of the bracts and initially they are vegetative in identity and form a few leaves, but eventually the axillary meristem too gives rise to an inflorescence meristem that grows like the main inflorescence. A detailed microscopic analysis of wild-type and mutant petunia has revealed the sequence of events which lead to this type of inflorescence architecture. First, the inflorescence simultaneously produces the two bracts before splitting into two halves, one of which becomes the determinate FM, the other continues as the inflorescence meristem.

ABERRANT LEAF AND FLOWER (ALF) is the petunia homologue of *LFY/FLO*, and its expression pattern in the inflorescence marks the formation of an FM, prior to the physical bifurcation of the meristem. *ALF* is not required for the bifurcation of the inflorescence as this occurs in *alf* mutants (Souer *et al*., 1998) and neither does it have aberrant leaves; however, *ALF*, like *FLO* and *LFY*, is required for establishing FM identity as *alf* mutant inflorescences do not bear flowers. The branching of the

Table 6.3 Inflorescence mutants of tomato

Gene	Phenotype	Identity	Reference
ANANTHA (AN)	*an* inflorescences proliferate indefinitely and do not make the transition to a floral meristem; structures are cauliflower-like	Not known	Allen and Sussex (1996)
BLIND (BL)	Inflorescences have fewer flowers and *bl* plants prematurely terminate their main shoot; has problems with initiating lateral meristems	R2–R3 type myb transcription factor	Schmitz *et al*. (2002)
FALSIFLORA (FA)	*fa* shoots do not make the transition from an inflorescence meristem to a floral meristem	Tomato *LFY/FLO* homologue	Molinero-Rosales *et al.* (1999)
JOINTLESS (J)	Inflorescence meristems revert to vegetative growth; identified as regulator of pedicel abscission	MADS box transcription factor	Mao *et al*. (2000)
SELF PRUNING (SP)	Has no effect on inflorescence; sympodial shoots terminate prematurely	*CEN/TFL1* homologue	Pnueli *et al*. (1998)
SINGLE FLOWER TRUSS (SFT)	Floral meristem identity regulator; *sft* inflorescences make 1–2 flowers then revert to vegetative growth	Not known	Molinero-Rosales *et al.* (2004)
TM29	Transgenic lines with reduced TM29 levels show floral reversion; required for maintenance of floral meristem identity	Encodes a MADS box proteins; SEPALLATA homologue	Ampomah-Dwamena *et al*. (2002)
UNIFLORA (UF)	*uf* mutants bear single flowers instead of developing an inflorescence	Not known	Dielen *et al*. (2004)

petunia inflorescence meristem is regulated by *EXTAPETALS* as *exp* mutants have an unbranched inflorescence that terminates in a single flower (Souer *et al*., 1998).

6.6.5 Capitulum development

The capitulum form of inflorescence is highly typical of Asteraceae, but inflorescences of a similar type are also common in numerous other families – in the Asteridae and even the Monocotyledonae (Harris, 1999). The monomeric unit of this structure is the floret, which constitutes a leaf-like bract and an axillary flower. The sunflower capitulum bears two types of florets, ray florets, which occupy the outer whorl of the inflorescence disc, and disc florets, which arise in spiral rows and occupy the inner whorl. Capitula also have involucral bracts, which function as equivalents of sepals and protect young buds as the capitulum develops. Capitulum types can be different depending upon the type of florets they bear (Leppik, 1977; Bremer, 1994). Accordingly, they are further classified as:

- radiate capitula, which have both disc and ray florets;
- discoid capitula, which bear only disc type florets on their heads and lack ray florets;
- disciform capitula, which have two forms of tubular disc florets and lack ray florets;
- ligulate capitula, with only ray florets.

Sunflower has an indeterminate capitulum or head, and it has been speculated that this type of morphology may be derived either after the contraction of a raceme or a racemose umbel (Harris, 1999). The ray florets of sunflower are sterile and have five petals, whereas disc florets are tubular and fertile. In *Senecio*, the genetic basis for two distinct floret types is known; in this plant, the development of ray florets is suppressed by a single co-dominant locus (Trow, 1912; Ingram and Taylor, 1982; Andersson, 2001). The locus regulating the rayed and non-rayed (discoid) forms of *Senecio* has been reported to be tightly linked to a homologue of the transcription factor *CYCLOIDEA* [*CYC*; see report in Eckardt (2001)]; further analysis should show if it is the *CYC* homologue that is responsible for the radiate and discoid capitular forms of *Senecio*. The development of the primary Asteraceae inflorescence has been well-studied and the commercial value of chrysanthemum and sunflower make them popular models for capitulum development. In plants with capitula, the switch from vegetative to reproductive development causes a change in the vegetative apex, which is a relatively small, highly domed and dense meristem, to a larger and flatter apex. An intermediate called the transition apex has been identified in some cases, and the transition apex differs from the vegetative apex by its larger size, broader shape and its lack of involucral bract primordia, floral primordia or receptacular bract primordia. When a transition apex is present, it is similar to the inflorescence apex; its cells divide to produce not organs, but a gradual increase in its size. Some species of the Asteraceae can retain this state indefinitely until the correct environmental cues trigger the flowering phase.

The sunflower floret primordium starts as a small outgrowth of the inflorescence meristem, which increases in size and is split into two equal domains – the adaxial and the abaxial halves – by a bisecting crease. The bilaterally symmetrical bract is derived from the peripheral abaxial domain, while the flower arises from the adaxial half. The *missing flowers* mutant of sunflower fails to develop flowers in the inflorescence, and the receptacles of mutant inflorescences bear absolutely no ray florets and have very few disc florets (Figure 6.4). The mutant plants also fail to produce axillary growth during the vegetative phase, presumably due to lack of axillary meristems (Fambrini *et al*., 2003). It has been hypothesised by Fambrini *et al*. (2003) that positional information for these fates is interpreted from a gradient along the abaxial and adaxial axis of the primordium, and that in the *missing flowers* mutant, adaxial domain fails to develop; consequently, the plants lack axillary shoots and inflorescences develop bracts, but the axils of these bracts lack flowers. It will be interesting to see if candidate genes that regulate polarity or axillary meristem development (Otsuga *et al*., 2001; Bowman *et al*., 2002; Schmitz *et al*., 2002; Greb *et al*., 2003;) in model plants play any role in the development of florets on the capitulum.

6.6.6 Arabidopsis inflorescence development

Arabidopsis inflorescence is a simple raceme that develops an elongated stem with a primary inflorescence meristem at its apex, with basal axillary inflorescences (coflorescences), and bractless floral primordia on the inflorescence stem. Its inflorescence architecture develops from the activities of three distinct meristems; the primary apical inflorescence meristem produces the main inflorescence axis, the secondary inflorescence meristems give rise to axillary meristems on the main shoot (branches) or in the axils of rosette leaves, and, subsequently, FMs produce bractless flowers on these inflorescence shoots. The absence of bracts subtending *Arabidopsis* flowers depends on the downregulation of the predicted transcriptional repressor *JAGGED* in cryptic bracts (Dinneny *et al*., 2004; Ohno *et al*., 2004). The meristem identity genes *TFL1, LFY* and *AP1* play a key role in developing this inflorescence architecture, and in addition to those described in Section 6.2, several other loci also contribute to the inflorescence architecture of *Arabidopsis* [Figures 6.2 and 6.5; Table 6.4]. Mutations in these loci have effects that range from a mild shift in architecture from a raceme to a more corymb-like inflorescence of *erecta* and *corymbosa2/hua enhancer-1* mutants (Torii *et al*., 1996; Suzuki *et al*., 2002), to the highly compressed inflorescence of *acaulis, compact inflorescence* and *fireworks* mutants (Figure 6.5). Some mutants, like *acaulis* and *pinoid*, affect specific aspects of plant development like internode growth, or the development of primordia, while others like *erecta* have an overall effect on morphology. *brevipedicellus (bp)* mutants also have reduced internode and pedicel length. These and their altered pedicel angle transform their inflorescence to a distinctive structure bearing clusters of flowers which point downwards (Figure 6.5); *BP* is encoded by *AtKNAT1*, a class 1 KNOTTED-1 like homeobox (KNOX) gene (Byrne *et al*.,

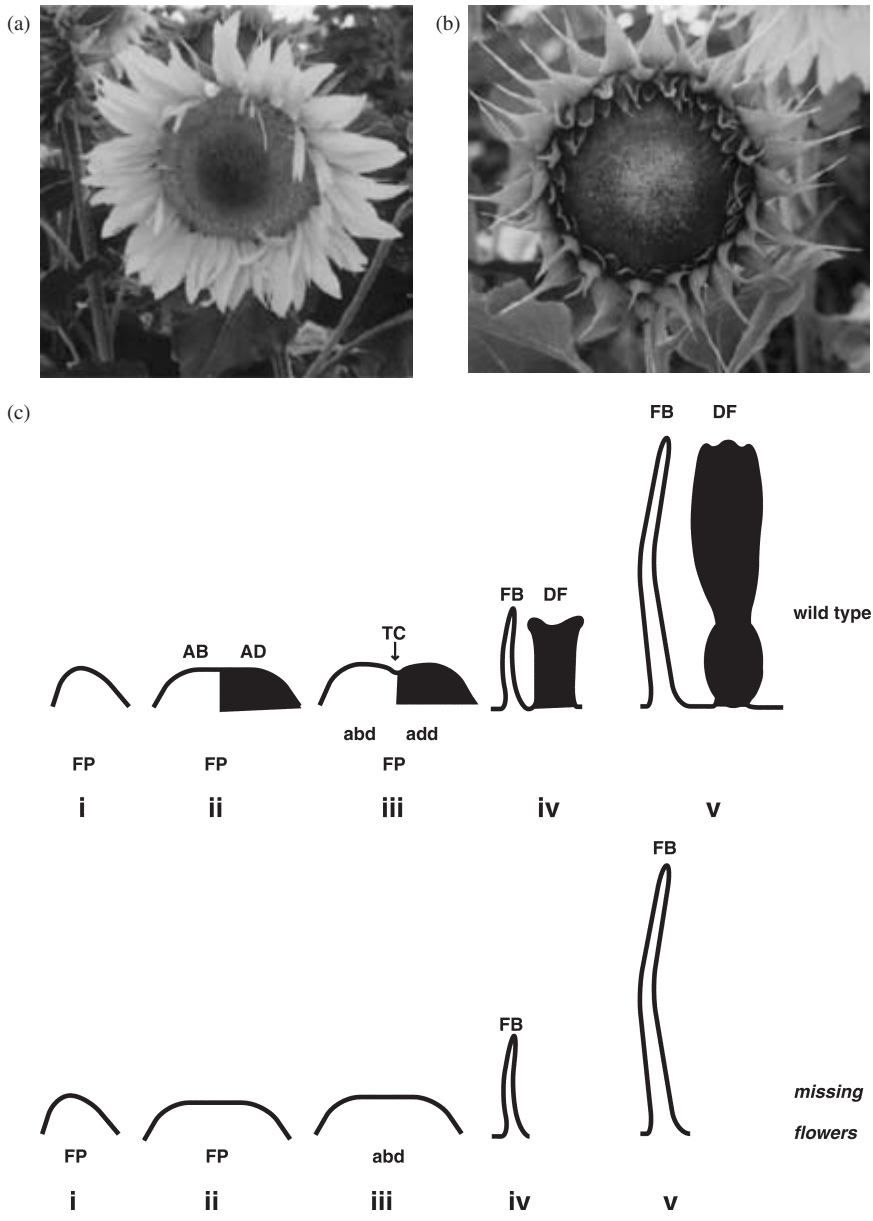

Figure 6.4 Capitulum of wild-type sunflower and the *missing flowers* mutant. Mature wild type sunflower inflorescence (a) shown with a mature *missing flowers* capitulum (b); panels (a) and (b) are reproduced with permission from Fambrini *et al*. (2003). Model proposed by Fambrini *et al*. (2003) for the development of adaxial and abaxial structures (c): the floret primoridium (FP) is initiated on the flank of the inflorescence meristem (i); subsequent to its enlargement (ii), the primordium is bisected by a crease (TC) into two domains with distinct fates (iii) – the abaxial domain (abd) develops into the bilaterally symmetrical bract (FB) and the adaxial domain (shown in black; add) develops into the flower (DF; iv and v). Fambrini *et al*. (2003) have suggested that the adaxial domain is not specified in the *missing flowers* mutant.

Figure 6.5 *Arabidopsis* mutants with defects in inflorescence architecture. Inflorescence architecture of (a) wild-type *Arabidopsis* (Landsberge *erecta* ecotype) and mutant *Arabidopsis* plants. The clustering of flowers on a *pinoid-2* mutant inflorescence is shown in (b), inset is a higher magnification of the same inflorescence. The pendant flowers and short floral internodes of *brevipedicellus* are shown in (c). *acaulis5* mutants fail to elongate their inflorescence shoot after flowering (d), while *tfl1* mutant inflorescences are determinate and terminate in a flower (e). The compact inflorescence of *cif* plant is due to lack of floral internode elongation (f). Figures shown in panels (d) and (e) are reproduced with permission from Hanzawa *et al*. (1997) and Shannon and Meeks-Wagner (1991), respectively.

2002; Douglas *et al*., 2002; Venglat *et al*., 2002). The pedicel growth and angle defects of *bp* plants are due to aberrant differentiation, growth and elongation of epidermal and cortical cell in the abaxial side of pedicels (Douglas *et al*., 2002; Venglat *et al*., 2002). Mutations in *BELLRINGER (BLR)*, which encodes a BELL class homeodomain protein that interacts with BP, also affects internode growth (Byrne *et al*., 2003; Smith and Hake, 2003). Based on the interaction and expression pattern of *BP* and *BLR*, it has been suggested that *BP* and *BLR* could pattern the floral internodes in the inflorescence (Smith and Hake, 2003). Mutations in

Table 6.4 *Arabidopsis* inflorescence architecture mutants

Gene	Phenotype	Identity	Reference
ACAULIS5 (ACL5)	Blocks inflorescence internode elongation	Homology to spermine and spermidine synthase; implicated in polyamine metabolism	Hanzawa *et al*. (1997, 2000)
ABNORMAL INFLORESCENCE MERISTEM1 (AIM1)	Inflorescence meristem is disorganized	Homology to multifunctional protein involved in β-oxidation of fatty acids	Richmond and Bleecker (1999)
BREVIPEDICELLUS (BP)	Mutant has downward-pointing siliques and has short internodes in L. *erecta*. Plays a redundant role in meristem development	*AtKNAT1* – Class I KNOX TALE homeodomain transcription factor	Byrne *et al*. (2002); Douglas *et al*. (2002); Venglat *et al*.(2002)
BELLRINGER (BLR)	Defective phyllotaxy and shoot growth, also affects replum development	BELL class homeodomain transcription factor	Bao *et al*. (2004); Bhatt *et al*., (2004); Byrne *et al*., (2003); Smith and Hake (2003); Roeder *et al*. (2003)
COMPACT INFLORESCENCE (CIF1 and CIF2)	Highly compacted floral internodes, flowers cluster at apex	Not known	Goosey and Sharrock, (2001)
CORYMBOSA2 (CRM2)	Inflorescence is corymb-like and plants are dwarfed	Allelic to *hua-enhancer1 (hen1)*, implicated in regulating levels of miRNA	Suzuki *et al*. (2002); Park *et al*. (2002)
ERECTA (ER) and ERECTA LIKE1 (ELK1) and ERECTA-LIKE2 (ELK2)	Erecta inflorescence is corymb-like; *erl2-1* enhances the inflorescence phenotype of er	Related proteins, encode leucine-rich repeat receptor-like serine/threonine kinase	Torii *et al*. (1996); Shpak *et al*. (2003)
FIREWORKS (FIW)	Undergoes premature senescence and flowers are clustered on inflorescence	Not known	Nakamura *et al*. (2000)

Table 6.4 *(Continued)*

Gene	Phenotype	Identity	Reference
PIN1	Floral primordia missing on inflorescence shoot, which is pin-like	Auxin *efflux* carrier; polar transport of auxin	Reinhardt *et al*. (2003); Friml *et al*. (2003)
PINOID (PID)		Ser/Thr kinase proposed role in auxin response	Benjamins *et al*. (2001); Christensen *et al*. (2000)
OVEREXPRESSION – *AtMYB13*	Mutant phenotype not known. Over-expression results in hook-like structures at pedicel branch points and the main shoot makes axillary inflorescences after initiation of solitary flowers on the main axis	Myb domain transcription factor	Kirik *et al*., (1998)
TERMINAL FLOWER2/LIKE HETERO-CHROMATIN PROTEIN1	Mutants are early flowering, dwarfed and inflorescence terminates with floral structure	Epigenetic repressor; has homology to Heterochromatin protein1	Gaudin *et al*. (2001); Kotake *et al*. (2003); Larsson *et al*. (1998)

COMPACT INFLORESCENCE (CIF) genes have a dramatic effect on the inflorescence architecture of *Arabidopsis*, resulting in floral clusters on inflorescence shoots as the floral internodes fail to extend (Goosey and Sharrock, 2001). The *cif* phenotype is due to two loci. One is a recessive mutation, which, in the presence of a dominant modifier in the Nossen-0 ecotype, produces the *cif* inflorescence phenotype; at present, the identity of the second locus is not known. The *fireworks (fiw)* mutant phenotype (Nakamura *et al*., 2000) is similar to that of *cif; fiw* shoots also bear a cluster of flowers at their apex as inflorescence stem elongation and flower formation cease prematurely both in the main stem and in the lateral inflorescences. *fiw* is unlikely to affect meristem determinacy as *fiw* plants do not produce a terminal flower. In addition to their inflorescence defect, *fiw* plants also undergo premature senescence of leaves. Mutations in several different genes can modify the inflorescence of *Arabidopsis* from a raceme, to a corymb-like structure. The Landsberg *erecta* ecotype of *Arabidopsis* has a mutation in the *ERECTA* gene, which results in a compact inflorescence (Torii *et al*., 1996). *ERECTA* belongs to a small family of related Leucine Rich Repeat receptor-like kinases and its loss makes the

Arabidopsis inflorescence more corymb-like in appearance. Two *ERECTA* paralogues, *ERECTA-like1 (ELK1)* and *ERECTA-like2 (ELK2)* also play redundant but distinct roles in inflorescence architecture (Shpak *et al*., 2004). Another mutant that also converts the raceme to a corymb-like structure is *corymbosa2 (crm2); CRM2* encodes a novel protein conserved amongst many eukaryotes (Suzuki *et al*., 2002). The corymb-like inflorescence of *crm2* mutants develops as there is a significant delay in the initiation of floral internode elongation and in the development of flowers. *crm2* is an allele of *hen1*, implicated in regulating genes via a miRNA-based mechanism (Park *et al*., 2002); this suggests a role for miRNAs in the regulation of inflorescence architecture.

Phytohormone biosynthesis and response also play a role in growth of the inflorescence shoot. Mutations in *AUXIN RESISTANT1 (AXR1)* and *AXR3* affect plant stature and branching of the inflorescence shoot (Rouse *et al*., 1998; Stirnberg *et al*., 1999), while gibberellic acid biosynthesis and response mutants, like *ga5* and *gibberellic acid insensitive1 (gai)*, also affect the overall height of the plant and floral internode growth (Kobayashi *et al*., 1994; Peng *et al*., 1997). In addition to these, auxin also plays a crucial role in the development of primordia on the inflorescence. Mutations in the auxin efflux carrier, *AtPIN1*, result in an inflorescence axis that is pin-like and lacking flowers (Galweiler *et al*., 1998; Oka *et al*., 1999; Reinhardt *et al*., 2000). The shoot phenotype of *acaulis (acl)* class of mutants is extreme and plants show normal inflorescence development and flower initiation but they fail to bolt because of a severe reduction in floral internode elongation (Hanzawa *et al*., 1997, 2000). One *ACL* class gene, *ACL5*, encodes a protein with homology to spermidine synthase and spermine synthase, enzymes of polyamine metabolism; how perturbing polyamine metabolism can totally block inflorescence stem elongation is not clear.

6.7 Evolution of inflorescence architecture

The analysis of mutants and their corresponding genes has been central to the analysis of inflorescence architecture in model plants, and now these studies have been extended to the evolution of inflorescence traits. Model plants have provided candidate genes useful in the comparative studies of inflorescence development. In parallel, the evolution of diverse inflorescence forms in related species has also been analysed by a combination of phylogenetic, developmental and molecular methods. These analyses help in increasing our understanding of how diverse inflorescence types evolved.

Studies on candidate genes have focused on mutants whose inflorescence development resembles natural variants within species. It has been hypothesised that genes identified by their mutant inflorescence phenotype in model species may also control similar inflorescence morphology in related plants (Doust and Kellogg, 2002). For example, the branched inflorescence phenotype of *ts4* mutant of maize resembles the inflorescence of *Sertaria italica* (Neuffer *et al*., 1997; Doust and Kellogg, 2002); therefore, it has been suggested that *TS4* could be a good candidate for a gene regulating this trait in *S. italica*. Similarly, mutants with altered spikelet number in maize could represent genetic loci that regulate variation in spikelet number

in other grasses. Although a mutant phenotype may resemble that of a related species/probable progenitor, the role of the candidate gene in the evolution of this inflorescence trait is likely to be relevant only if the final inflorescence structure arises from similar developmental events. Consider the phenotype of *Sos1* allele of maize, and teosinte – the probable progenitor of maize. Both have an inflorescence bearing single spikelets rather than a pair of spikelets. However, the development of a single spikelet is the outcome of distinct developmental events; in *Sos1*, only a single spikelet primordium is formed, while for teosinte, both pedicillate and sessile spikelets are formed and the pedicillate spikelet is aborted later in development (Doebley *et al*., 1995). In this instance, at least *Sos1* was not involved in the *evolution* of this trait in maize.

In a study of the evolution of the inflorescence form in the bristle grass clade, Doust and Kellogg (2002) compared the development of inflorescence structures in these morphologically diverse species, in parallel with a phylogenetic analysis. The bristle grass clade includes panicoid species in which some inflorescence branch meristems are transformed to setae or bristles. These studies did not focus on the mature form of the inflorescence; rather, they looked at how these inflorescence structures developed. The inflorescences found in members of this clade vary from some that are long to those that are more compact; they also range from few branches to those that have highly branched inflorescence forms. The authors found that the diverse morphology of mature inflorescences in the bristle grass clade is an outcome of a combination of changes that occur during different stages of development. For example, changes that affect the branching and differentiation of primordia happen early in the development of the inflorescence structure, while changes in the inflorescence form that involve the elongation of the axis happen later (Doust and Kellogg, 2002). Thus, the differences in the mature morphology of these inflorescences reflect a combination of changes that occur during different developmental stages, and relatively few developmental changes account for the inflorescence types of the bristle clade (Doust and Kellogg, 2002).

The candidate gene approach has been particularly informative, especially when genes with central roles in meristem identity have been the focus of these analyses. The conserved function of meristem identity genes like *TFL1* and *LFY* in regulating meristem identity means that they are prime candidate regulators of shoot and inflorescence form in diverse plants. Baum and colleagues analysed the role of the FM regulator *LEAFY* in the control of rosette flowering in Brassicaceae (Shu *et al*., 2000; Yoon and Baum, 2004). As reduced *LFY* function results in a shift from floral identity towards inflorescence meristem identity, and ectopic *LFY* can lead to rosette flowering (Weigel and Nilsson, 1995), *LFY* orthologues were cloned from three different rosette flowering species. *Ionopsidium acaule, Idaho scapigera* and *Leavenworthia crassa* are rosette flowering species, and bear flowers in the axils of rosette leaves; thus, their inflorescence architecture is unlike the raceme of *A. thaliana* (Figure 6.6), which bears solitary flowers on an extended inflorescence stem. Yoon and Baum (2004) used a transgenic strategy to test if the FM identity gene *LEAFY* played any role in the evolution of the rosette flowering trait in these

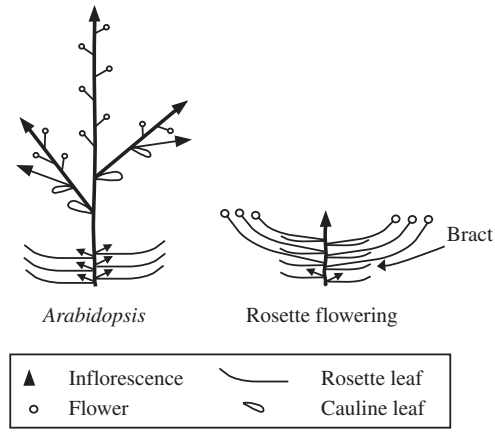

Figure 6.6 A schematic representation of inflorescence and rosette flowering architecture based on images from Yoon and Baum (2004). Leaves are represented by curved lines, cauline leaves on *Arabidopsis* inflorescence subtend secondary inflorescences, and bracts on rosette flowering species directly subtend flowers. Flowers are shown as circles while shoot meristems are shown as triangles.

plants. They tested whether *LFY* orthologues from *I. acaule, I. scapigera (IscLFY1)* and *L. crassa (LcrLFY)* could alter *Arabidopsis* inflorescence structure to a rosette type. Results indicated that an introduction of *IscLFY1* and *LcrLFY* did alter the shoot architecture of *Arabidopsis*, but did not conclusively prove that *IscLFY1* and *LcrLFY* contribute to rosette flowering in *I. scapigera* and *L. crassa* (Yoon and Baum, 2004).

The analysis of quantitative trait loci regulating inflorescence and shoot development in several model plants has also been very useful in the identification of candidate genes (Lan and Paterson, 2000; Ungerer *et al*., 2002). In the future, the comparative analysis of inflorescence development in related species should increase our understanding of how diverse inflorescence forms develop.

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7 Root architecture

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7.1 Introduction – an evolutionary perspective

Root system plasticity is an essential ability of plants that has evolved during the past 400 million years, which facilitates adaptation to highly variable physical and chemical soil environments. At the structural and functional level, root plasticity became increasingly important when primitive plants such as mosses and ferns initiated the conquest of land and became independent of aquatic environments. Plant root systems have, therefore, experienced a progressive transformation during evolution. The earliest land plants probably lived at the interface between land and water bodies, in extremely wet environments, and grew a photosynthetic stem above the shallow freshwater in which they lived. The root systems of these primitive plants were probably rather simple, considering that they did not face problems of acquisition of water or of efficiently anchoring themselves to the soil. An increasing number of land plants began to stabilize the land with their primitive roots (rhizoids), promoting the development of more sophisticated vegetation, as soil replaced sand (Raven and Edwards, 2001). Since those primitive beginnings, the limited availability of certain mineral nutrients in the soil represented an important challenge for the survival of plants and became a major adaptive force driving the evolution of complex root systems.

As plants started becoming independent of very moist environments, it is easy to envisage that root branching and root hair development were the steps which followed in the evolution of plant roots in order to secure maximum efficiency of nutrient and water uptake, and a strong anchor to the soil. The establishment of genetic programs coordinating the different elements that determine root architecture – namely root growth, root branching and epidermal cell modification – gave rise, through plant evolution, to the diverse root systems and different spatial configurations present in today's plants.

Mutualistic associations between fungi and roots also played an important role in the early evolution of land plants. Mycorrhizal fungi benefited their host plants by increasing the ability of roots to capture essential nutrients, especially phosphorus. Given the poorly developed soils available at the time of the first colonization, the association of roots with mycorrhizal fungi may have been a critical step in

promoting colonization of land by plants. Currently, mycorrhizal symbiosis occurs in the vast majority of vascular plants, both wild and cultivated. Later on in evolution, around 70 million years ago, soil bacteria of the genus *Rhizobium* also made use of part of the plant machinery used by fungi to establish their symbiosis with legume roots (Marx, 2004).

The aim of this chapter is to provide a review of root system architecture and recent knowledge about the molecular mechanisms regulating root growth and differentiation. We begin with a discussion of the various types of root systems; then, we review recent advances in the knowledge of molecular, genetic and cellular processes that modulate embryonic and postembryonic root development in the model plant *Arabidopsis thaliana*; we proceed to focus on root specializations that maximize nutrient extraction (notably proteoid roots) and the signaling effects that certain nutrients exert on root growth; and finally, we present recent findings in the understanding of the mechanisms that enable root symbiosis with mycorrhizal fungi and *Rhizobium*, which play critical roles in phosphorus and nitrogen acquisition by plants.

7.2 Basic root systems

In angiosperms, the first structure that emerges immediately after seed germination is the radicle or embryonic root. This gives rise to the primary root from which lateral roots emerge to form a root system with the capacity to explore the soil by extensive iterative branching processes. According to Lynch (1995), the architecture of the root system is determined by (i) the particular morphology of the root, including the arrangement of individual cell layers and the number and length of root hairs and lateral roots; (ii) the root system topology – how axes of individual roots are angled with respect to each other during the branching process; and (iii) the distribution of roots in a positional gradient. Taking these considerations into account, a picture emerges in which the primary root growth, together with its branching patterns, gives rise to the principal types of root systems of higher plants: taproot, fibrous and food storage root systems.

7.2.1 Taproot systems

The primary root becomes a taproot when it grows continuously downward into the soil reaching a greater depth than that of the lateral roots. Lateral roots that are formed directly from the taproot are called secondary roots, which in turn give rise to further branches called tertiary roots. Reiteration of such a developmental branching program can generate a root system with great complexity. Most gymnosperms and dicotyledonous plants develop a taproot system. As shown in Figure 7.1a, the taproot system consists of a vigorous main root (taproot) that grows deep into the soil in order to reach water and nutrients. The taproot, together with its branches, provides strong support for the aerial parts of the plant.

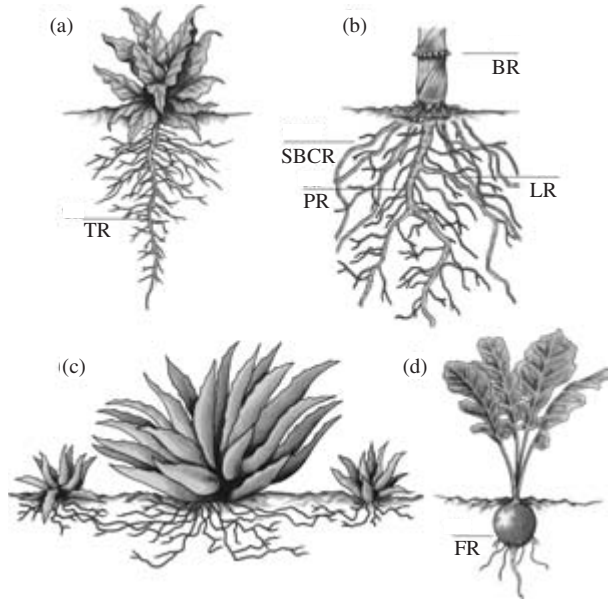

Figure 7.1 Root systems: (a) tap root system, (b) fibrous root system, (c) root system of cacti and (d) storage root system. Abbreviations: TR, tap root; BR, brace root; SBCR, stem-borne crown roots; PR, primary root; LR, lateral root; FR, fresh root. (Drawings by Paul Tarin.)

The plant *A. thaliana* has been used as an example of a model taproot system. Under optimal environmental conditions, the primary root of *Arabidopsis* grows steadily downward due to the ability of the root apical meristem cells to divide continuously. In this growth pattern, also called indeterminate (Veit, 2004), the root apical meristem plays a crucial role in postembryonic development by maintaining a steady stream of precursor cells and by coordinating their subsequent differentiation (Van den Berg *et al*., 1997; Umeda *et al*., 2000). Indeterminate growth can be altered by low nutrient or water availability. Under adverse conditions, meristem cells can reduce or stop cell division, leading to an arrest of primary root growth accompanied by a stimulation of lateral root emergence. This gives rise to a different root system architecture than that observed under normal growth conditions (López-Bucio *et al*., 2003).

7.2.2 Fibrous root systems

In monocotyledonous plants, the primary root has a short life span that contrasts with that of the taproot. Fibrous root systems sometimes develop from stem-borne roots that grow above or within the soil. The postembryonic stem-borne root system gives rise to branches that build a root system. Maize (*Zea mays*) is a typical example of a plant that produces a fibrous root system (Figure 7.1b).

In maize plants, the first stage of root development involves the formation of the primary root and a variable number of seminal roots. The second stage involves the initiation of stem-borne roots that arise from stem nodes. Stem-borne roots formed at consecutive underground nodes are called crown roots, whereas the roots formed at consecutive aboveground nodes of the stem are called brace roots (Figure 7.1b) (Hochholdinger *et al*., 2004). Although the brace roots need to reach the soil to perform their functions, all the root types described previously have the capability to form lateral roots and root hairs. One important difference of fibrous systems in comparison to taproot systems is that in fibrous systems, no root has preferential growth over the others. In fact, later on in development, the post-embryonic stem-borne root system becomes dominant and is responsible, together with its lateral roots, for the major portion of water and nutrient uptake (Hochholdinger *et al*., 2004).

Most stem-borne roots usually grow underground. However, some plants develop roots produced from aboveground structures; these roots are generally named aerial roots or prop roots, as is the case of the brace roots of maize. Prop roots have the specific function of stabilizing the main stem and they are also capable of branching and absorbing nutrients and water.

As in other plants, the fibrous root system architecture of maize is controlled at the genetic level. A number of maize mutants affected specifically in root development have been identified. The recessive mutant *rt1* forms no, or fewer, crown and brace roots, while the primary and seminal roots are not affected (Hochholdinger *et al*., 2004). In the recessive mutant *des21*, lateral seminal roots and root hairs are absent (Gavazzi *et al*., 1993). Root hairs are lacking in the recessive mutants *rth1–3* (Wen and Schnable, 1994). The mutants *lrt1* and *rum1* are affected before lateral root initiation and mutants *slr1* and *slr2* are impaired in lateral root elongation (Hochholdinger and Feix, 1998; Hochholdinger *et al*., 2004).

In *lrt1*, lateral roots cannot be induced by auxin. However, inoculation with the vesicular arbuscular fungus *Glomus mosseae* or growth in a high phosphate soil induces lateral roots in this mutant (Paszkowski and Boller, 2002). These findings suggest that many root developmental traits in maize are under monogenic control, and that some of these genes may act in a pathway that integrates nutrient sensing signals with particular developmental processes. Maize mutants are now the starting points for identifying molecular mechanisms involved in root formation.

In addition to maize, diverse tropical trees such as *Ficus benghalensis* and the coastal dwelling mangrove *Rhizophora mangle* form prop roots. Roots require oxygen for respiration. Low oxygen levels in the rhizhosphere, caused by water logging or complete submergence, is a serious environmental stress that affects plant distribution in natural habitats. In the Central Amazon floodplain, which represents one of the largest inundation areas in the world, covering more than 300 000 km^2, many tree species show root developmental adaptations to anaerobic conditions caused by inundation. Some of the trees, such as *Tabernamontana jurana* and *Salix martiana* respond to low oxygen conditions by the formation of adventitious roots, the presence of air spaces in the root cortex and the development

of apoplastic barriers in the root exodermis. The roots of mangroves, *Avicennia germinans* and *Laguncularia racemosa*, produce pneumatophores (air roots), which are root extensions with negative gravitropism. These grow upward out of the water and provide adequate aereation. Therefore, the roots of such trees serve not only to anchor but also to aerate the root system.

7.2.3 Roots of desert plants

Agaves and cacti are succulent desert plants that grow in arid and semiarid regions under conditions where water is scarce and temperature variations are extreme, thus making water uptake a crucial challenge for their root systems. Although some cacti such as *Lophophora, Pterocactus* and *Peniocereus* have modified taproots for water storage, most agaves and cacti form shallow root systems (Figure 7.1c). These systems more efficiently explore the upper layers of soil, foraging for nutrients and acquiring water when occasional rains occur. There are obvious differences between the root systems of cacti and the taproot and fibrous root systems of other angiosperms. In the case of the taproot system, the primary root keeps growing because of the indeterminacy of its meristem, whereas agaves and cacti develop a short-lived primary root. The short life of the primary root is due to a particular growth pattern in which root meristematic cells enter a determinate developmental program. In this program, root meristematic cells divide for few days after germination and then differentiate (Dubrovsky, 1997). Following primary root growth arrest, pericycle cells are activated to form lateral roots. If we consider that the cessation of primary root growth allows the rapid formation of lateral roots that grow in the shallow layers of soil, then the water and nutrient uptake efficiency of the newly formed root branches may increase the survival potential of young plants that experience drought for prolonged times.

Although root systems of cacti are characterized by short-lived primary and lateral roots, they do not develop typical fibrous root systems. In contrast, there are several individual examples such as the cactus *Copiapoa coquimbana*, which develops a root system in which a single main root grows vertically, while several shallow roots grow horizontally in all directions. Many agave species develop a root system composed of multiple shallow roots that grow vertically and give rise to laterals that usually grow horizontally and branch in the same direction producing a root system that extends radially.

7.2.4 Food storage roots

The root systems of some species have the special capability of storing important substances for growth including water, minerals, carbohydrates and vitamins. These roots are commonly named food storage or fleshy roots (Figure 7.1d). Examples of plants with fleshy roots are carrot (*Daucus carota)*, sweet potato (*Ipomoea batatas)* and sugarbeet (*Beta vulgaris)*. The main structural characteristics of storage roots are the abundance of parenchyma cells and the presence of a permeable

vascular tissue. The development of storage roots is essentially similar to that of non-fleshy roots, except that the main root grows in diameter through generation of cells derived from additional concentric layers of cambium, resulting in formation of an extremely thick tissue, typically, with high concentrations of carbohydrates.

7.3 Regulation of root architecture

The main functions of the root system are to provide an anchor and support for the plant, to seek out and absorb water and nutrients from the soil and to store the products of photosynthesis from the shoot system. These functions are facilitated by growth of the primary and lateral roots through continued cell division and cell expansion in the root tips, which are also regions of gravity and moisture perception. The spatial configuration of the root system varies among plant species. Some root systems can penetrate to remarkable depths into the soil, for example, roots of the desert shrub mesquite (*Prosopis juliflora*) have been found at depths of over 50 m below the soil surface. Other root systems have increased root biomass when compared to shoot biomass. In plants of winter rye (*Secale cereale*), the surface area of the root can be 130 times that of the shoot.

Although different root types can initiate from different tissues during embryonic and postembryonic development, common developmental features can be seen in the mechanisms that underlie histogenesis and radial patterning processes. These similarities include the approximate location of apical initial cells within the meristem, their position-dependent identity and their infrequent and polarized division (Van den Berg *et al*., 1995; Nakajima and Benfey, 2002). This basic pattern of meristem structure is an important target in the modification of root architecture beyond the basic taproot or fibrous patterns. As described later, the root apical meristem plays a fundamental role acting as sink and source of signals that regulate root system architecture and modulate the adaptation to environmental stress.

7.3.1 Embryonic root development

In most angiosperms, the primary root meristem is formed early during embryogenesis. Figure 7.2 shows the different developmental stages of embryonic root development. During embryo development, the zygote undergoes an asymmetric division to form an apical and a basal cell. Radicle formation proceeds through regular, predictable cell division (Scheres *et al*., 1994). The apical cell undergoes three rounds of mitoses to form an eight-celled, two-tiered proembryo. The upper tier of the proembryo gives rise to the shoot meristem and most of the cotyledons, while derivatives of the lower tier contribute to the cotyledons, the hypocotyl and most of the root. The basal cell divides to form the suspensor. The uppermost cell of the suspensor is called the hypophysis. The origin of the quiescent centre (QC) and the columella of the root cap can be traced back to the single hypophysis cell.

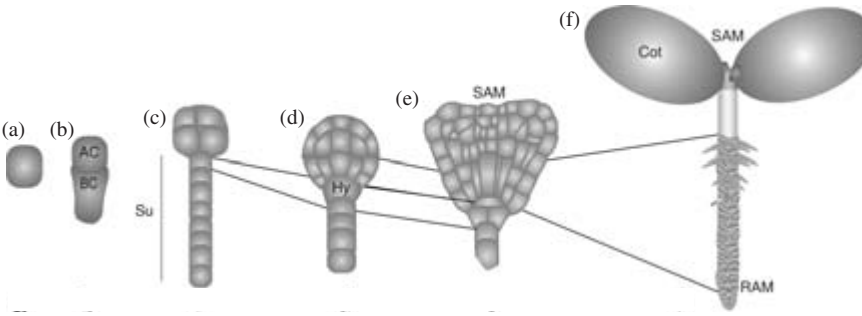

Figure 7.2 Embryonic root development. (a) zygote, (b) two-cell stage, (c) eight-cell stage, (d) globular stage, (e) heart stage and (f) seedling. Abbreviations: AC, apical cell; BC, basal cell; Su, suspensor; Hy, hypophysis; SAM, shoot apical meristem; Cot, cotyledon; RAM, root apical meristem.

In conclusion, it can be said that the radicle is derived from two different cells whose origins can be traced to the very first division of the zygote.

7.3.1.1 Auxin regulation of embryonic root development

In *Arabidopsis*, auxin regulation of root meristem establishment begins in early embryogenesis. Dynamic gradients of auxin accumulation and response during embryogenesis, which are mediated by cellular efflux are required for proper formation of embryo structures (Friml *et al*., 2003). An important role for auxin in elaborating the embryonic axis is supported by both pharmacological and genetic evidence. First, developmental alterations can be induced in embryos by blocking auxin movement with substances that inhibit auxin transport (Hadfi *et al*., 1998) and second, mutations in three *Arabidopsis* auxin-related genes, *MONOPTEROS (MP), BODENLOS (BDL)* and *GNOM (GN)*, cause defects in axis elaboration.

During embryogenesis in *monopteros* mutants, the basal pole of the embryo, including the radicle, fails to form, indicating that *MP* gene function is required for proper root development. The defect in *mp* mutants is evident as early as the eight-cell stage of embryogenesis. At the triangular stage, cells of both the lower tier and the hypophysis divide aberrantly. *MP* encodes a member of the auxin response factor (ARF) protein family (Hardtke and Berleth, 1998). ARF transcription factors are proteins that bind and regulate the transcription of auxin responsive promoters (Ulmasov *et al*., 1997).

The *BDL* gene is involved in auxin-mediated processes of apical basal patterning. *bodenlos* mutants lack the entire root, which suggests that auxin-promoting effects influence an early stage in normal root development. The earliest defect observed in *bodenlos* mutants is an abnormally oriented division plane of the apical daughter cell of the zygote, whereas, later in development, the uppermost derivative of the basal daughter cell of the zygote fails to give rise to the QC of the root meristem and the central root cap. *BDL* encodes the transcriptional repressor IAA12 (Hamman *et al*., 2002).

Mutations in *GN* cause defects that are highly reminiscent of the effects of blocking auxin transport. *GN* encodes a guanine-nucleotide-exchange factor of certain GTPases, which are essential regulators of vesicle trafficking in many organisms. GNOM has an essential role in the maintenance of endosomal integrity and function. This led Steinmann *et al*. (1999) to suggest that GNOM could regulate the vesicle trafficking required for the coordinated polar localization of auxin efflux carriers that in turn determine the direction of auxin flow.

7.4 Parts of the root system

7.4.1 Primary root tip

The first root structure that originates from the embryo is the radicle. In germinating gymnosperms and dicotyledonous seeds, the radicle develops as the primary root, which grows directly downward as the taproot and initiates lateral roots. Each root grows in length through proliferative activity of the root apical meristem. Differentiation processes produce the tissues and cell types present in the mature root. Because of the restriction of certain developmental events to particular regions, several root regions with particular morphological characteristics can be recognized: the cell division, cell elongation, cell differentiation and cell maturation zones. Figure 7.3a shows the different morphological regions of the *Arabidopsis* primary root. However, a much more complex map of the *Arabidopsis* root results from comparisons of gene expression. Gene expression studies identified 15 different root zones that correspond to cell types and tissues at progressive developmental stages. These expression patterns traverse traditional anatomical boundaries and show putative hormone activity centers (Birnbaum *et al*., 2003). Cell-type-specific gene expression profiles facilitate the identification of the determinants of cell fate.

The root apical meristem contains a distinct central region of mitotically inactive cells – the QC – whose apparent function is to inhibit the differentiation of a group of initial or stem cells that undergo cell divisions to continuously produce each of the root cell types (Van den Berg *et al*., 1997). In *Arabidopsis*, the QC is composed of a group of four cells in which cell division is essentially nonexistent. QC cells have a characteristic ultrastructure and express distinct markers compared with dividing meristem cells (Figure 7.3b).

Proximal to the QC, the rate of cell division increases rapidly. This can be seen in *Arabidopsis* root meristems that express a fusion construct between mitotic cyclin and the GUS reporter gene *(CycB1,1 : GUS)* in which dividing cells are stained (black dots) (Figure 7.3c). Auxin has a central role in the establishment and elaboration of pattern in root meristems. By using an *Arabidopsis* line that harbors an auxin-responsive promoter linked to GUS *(DR5::uidA)*, Sabatini *et al*. (1999) showed that auxin is accumulated in the root tip, with an apparent high concentration (auxin maximum) in the columella initial/QC region (Figure 7.3d). Treating *Arabidopsis* roots with NPA – an auxin transport inhibitor – not only shifts

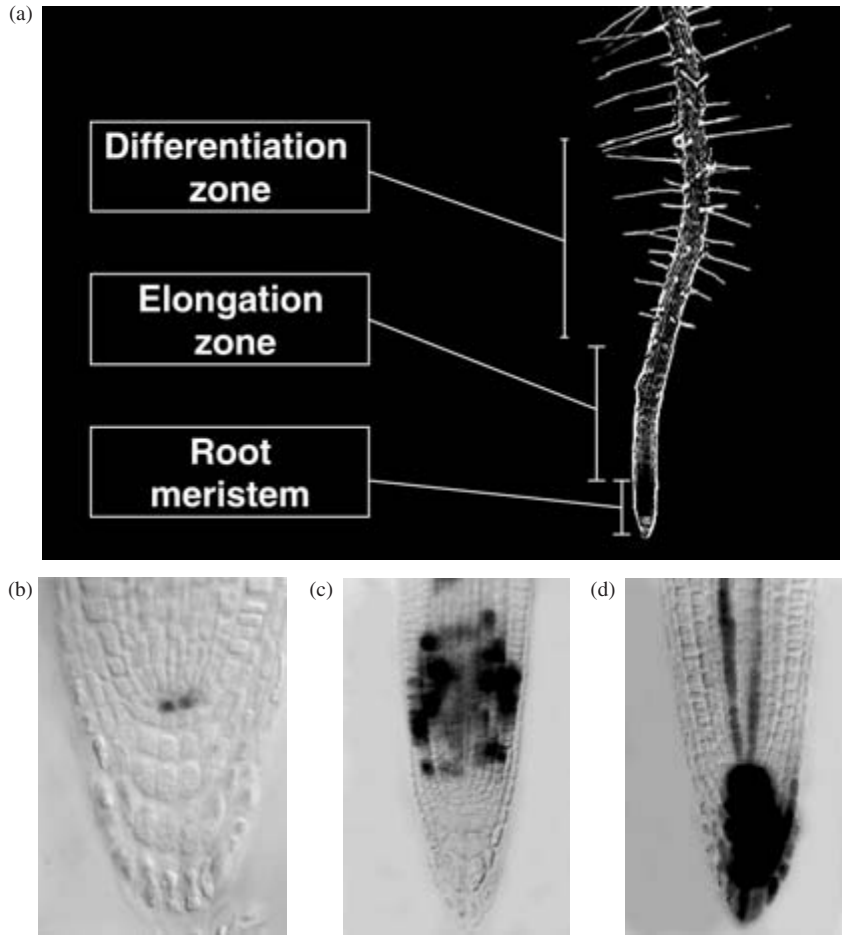

Figure 7.3 *Arabidopsis* primary root. (a) Parts of the primary root; (b) expression of the *QC46:uidA* marker in the quiescent centre; (c) expression of *CycB1,1:uidA* in the primary root meristem; and (d) expression of *DR5::uidA* in the primary root tip.

the auxin maximum to a more basal region of cortical cells, but also leads to the acquisition of QC identity in former epidermal, endodermal and cortical cells (Sabatini *et al*., 1999). This suggests that differential auxin distribution in root tips is required for specification of cell types.

In the root apical meristem, the daughters of initial cells undergo a small number of cell divisions and undertake progressive elongation and differentiation. A stereotyped division of initial cells and the subsequent acquisition of cell fate generate the radial organization of roots. Mutations that disrupt patterning of the ground tissue and vascular cylinder have been identified. In both the *short-root (shr)*

and *scarecrow (scr)* mutants, instead of cortex and endodermis, there is a single mutant layer between the epidermis and the stele. This suggests that both *SHR* and *SCR* genes are required for the longitudinal division of the initial cell that gives rise to endodermis. Both genes encode members of the GRAS family of transcription factors (Helariutta *et al*., 2000).

The root elongation process is mediated by increases in cell number and cell size: cell division and elongation result in the root tip being pushed forward into the soil. Elements of the cytoskeleton play important roles in the control of direction(s) of cell growth (Dolan and Davies, 2004).

7.4.2 Internal root structure

The development of plant organs requires the establishment of symmetry. Radial symmetry in roots is set up during embryogenesis and maintained during postembryonic growth. In mature roots, tissues are arranged in concentric layers (Figure 7.4a). The external layer is the epidermis, which usually consists of a single layer of cells composed of two cell types, those that form root hairs, termed trichoblasts and those lacking root hairs termed atrichoblasts (Figure 7.4a). Root hairs are tubular outgrowths that extend by tip growth processes similar to those in pollen tubes and fungal hyphae. The high numbers of root hairs greatly increase root surface area, which helps to optimize water and nutrient uptake. Their small diameter relative to the root axis further enables uptake processes due to improved close contact with fine soil particles and increased exploration of soil spaces. Internal to the epidermis is the cortex, which is usually several cell layers in thickness, but in the case of *Arabidopsis*, consists of only one cell layer. Most cortical cells function as storage cells for the plant and have been related to air distribution within the root because of abundant intercellular spaces. Deeper into the root cylinder we find the endodermis, which is characterized by a narrow band that extends along the radial and transverse walls. This band, termed the casparian strip, is chemically distinguishable and highly hydrophobic because it is impregnated with suberin and lignin. The main function of the casparian strip is to prevent direct apoplastic movement of water and nutrients between the cortex and vascular cylinder. Internal to the endodermis is the vascular cylinder itself composed of the pericycle and the transport tissues – xylem and phloem. The outermost of these layers is the pericycle, composed of cells that can re-initiate cell division to produce lateral roots. All cells in the pericycle appear to be capable of lateral root initiation; but, under normal circumstances, only the pericycle cells nearest the internal xylem poles perform this function. In the centre of the root, the vascular tissue has bilateral symmetry with water-conducting xylem on the axis of symmetry, flanked on both sides by the sugar-transporting phloem. Xylem cells (tracheary elements) are characterized by specific cell wall thickening that form helicoidal-shaped rings. The principal cell types in the phloem are sieve tube elements, which are the conducting cells and the associated companion cells. The end walls of the sieve tube elements contain perforated sieve plates that permit movement of materials from one element to the next.

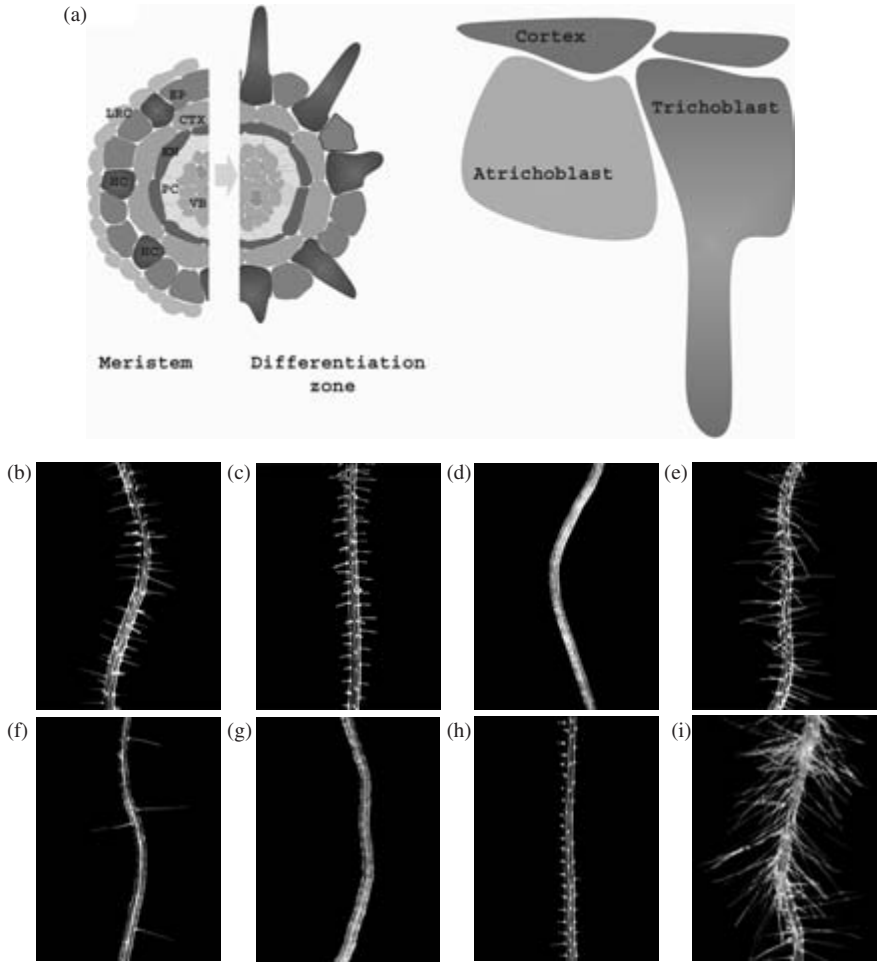

Figure 7.4 Root hair development. (a) Radial structure of the *Arabidopsis* root at the meristematic and differentiation zones. (b)–(i) Photographs of wild-type and root hair mutants: (b) col 0, (c) *rhd2*, (d) *rhd6*, (e) *gl2*, (f) *cpc*, (g) *slr*, (h) *doc1* and (i) *ctr1*. Abbreviations: LRC, lateral root cap; HC, hair cell; EP, epidermis; CTX, cortex; EN, endodermis; PC, pericycle; VB, vascular bundle.

The root apical meristem usually occupies a subterminal position at the root tip because, in most species of vascular plants, it is covered by the root cap. The root cap has important functions that allow proper root growth by protecting the root apical meristem, sensing environmental signals, creating a chemical microenvironment through root exudation and facilitating soil penetration. The root cap is composed of two cell types – the lateral root cap and the columella. Columella cells differentiate and produce large starch grains in their plastids, believed to be the statoliths whose

gravitational sedimentation triggers root graviperception. Lateral root cap cells produce and secrete mucilage, proteins and secondary metabolites. Root cap cells are continuously replaced as they are removed or sloughed off as border cells. Although much less studied than gravity response mechanisms, many other environmental factors such as light, touch and water are also perceived by the root cap (Tsujeki and Fedoroff, 1999).

7.5 Genetics of postembryonic root development

7.5.1 Root hairs

Root hairs are present in the roots of representatives of all major groups of vascular plants. Depending on the species and environmental factors, such as nutrient availability, soil pH and/or the presence of rhizosphere microorganisms, root hair length varies between 80 and 1000 μm with diameters in the range of 10–20 μm. Under favorable environmental conditions, root hair formation takes place at the differentiation zone of the root, where trichoblasts change their growth from normal apposition across much of the cell surface and initiate a tube-like protuberance perpendicular to the epidermal surface (Figure 7.4a).

Along with their crucial role in water and nutrient uptake, root hairs play a significant role in the interaction between plants and nitrogen-fixing microorganisms (e.g. *Rhizobium* and *Frankia*) and symbiotic mycorrhizal fungi. The study of root hairs has experienced notable growth in the last ten years, with research mainly directed toward dissection of the molecular basis of root hair origin and function.

Most of the advances in understanding genetic control of root hair development derive from *A. thaliana* mutants. The first screening for *Arabidopsis* root hair mutants identified over 40 mutants with defects in hair initiation and growth (Schiefelbein and Somerville, 1990). Four of these were characterized in detail and defined as nuclear recessive mutations. The gene product of one of these mutants, *RHD1*, is involved in the early stage of root hair initiation. *rhd1* mutants form bulbous root hairs with limited elongation. *rhd2, rhd3* and *rhd4* mutants form short root hairs (Figures 7.4b and c), which suggests that these mutated genes normally participate in root hair elongation. More recently, Parker *et al*. (2000) identified loss-of-function mutations in eight new genes required for root hair growth in *Arabidopsis*, *SHAVEN1, 2* and *3*; *CENTIPEDE1, 2* and *3*; *BRISTLED1* and *SUPERCENTIPEDE*. The authors also combined mutations in 79 pairs of genes involved in root hair development and formulated an updated model of genetic regulation of root hair formation (Parker *et al*., 2000). In this model, root hair development is divided into four cellular events, cell fate and hair initiation, swelling, transition to tip growth and tip growth. The selection of an initiation site within the hair cell depends on the gene *RHD6* and is influenced by the plant growth regulators, auxin and ethylene. The *rhd6* mutant almost completely lacks root hairs (Figure 7.4d).

The hair/non-hair cell fate is determined at early stages of epidermis cell layer differentiation. Soon after epidermal cells are produced by the division of their

precursor stem cells in the meristem, those destined to become trichoblasts can be identified by cytological differences, including a reduced cytoplasmic vacuolization of atrichoblasts as compared to trichoblasts. This early determination involves cell-to-cell communication not only between immature hair and non-hair cells but also with the cortical cells adjacent to them. For example, surgical detachment of epidermis from cortex leads to increased trichoblast frequencies, suggesting loss of a negative regulatory influence, perhaps due to symplasmic isolation (Barlow, 1984). However, little is known about the specific signals that mediate this communication.

Fundamental gene products associated with root hair cell determination include the homeodomain protein GLABRA 2 (GL2) (Masucci *et al*., 1996), the MYB transcriptional factor WEREWOLF (WER) (Lee and Schiefelbein, 1999) and TRANSPARENT TESTA GLABRA (TTG), which encodes a WD-repeat protein. Mutations in any of these genes cause formation of extra root hairs suggesting that they negatively regulate root hair formation. Figure 7.4e shows ectopic root hair formation in *gl2*. Recently, Bernhardt *et al*. (2003) reported two additional transcriptional factors that determine the non-hair cell type – the bHLH proteins GLABRA 3 (GL3) and ENHANCER OF GLABRA 3 (EGL3). Roots of each of these mutants fail to specify the non-hair cell fate and also produce ectopic root hairs. In fact, *GL2* expression is drastically reduced in *ttg* and *gl3* mutants and completely abolished in the *ttg* and the *gl3–egl3* double mutant. Taken together, these results indicate that WER, GL3, EGL3 and TTG act positively, regulating *GL2* expression and that all these genes affect the non-hair cell fate.

In contrast to GL2, GL3, EGL3, TTG and WER, the small one-repeat MYB proteins CAPRICE (CPC) and TRYPTICHON (TRY) help to determine the root hair cell fate. Mutants in either *CPC* or *TRY* show a reduction in root hair formation (Figure 7.4f), implying that CPC and TRY positively regulate trichoblast differentiation (Wada *et al*., 1997).

Once trichoblast cell fate is established, the root hair growth process is initiated by cell wall expansion to form a bulge. The swelling process implies cytoskeletal rearrangements and the activity of cell wall modifying enzymes such as cellulases and expansins. The transition to tip growth requires the proteins *RHD2*, *SHV1*, *SHV2* and *SHV3*.

Auxin and ethylene signaling affect root hair growth. Addition of auxin and/or the ethylene precursor ACC to the growing media induce either elongation or formation of extra root hairs in diverse plant species. Auxin and ethylene-resistant mutants show either absence or reduction in hair growth (Figures 7.4g–i). Mutations in *AUXIN RESISTANT 2* and *SOLITARY ROOT* genes encoding AUX/IAA proteins affect in root hair formation, mainly at the initiation stage (Figure 7.4g). Mutations at the *TIR3/DOC1* locus, which encodes BIG, a regulatory protein involved in auxin transport, diminish root hair elongation (Figure 7.4h), suggesting that auxin produced in the shoot system must reach epidermal cells to promote root hair growth. Conversely, *Arabidopsis* mutants that overproduce ethylene, such as *eto1*, or that manifest constitutive ethylene responses, such as *ctr1*, produce roots with ectopic root hairs, longer than those in wild-type plants (Figure 7.4i).

7.5.2 Lateral roots

Lateral root formation plays a crucial role in plant development by permitting the construction of branched root systems. Root branches can occur at different sites on the plant root – some as part of normal development and others as responses to environmental factors. Typically, lateral roots are formed successively in an acropetal sequence so that the youngest lateral root is nearest the tip of the parent root whereas the oldest is located close to the root/hypocotyl junction. The process of lateral root formation consists of two major steps: cell cycle reactivation in the pericycle and establishment of a new meristem (Laskowsky *et al*., 1995; Malamy and Benfey, 1997). Lateral roots are initiated by the local activation of pericycle cells at the xylem poles. Mature pericycle cells, once stimulated, dedifferentiate and proliferate to form a lateral root primordium (LRP). The LRP grows through the overlying cell layers of the parent root and eventually breaks through the epidermis and emerges. The first formative divisions in the pericycle depend on the transport of auxin from the root tip in the direction of new LRP axis, whereas shoot-derived auxin regulates the later emergence of lateral roots (Casimiro *et al*., 2001; Bhalerao *et al*., 2002). It remains largely unknown how plants control the reactivation of the cell cycle during development, but it is generally accepted that plant hormones may play a central role.

Many plants can form roots adventitiously on the stem. These roots are formed in less precisely defined locations of the shoot system. In some cases, adventitious roots can be part of the normal developmental program of the plant, but, in most species, they are formed in response to wounding and decapitation of the primary or other roots as occurs when a stem cutting is used for plant propagation.

To understand how lateral root formation is regulated, Celenza *et al*. (1995) performed the first screens aimed at identifying *Arabidopsis* mutants that fail to form lateral roots or that have an increased number of lateral roots. Mutants completely lacking lateral roots were apparently rare, indicating that the genes regulating this process may be either redundant or essential for plant viability. This screening yielded the *aberrant lateral root formation (alf), alf1-1, alf3-1* and *alf4-1* mutants. The *alf1-1* line shows hyperproliferation of lateral roots and has been found to be allelic to the auxin overproducing mutants, *superroot* and *rooty* (Boerjan *et al*., 1995; King *et al*., 1995). The *alf4-1* mutation prevents the initiation of lateral roots, and *alf3-1* is defective in the maturation of lateral roots. The *alf3-1* mutant can be rescued by IAA, whereas the *alf4-1* mutant is not rescued by auxin application (Celenza *et al*., 1995). The gene product responsible for the *alf3* phenotype remains elusive; however, it is likely that it regulates the cell cycle in response to auxin levels, or is required for the transport of auxin to developing LRPs (Celenza *et al*., 1995). *ALF4* has been cloned and encodes a large nuclear protein that is expressed in most tissues of the plant. Auxin does not affect ALF4 levels, intracellular location or tissue distribution. This suggests a model in which ALF4 is required to maintain the developmental plasticity of the pericycle, so that this tissue can be activated to form lateral roots in response to internal or external signals (Di Donato *et al*., 2004).

Auxins and cytokinins are plant growth regulators that play major roles in lateral root development. Exogenous application of auxin to plant roots and overproduction of auxin by transgenic approaches have been found to elicit root branching in several plant species. In contrast, cytokinins are negative regulators of root growth and lateral root formation. The most dramatic phenotypes in lateral root formation are caused by mutations in genes involved in auxin transport/signaling (see later).

7.5.2.1 Role of auxin in lateral root development

Normal auxin transport and response are required for lateral root formation. Mutations in the putative auxin-influx carrier AUX1 show a 50% reduction in lateral root number. AUX1 regulates lateral root development by facilitating the export of IAA from newly developing leaf primordia to the root system, promoting IAA uploading in the primary root apex, and distributing IAA into the developing LRP (Hobbie and Estelle, 1995; Marchant *et al*., 2002). The *transport inhibitor response (tir)* mutants were isolated in a screen to identify mutants defective in auxin transport. From this screening, 16 independent mutants that defined 7 genes were isolated. Some of these mutants were also found to be resistant to auxin, indicating that their primary defect was in auxin responses. Recessive mutations in one of these genes, *TIR3*, result in altered responses to the auxin transport inhibitors NPA and CPD, a reduction in polar auxin transport and a variety of morphological defects that can be ascribed to changes in indole-3-acetic acid distribution. *tir3* is strongly deficient in lateral root production – a process that is known to depend on polar auxin transport from the shoot into the root. Recently, Gil *et al*. (2001) reported that *tir3* is allelic to *doc1* – a mutant previously isolated in screens for plant responses to light signaling. *tir3/doc1* was found to encode a protein of extraordinary size (560 kd), renamed BIG, which contains several putative zinc-finger domains. These domains are present in transcription factors and other proteins with regulatory function in animals and plants. The precise function of BIG in regulating auxin transport and lateral root formation remains to be elucidated.

The *tir1* mutant was found to be defective in a number of auxin-responses, including lateral root formation, with both untreated and auxin-treated *tir1-1* seedlings producing significantly fewer lateral roots (Ruegger *et al*., 1998). Overexpression of TIR1 was found to mimic the effect of growing plants in the presence of auxin, indicating an increase in auxin response in these transgenic plants (Gray *et al*., 1999). It is evident from the analysis of *tir1* that a functional auxin-response pathway is required for correct lateral root formation. *TIR1* itself is expressed in the vascular tissue behind the primordium tip, and in immature lateral root meristems. Since *TIR1* is involved in the auxin response, this supports the hypothesis that auxin is required to stimulate pericycle cells to initiate LRP formation.

The *axr1* and *axr4* mutants were identified in a screen for auxin resistance. The *axr1/axr4* double mutants respond more slowly to gravity, and form fewer lateral roots than either single mutant alone. These results suggest that the two mutations have additive or even synergistic effects. The *AXR1* and *AXR4* gene products may, therefore, act in separate pathways of auxin response, or perhaps perform partially

redundant functions in a single pathway. The *AXR1* gene encodes a protein with homology to the ubiquitin activating enzyme E1 (Leyser *et al*., 1993). The *axr4/auxl-7* double mutant has the same sensitivity to auxin as the *auxl-7* mutant but forms far fewer lateral roots than either single mutant. The conclusion of this work is that the *AXR4* gene product, along with those of the *AXR1* and *AUX1* genes, is important for normal auxin sensitivity, gravitropic response in roots and lateral root formation (Lincoln *et al*., 1990; Hobbie *et al*., 1995).

Some of the genes that translate the auxin signal to activate lateral root formation are transcription factors. Rogg *et al*. (2001) isolated a dominant auxin-resistant *Arabidopsis* mutant *(iaa28)* that is severely defective in lateral root formation and shows diminished adult size and decreased apical dominance. *iaa28* is resistant to inhibition of root elongation by auxin, cytokinin and ethylene, but responds normally to other phytohormones. *IAA28* is a previously uncharacterized member of the *Aux/IAA* gene family that is preferentially expressed in roots and inflorescences, and in contrast to other *Aux/IAA* genes, its transcription is not induced by exogenous auxin. Studies of the gain-of-function *iaa28* mutant suggested that IAA28 normally represses transcription, perhaps of genes that promote lateral root initiation in response to auxin signals. Another member of the Aux/IAA family, the *solitary root (SLR)* gene was found to regulate lateral root initiation. In *slr* mutants, the primary root forms normally but LRP are totally absent, suggesting a primary defect in pericycle cell activation. *SLR* encodes *IAA14*, a member of the large family of transcription factors that are believed to mediate specific responses to auxins (Fukaki *et al*., 2002).

The *NAC* gene family is specific to plants and consists of proteins with highly conserved *N*-terminal domains that are hypothesized to function in transcriptional regulation. Xie *et al*. (2000) showed that *NAC1*, a new member of the *NAC* family, is induced by auxin and mediates auxin signaling to promote lateral root development. *NAC1* is a transcription activator consisting of an *N*-terminal conserved NAC domain that binds to DNA and a *C*-terminal activation domain. This factor activates the expression of two downstream auxin-responsive genes, *DBP* and *AIR3*. Transgenic plants expressing sense or antisense *NAC1* cDNA show either an increase or reduction of lateral roots, respectively. TIR1-induced lateral root development is blocked by expression of antisense *NAC1* cDNA, and *NAC1* overexpression restores lateral root formation in *tir1*, indicating that NAC1 acts downstream of TIR1. All this information suggests that a myriad of transcription pathways play a critical role in root branching, some of which may act by translating the auxin signal. However, it is currently unknown how these genes function in lateral root initiation and how their activity is influenced by environmental cues.

7.6 Regulation of root system architecture by nutrient signals

All terrestrial plants must obtain inorganic nutrients from the soil to ensure successful growth and development of both vegetative and reproductive tissues. The developmental plasticity of the root system is of fundamental importance to

maximize nutrient and water acquisition. There are two major contrasting types of response to nutrient availability changes. Under conditions of local nutrient abundance, root proliferation within that volume of soil is often greatly enhanced, allowing the plant to opportunistically capture additional resources. However, under nutrient deficient conditions, insufficient availability within the normal soil volume explored by the roots leads to an overall enhancement of root growth. This is often at the expense of investment in shoot growth, as indicated by typically lowered shoot:root ratios.

Agronomists have observed that genotypic differences in P uptake from P-deficient soils may be due to better root growth, increased root branching or the internal P utilization efficiency for root dry matter production. Instead, when roots encounter a nutrient-rich zone in the soil, they also often proliferate within it. Patches rich in nitrogen and phosphorus elicit lateral root growth and often enhance the plant's ability to take up nutrient ions. These plastic root system responses have been proposed to be the major mechanism by which plants cope with the naturally occurring heterogeneous supplies of nutrients in the soil (Hodge, 2004). Lateral root formation is also affected by the general availability of nutrients, being favored by nutrient-poor conditions and inhibited in nutrient-rich conditions. Thus, one way of improving plant nutrition can be the manipulation of the pathways that translate nutrient sensing to root branching. Molecular mechanisms by which roots sense the availability of nutrients and translate the nutrient signals into developmental processes are beginning to be understood (López-Bucio *et al*., 2003).

7.6.1 Effects of nutrient availability on root hair formation

One of the most conspicuous effects of low nutrient availability on root development is the induction of epidermal cell layers to form root hairs. Conditions of low P and Fe availability induce the formation of roots with a greater density of longer root hairs. The elongation of root hairs is regulated by P availability in a dose-dependent manner. Recent work in *Arabidopsis* shows that P deprivation not only affects root hair elongation but can also produce up to a five-fold increase in root hair density. This effect is due to an increase in the number of epidermal cells that differentiate into hair-forming cells (trichoblasts) (Ma *et al*., 2001). When iron is limiting, root hair formation and elongation rates also increase. The extra root hairs that result from limiting iron availability are often located in positions that are occupied by non-hair cells under normal conditions (Schmidt and Schikora, 2001). Although similar, the changes in root hair morphology in response to P and Fe have been found to be under control of different signaling pathways. Epidermal differentiation that is induced by low Fe concentrations requires ethylene. This response is inhibited in mutants that are defective in ethylene signaling and in the auxin resistant *Arabidopsis* mutants *aux1, axr1* and *axr2*, suggesting possible cross-talk between the ethylene and auxin pathways. Epidermal changes that are induced by low P availability are not affected in the ethylene and auxin mutants implying that this response is controlled by an independent signaling pathway (Schmidt and Schikora, 2001).

7.6.2 Effects of nutrient availability on root branching

Plants extend their root systems by producing lateral roots. Root branching offers important opportunities for soil exploration and interaction with beneficial soil microorganisms. A number of plant species that are well adapted to grow in infertile soils with limited amounts of available nutrients exploit the soil environment better through changes in root branching patterns. In particular, white lupin *(Lupinus albus)* grows and proliferates in soils with limited P availability, by the formation of cluster roots. Cluster roots, also termed proteoid roots because of their prevalence in the Proteaceae, consist of groups of small lateral roots that arise from the pericycle. These roots develop more extensively when lupin plants are exposed to limiting P conditions and are specialized in P uptake. Cluster roots have determinate growth, that is, their root meristematic cells divide only for a limited period and then differentiate. After just a few days of growth, proteoid roots become exhausted and form large numbers of root hairs. The increased P-uptake capacity of cluster roots relative to roots that have normal growth is provided by their increased absorptive surface, increased exudation of organic acids and phosphatase and possibly greater expression of P transporters (Neumann and Martinoia, 2002).

In *Arabidopsis*, the formation of lateral roots is greatly influenced by phosphate and nitrate availability. The root architecture of *Arabidopsis* plants that have been grown in low P medium resembles that of the cluster roots of lupins: lateral roots arise in close proximity to each other and are densely covered by root hairs (Figure 7.5). Our group and others have reported that low P availability favors lateral root growth over primary root growth. When quantified, the lateral root density significantly increased in plants grown in a limiting (1 μM) P concentration as compared to plants supplied with optimal (1 mM) P (Williamson *et al*., 2001; López-Bucio *et al*., 2002). Root hairs are longer under low P conditions consistent with their role in P uptake.

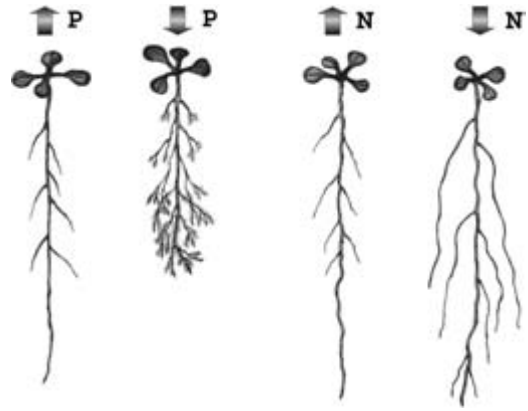

Figure 7.5 Effects of phosphorus (P) and nitrogen (N) on *Arabidopsis* root system architecture.

Changes in nitrate and phosphate availability have contrasting effects on lateral root formation and elongation (Figure 7.5). In *Arabidopsis*, increasing nitrate availability reduces primary root elongation, whereas an increase in P supply has the opposite effect. Lateral roots of *Arabidopsis* show two contrasting responses to high nitrate. Uniformly high nitrate (10 mM) in the medium reduces lateral root elongation throughout the root system, whereas in plants grown on a low nitrate concentration (10 μM), exposure of a section of the primary root to high nitrate induces a local stimulation of lateral root elongation (Zhang and Forde, 1998; Linkohr *et al*., 2002). An important component of the signaling pathway that regulates the nitrate-induced changes in root architecture has been identified. The *Arabidopsis NITRATE-REGULATED1 (ANR1)* gene encodes a NO_3^- -inducible MADS-box transcription factor, isolated in a screen designed to identify genes whose expression is induced by the presence of patches of high nitrate. The lateral roots of *ANR1*-antisense *Arabidopsis* plants do not respond to localized NO_3^- availability, suggesting a role for *ANR1* in the root response to nitrate (Zhang and Forde, 1998).

Understanding how low P-induced determinate root development is translated into increased P uptake capacity will be helpful in establishing more efficient P fertilizer strategies in crops and in gaining insight for a future manipulation of lateral root development in transgenic plants. In a similar way, identification of novel genes that participate in the signal transduction pathways that interpret the environmental availability of nutrients to modify root development will be crucial to manipulate nutrient uptake efficiency in economically important plant species.

7.6.3 Lipid-derived molecules that regulate root development

Phospholipid-derived molecules play an important role in root development because they are intracellular messengers that mediate a multitude of cellular processes, including cell elongation, cytoskeletal rearrangements and membrane trafficking. Signaling lipids in plants include glycerolipids, sphingolipids, fatty acids, sterols, *N*-acetyl ethanolamines and alkamides (Chapman, 2000; Dunn *et al*., 2004; Wang, 2004). The production of lipid mediators is controlled by various lipid-modifying enzymes such as phospholipases, lipid kinases and phosphatases. Phosphatidic acid (PA) and alkamides have recently emerged as important mediators in the regulation of root system architecture.

7.6.3.1 Phosphatidic acid

Ohashi *et al*. (2003) were the first to demonstrate that regulation of phospholipase D plays an important role in root hair development. They showed that the homeodomain transcription factor *glabra2* (GL2) binds to promoter sequences of the *PHOSPHOLIPASE Dδ1 (PLDδ1)* gene of *Arabidopsis*, thus repressing its transcription. Downregulation of *PLDδ1* in transgenic *Arabidopsis* plants by RNA interference caused formation of globular-shaped root hairs, which occur at random positions,

whereas overexpression of *PLDδ1* led to ectopic root hair formation. These results suggest that GL2 regulates root hair formation, at least, in part, by acting as a transcriptional repressor of the *PLDδ1* gene. Additional evidence for a critical role of PLD in root hair patterning comes from pharmacological experiments. Root hair formation is abolished in *Arabidopsis* seedlings treated with 1-butanol – an agonist of PLD that inhibits PA formation (Ohashi *et al*., 2003). In *Arabidopsis*, there are 12 PLDs, all of which catalyse the hydrolysis of the phosphodiester bond of phospholipids such as phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEA) and phosphatidylglycerol (PtdGly) – a reaction that generates PA and a free head group (choline, ethanolamine and glycerol). Since *PLDδ1* produces PA using PtdCho as a specific substrate, it is possible that GL2 regulates root hair development by modulating PA production.

Support for a role for PA and *PLDδ1* in the regulation of root hair development, as well as in cell elongation and viability came from the study of *xipotl*, an *Arabidopsis* mutant affected in a gene encoding an *S*-adenosyl-L-methionine : phosphoethanolamine *N*-methyltransferase (PEAMT), the enzyme responsible for phosphocholine (PCho) biosynthesis (Cruz-Ramírez *et al*., 2004). *xpl1* mutants have decreased levels of PCho and PtdCho in their roots and showed a short-root phenotype with alterations in root development including reduced root hair elongation and epidermal cell death in the root elongation zone (Figure 7.6). Treatment of *xpl1* seedlings with PA eliminated epidermal cell death and restored normal root hair formation, thus confirming an important role for PA, produced by *PLDδ1* from PtdCho, for normal root hair development.

7.6.3.2 Alkamides and N-acylethanolamines

In the last year, two groups of single chain amides have been reported to alter several aspects of root development, alkamides and *N*-acylethanolamines (NAEs). Alkamides form a group of molecules comprising over 200 related compounds widely distributed in plants. The general structure of alkamides originates from the condensation of an unsaturated fatty acid and an amide. Alkamides are structurally related to NAEs, which are produced by hydrolysis of the membrane phospholipid *N*-acylphosphatidylethanolamine (NAPE) by phospholipase D (Chapman, 2000, Blancaflor *et al*., 2003; Ramírez-Chávez *et al*., 2004). In animals, this reaction is part of the endocannabinoid signaling pathway which regulates a variety of physiological processes, including cell proliferation, neurotransmission and embryo development (Howlett and Mukhopadhyay, 2000; Wilson and Nicoll, 2002). In plants, NAEs are present in different tissues, being quite abundant in desiccated seeds where their levels decline during seed imbibition and germination (Chapman, 2000).

Blancaflor *et al*. (2003) evaluated the effects of high levels of *N*-lauroylethanolamine on early root development of *Arabidopsis*. In young seedlings, this molecule was found to inhibit root elongation, increase radial swelling of root tips and alter root hair development. Older seedlings showed increased lateral root formation. These developmental effects were related to altered cell division, endomembrane organization and vesicle trafficking, suggesting that

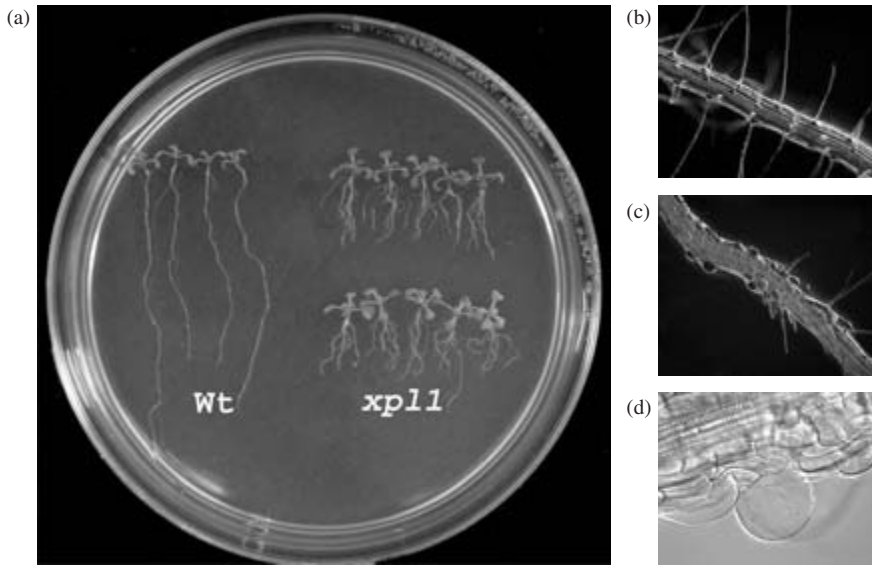

Figure 7.6 Root developmental alterations in *xipotl (xpl) Arabidopsis* mutant. (a) Photograph of 12-day-old wild-type and *xpl* plants growing side-by-side over the surface of an agar plate. (b) Wild-type root hairs, (c) *xpl* root hairs and (d) close up of *xpl* root hairs.

N-lauroylethanolamine may play a role in these fundamental processes. More recently, it was reported that alkamides isolated from *Heliopsis longipes* promote growth and alter root development in plants. In *A. thaliana*, the effects of three alkamides (*N*-isobutyl-2E,6Z,8E-decatrienamide *N*-isobutyl-2E-decenamide and *N*-isobutyl-decanamide) included greater formation and emergence of lateral roots and increased root hair elongation (Ramírez-Chavez *et al*., 2004). Low concentrations of alkamides stimulated primary root elongation, whereas higher concentrations inhibited primary root growth through inhibited cell division and elongation. Although the effects of alkamides are similar to those produced by auxins on root system architecture, two lines of evidence suggested that the ability of the root system to respond to alkamides is independent of auxin signaling. First, alkamides are unable, even at high concentrations (10^{-4} M), to activate the expression of the auxin-inducible markers *DR5::uidA* and *BA3::uidA* and second, the auxin-resistant mutants *aux1-7, eir1-1* and *axr4-2* are equally as sensitive to alkamides as wild-type plants. Taken together, these data suggest that alkamides and possibly NAEs are part of a novel group of plant growth promoting substances that may regulate root development through particular signal transduction cascades yet to be identified.

7.7 Mutualistic associations between roots and soil microorganisms

During their life cycle, the roots of plants grow through the soil in search of water and nutrients. They also influence the soil environment by releasing large amounts

of substances, collectively referred to as root exudates. Root exudates include sugars, amino acids, organic acids, phenolic compounds, vitamins and various secondary metabolites. Depending on the plant species, plant age and environmental conditions, as much as 40% of all photosynthetically fixed carbon is transferred to the rhizosphere, stimulating or inhibiting microbial populations and their activities (Bowen and Rovira, 1999). Root activities, therefore, play a central role in determining the ecology of the rhizosphere, defined as the part of the soil ecosystem where plant roots and soil organisms interact with each other. Once established, the microorganisms influence root development and function by forming symbiotic associations, producing plant growth regulators or through competition with neighboring organisms. Plants are known to establish two important symbiotic relationships – with mycorrhizal fungi and nitrogen fixing bacteria.

The roots of most plants are colonized by mycorrhizal fungi that help them acquire phosphate and other nutrients from the soil. Two major types of mycorrhizal fungi exist: ectomycorrhiza, which associate externally with root cells, and endomycorrhiza, which penetrate root cells. Endomycorrhiza form the so-called 'vesicular–arbuscular' symbiosis – a name given because of characteristic structures formed in the symbiotic root (Barker *et al*., 1998). Arbuscules are intricately branched fungal hyphae surrounded by host plant plasma membranes that form within cortical cells. Vesicles are intracellular fungal storage structures that contain lipids and nuclei and are though to act as propagules.

In *Rhizobium*-legume symbiosis, rhizobial bacteria colonize the roots of legumes such as peas, soybeans and alfalfa, where they convert N_2 into organic forms that are used by the plant to sustain its growth. In return, the plant supplies both fungi and bacteria with carbon compounds for their nutrition. In the last decade, great progress has been achieved in understanding the genetic interplay involved in plant–microbe symbiosis. In the next section, some of the most relevant recent findings in this field are discussed.

7.7.1 Signaling in plant–microbe interactions

Plant–microbe symbioses have an ancient history. Fungal–plant symbioses are much older than rhizobia–legume associations. Detection of fungi in fossilized plants indicates that their associations date back to the first land plants that inhabited our planet, some 400 million years ago. *Rhizobium*–legume association dates back to 70 million years. VA mycorrhizal fungi show little host specificity; however, the rhizobacterium is only capable of productively interacting with a limited number of plant species. Nodulation of legume roots follows a very specific pattern. The infection process involves chemotaxis of the organism toward the roots, apparently in response to flavonoids exudated by roots. In response to flavonoids, the rhizobia produce lipo-oligosaccharides (termed Nod factors) that function as regulatory signals for the production of nodules. The formation of nodules is initiated when the bacteria enter the plant through root hairs. These root hairs then develop into curled structures and the bacteria induce cell division in localized regions of the cortex.

Infection occurs via infection threads that carry the bacteria into envelopes derived from host cell plasma membrane. Proliferation of the membrane-enclosed bacteroids – the name given to the nitrogen-fixing rhizobia – and cortical cells of the root result in the formation of tumorlike growths known as nodules. Nodule organogenesis may be separated from infection as shown by certain rhizobia strains that secrete *trans*-zeatin and elicit nodules without infecting the tissue. In addition, certain genotypes of *Medicago sativa* can spontaneously produce nodules (Truchet *et al*., 1989). Thus, nodule development is a plant-developmental program, which shares similarities with lateral root development because both occur postembryonically from preexisting roots. However, the question remains whether the similarities and differences in the ontogeny of these two organs require the same or different molecular signals. Although Nod factors provoke nodule initiation, it is known that plant hormones such as cytokinins and auxins are involved in nodule development. The fact that certain nodulin genes (genes expressed specifically in nodules compared with roots) can be induced in roots by cytokinin or in pseudonodules produced by auxin transport inhibitors, provides molecular evidence for such a hypothesis (Hirsch *et al*., 1997). Recent expression studies of *aux1*-like genes in *Medicago truncatula* suggest that auxin transport is required during two steps in early lateral root and nodule development: initiation of the primordia and differentiation of the vasculature (De Billy *et al*., 2001). There are, however, two important differences between nodules and lateral roots; first, lateral roots arise from pericycle cells whereas nodules derive from the cortex, and second, lateral roots contain a single central vascular bundle, whereas nodules contain several peripheral vascular strands.

Into the nodule, the bacteria fix nitrogen while receiving carbon compounds from the plant. Genetic studies indicate that legume root cells carry receptors that recognize and bind the appropriate Nod factors. Much of the progress in this field has been achieved through isolation of *M. truncatula* mutants. One group of mutants defined three genes required for early responses to Nod factors, *DMI* (for *Does not Make Infections*) *1, 2* and *3*. Interestingly, these three genes are also required for infection of *Medicago* plants by VA fungi, suggesting that signaling to the plant by both the rhizobia and VA fungi likely shares components of the same pathway. The *DMI2* gene has been found to encode a receptor-like kinase with leucine rich repeats (Endre *et al*., 2002). Receptor-like kinases are located in the plasma membrane with one domain extending to the outside of the cell where it can bind signaling molecules, and an interior domain that appears to have kinase activity. *DMI1* encodes a novel protein with similarity to the ligand-gated cation channel domain of archaea. This protein is highly conserved in angiosperms and are ancestral to land plants, thus suggesting that DMI1 represents an ancient plant-specific innovation potentially enabling root-fungal and rhizobial associations (Ané *et al*., 2004). Whether DMI1 functions as a ligand-gated cation channel remains to be determined, however, biochemical information reveals that DMI1 is required for Nod-factor-induced calcium oscillations in *Medicago* root hair cells involved in the earliest steps of nodulation. Compelling evidence for an important role of calcium in mediating the nodulation response came with the identification of the *DMI3* gene. DMI3, which

acts immediately downstream of a rising calcium concentration in the nodulation signaling pathway, encodes a calcium/calmodulin dependent protein kinase (CCaMK). Members of the CCaMK group have been described in a number of monocotyledonous and dicotyledonous plants and in the moss *Physcomitrella*. Widespread occurrence of DMI3 in the plant lineage fits well with the hypothesis of an ancient origin for VA mycorrhizal symbiosis and of the use of this pathway by *Rhizobium* to induce nodulation. Interestingly, the CCaMK family has no known member in the sequenced genome of *Arabidopsis*, a plant that, like most members of the Brassicaceae family, is unable to establish symbiosis with mycorrhizal fungi.

7.8 Conclusions

Molecular research is increasing our knowledge of the signal pathways involved in root system architecture. Much progress has been made using model plants such as *A. thaliana, L. albus* and *M. truncatula*. The view that emerges is that postembryonic root development is controlled by complex genetic programs, which allow plant roots to respond to a network of environmental factors by changing their morphology, physiology and metabolism. The distinctive patterns of organogenesis in the root system involve complex hormonal interactions where auxins are pivotal players. Many genes that control essential steps in meristem maintenance, root hair growth and lateral root development have been identified. These encode both structural and regulatory proteins of two main types: transcription factors and protein kinases. Molecules derived from phospholipid hydrolysis, such as PA and alkamides can regulate several aspects of plant development ranging from cell integrity to whole root architecture. Notable progress has been made in deciphering the molecular determinants that regulate symbiosis between plants and beneficial microorganisms. One of the genes discovered encodes a calcium- and calmodulin-dependent protein kinase, and the other a ligand-gated cation channel. Thus, calcium signaling appears to be critical for plant–microbe symbioses. Taken together, the information available suggests that plasticity of the root system can be viewed as an adaptive mechanism to increase nutrient capture in adverse soil conditions.

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8 Woody tree architecture

Frank Sterck

8.1 Introduction

The evolutionary invention of lignin some 300 000 000 years ago triggered the evolution of the wide variety of woody architectures encountered today. Gymnosperms were among the first woody plants. Today, the conifers are the most important group of gymnosperms (~550 out of 600 species) and they dominate large parts of temperate and boreal zones. Approximately 200 000 000 years later, angiosperms emerged within the plant kingdom, radiated in a variety of life forms – including woody shrubs, trees, palms and lianas – and currently embrace about 230 000 species. Nowadays, trees surpass other woody life forms in diversity, biomass, dominance, carbon fluxes and architectural complexity. The key to their success is their ability to grow to a great size, win the struggle for light and survive for decades, centuries or even millennia in a constantly changing environment. Their success led to the success of many other plants (e.g. lianas, epiphytes), animals and man by providing arboreal life space, resources, food and shelter.

Trees build their woody architecture with the same 'building blocks' as other plants do. These building blocks are named modular units and are organized in a hierarchical way. The most elementary unit is the metamer, or phytomer (White, 1979; Room *et al*., 1992): it consists of an internode, a node, one or more leaves at the node, one or more meristems in the axil of the leaf and, initially, an apex (Figure 8.1). When a meristem faces periods of dormancy it appears as a bud. Whenever active, such a bud produces a sequence of meristems simultaneously, called an extension unit or growth unit (Figure 8.1, Hallé *et al*., 1978; Bell, 1991). Extension units are most obvious in deciduous trees of seasonal habitats, but they are also frequently encountered in evergreen trees of less seasonal habitats. The axis produced by one apex consists of one or more metamers and/or extension units, and is called a module or sympodial unit (Bell, 1991). To this point, trees do not differ from most other plants. Where they differ from other plants is by organizing the building blocks into stable, large, complex, vertically oriented and heavily branched structures.

Like other plants, trees have vascular cambium that produces phloem (part of the 'bark') to the exterior and xylem (wood) to the interior. In the phloem, sugars and other organic compounds are transported through specialized conduits – the sieve tubes. In the xylem, water and dissolved minerals are transported from the roots to the leaves and other plant parts. Trees produce more xylem relative to

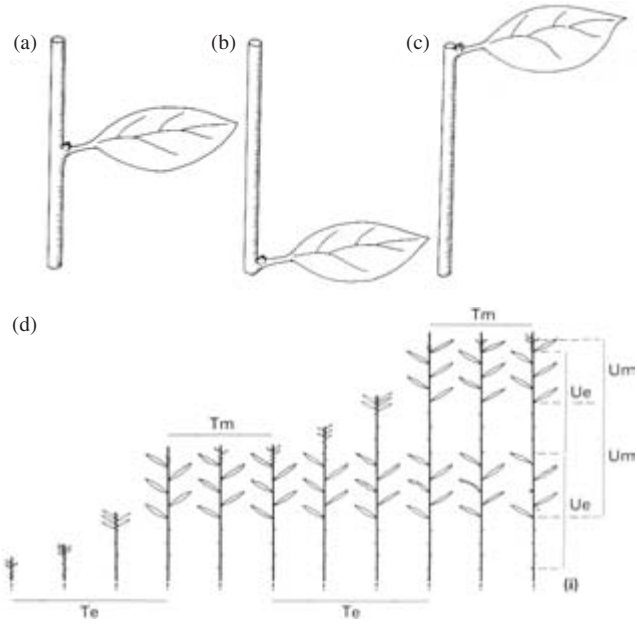

Figure 8.1 (a)–(c) Different representations of a metamer or phytomer, an internode, and a node with appending leaf and axillary meristem. (d) The development of extension units (Ue), the part of a shoot that is expected to result from a period of extension (and not differentiation). Te = time of extension; Tm = time of morphogenesis; Ue = unit of extension; Um = unit of morphogenesis [Figure 283a, b, c, & i (p. 283, Bell, 1991), line drawings by Alan Bryan (Bell 1991), by permission of Oxford University Press].

phloem than do other plants, accumulate xylem over time and may thus produce thick woody branches and stems. In conifers, the xylem consists of longitudinal tracheids that are connected by pits. In such pits, the secondary cell wall is absent and the membranes (modified primary cell walls) are differentiated in a porous margo and a central non-porous torus. The porous margo aperture facilitates the water transport between adjacent cells, and the torus may seal this aperture to prevent an embolism from spreading from one cell to the other (Zimmermann, 1983). Tracheids serve as water transport channels and their solid secondary cell walls provide mechanical strength. Xylem parenchyma is mainly responsible for radial transport and storage of carbohydrates. In angiosperms, vessels evolved with scalariform perforation plates resembling scalariform pitted tracheids. Subsequently, perforation plates became simpler and vessel members became shorter and wider (Figure 8.2), thus improving the water transport efficiency. Concomitantly, narrow fibers with few pits and thick secondary walls evolved. These fibers provide mechanical strength and, with the parenchyma, play a role in the storage of water, carbohydrates and minerals. Angiosperms further faced the evolution of more specialized cell types in the xylem than did gymnosperms, but

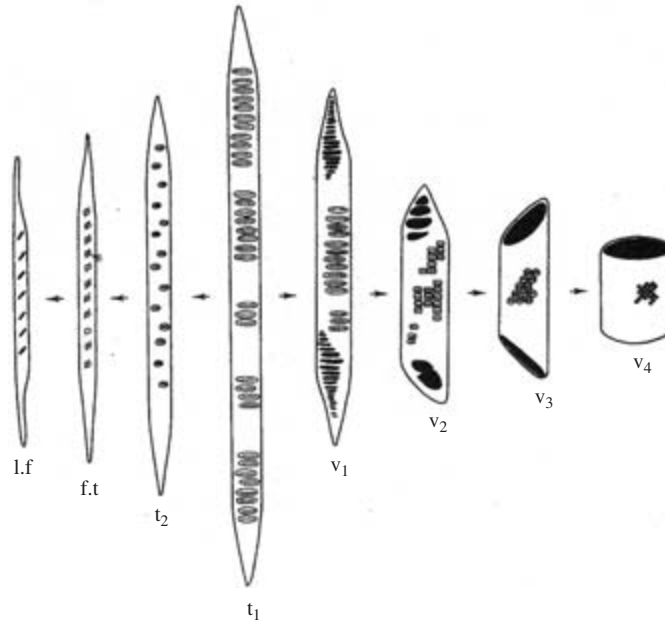

Figure 8.2 Evolutionary differentiation of primitive tracheids (t1) to more specialized tracheids (t2), fibers (fiber tracheid, f.t., libriform fiber, l.f.) and vessels (v1 to v4) [adapted from Bailey and Tupper (1918)].

such specializations are beyond the scope of this chapter (e.g. Panchin and de Zeeuw, 1980).

The modular structure and wood properties enable a tree to grow from a structurally simple seedling to a complex, large, heavily branched structure with a single stem. Several disciplines are concerned with questions of 'woody tree architecture'. This chapter starts with a short overview of anatomical aspects, followed by an introduction of key processes (apical dominance, apical control) and physical constraints (allocation, stability margins). A subsequent discussion of major woody architectural patterns covers (i) different tree species, (ii) different trees of the same species and (iii) different shoots of the same tree. Whenever possible, these patterns are linked with the underlying processes and constraints. Attention is given to the effects of light availability and physical damage on tree architecture, because (i) light and damage are the most obvious factors affecting tree architecture, and (ii) foresters manipulate trees by modifying the light environment (thinning, liberation) and by damaging trees (pruning). Information is provided on the limits of maximum tree height. The chapter attempts to take a general, worldwide approach, to integrate rather disparate fields of research, and to focus on general patterns rather than on particular, local situations.

8.2 Anatomy

8.2.1 Vascular differentiation

The vascular system continues to connect leaves with root tips as long as the tree grows. Three types of vascular differentiation can be distinguished (e.g. Sachs, 1981): (i) Primary differentiation occurs subsequent to cell division and cell growth, in the root and shoot apices, resulting in production of the procambium, primary xylem and phloem, and increasing the length of the shoot and root. (ii) Secondary differentiation is the result of vascular cambium activity which produces new secondary xylem at the inner side and secondary phloem to the outer side. (iii) Regenerative differentiation repairs the vascular system after wounding, but also after branches have dropped off. Secondary differentiation of vascular tissues is the main contributor to the radial growth of stems and branches, and is discussed in more detail here.

Secondary vascular differentiation depend on various signals (e.g. Roberts *et al*., 1988), of which auxin – produced by buds, growing shoots and leaves (Thimann and Skoog, 1934; Wangermann, 1967; Aloni *et al*., 2003) and transported basipetally toward the roots – is the key trigger. Cambial activity and vascular differentiation roughly coincides with bud break in spring, and proceeds from the bud downward (Sachs, 1991). Sachs (1981, 2000) has assembled considerable support for his hypothesis that cells respond to auxin flux by gradually differentiating, and thus becoming the preferred channels for this flux. The differentiating cells thus lead the auxin flux in the longitudinal direction and, accordingly, gradually develop (secondary) vascular strands in basipetal direction (Sachs, 1981). Uggla *et al*., (1998) suggest that the cambium is the major pathway for auxin transport, from where the signal may spread in a radial direction. The longitudinal auxin flux triggers the cell division and differentiation into xylem and phloem. Roots play a role as signal sink, and thus contribute to the orientation of the flux of auxin and vascular differentiation. In addition, other signals, especially cytokinins, promote vascular development (Aloni, 1987, 2001).

In angiosperm trees, vessels become wider but less frequent in the basipetal direction (Sanio, 1872; Bailey, 1958; Digby and Wareing, 1966). The suggestion that vessel size correlates positively with auxin concentration (Digby and Wareing, 1966; Larson, 1969) is not supported by the decreasing auxin concentration and increasing vessel size in that direction (Aloni and Zimmermann, 1983; Aloni, 1987). Aloni and Zimmermann (1983) hypothesized that decreasing auxin levels in the downward direction reduces the differentiation rate of vessels, and that cells, thus, have more time to expand in radius as it takes longer to deposit the inflexible secondary cell wall. Hence, decreasing auxin concentration from leaves to roots leads to an increase in vessel size (both radius and length) in that direction, with vessel density decreasing at the same time. Aloni (1987) suggests that only tall plants such as trees and lianas can build up considerable auxin gradients that can lead to wider vessels in lower densities. This suggestion is still a topic of debate [see e.g. Little and Pharis (1995)], but it agrees with the idea that shrubs and other

small plants, with relatively small vessels (Wheeler, 1991), cannot build up sufficiently strong gradients in auxin to produce vessel tapering.

In temperate forest trees, the distribution of vascular tissues follows a predictable pattern from the wood produced at the start of the growing season (early wood) to the wood produced at end of the growing season (late wood; Kramer and Kozlowski, 1979): vessel members and/or tracheids become narrower, and tracheids and fibers produce thicker cell walls. Ring-porous (angiosperm) trees show a strong and abrupt decrease in vessel size, but vessel size also reduces in diffuse-porous angiosperm trees while tracheid size decreases in conifers. Several hypotheses have been put forward to explain these annual patterns.

Gordon and Larson (1968) suggested that developing shoots act as strong sinks for available resources in the early season, and thus leave limited resources for cell wall production in the wood. In the late season, when new shoots are fully developed, competition for resources may be less intense and more resources are allocated to cell wall formation of late wood. Alternatively, Denne and Wilson (1977) and Wodzicki (1971) suggest that cell wall thickness increased in response to higher auxin levels later in the season. Aloni (1991) suggests that the cambium in ring-porous trees is highly sensitive to low concentrations of auxin, leading to early reactivation of cell division in the vascular cambium and to the production of wide vessels before the full onset of leaf production (Suzuki *et al*., 1996; Aloni, 2001). The higher auxin levels produced by the leaves later in the season may inhibit, either directly or indirectly, the further production of wide vessels. Aloni (2001) suggests that the cambium in diffuse-porous trees is less responsive to auxin and, therefore, that the vessels of diffuse-porous trees do not vary that much in size and develop 2–7 weeks after the onset of leaf expansion (Suzuki *et al*., 1996). It should be emphasized that some of these ideas remain hypothetical, and await further empirical support.

8.2.2 Radial patterns

Worldwide, trees share a number of radial vascular patterns. First, from the pith to the bark, the wood changes from so-called ‘juvenile wood’ to ‘adult wood’. Juvenile wood (though the oldest in age!) contains smaller tracheids, vessel members and fibers, with smaller secondary walls, and has a lower density than adult wood. Adult wood is considered superior in terms of mechanical strength and wood quality (e.g. Pashin and de Zeeuw, 1980). Second, older stem and branches show a transition from sapwood to heartwood in the oldest xylem. The heartwood has no living cells, does not transport water, contains hardly any sugar or starch and may accumulate secondary chemicals (Hillis, 1987). The sapwood contains living cells (at least parenchyma) and usually contributes to the water flux from root to leaves. Third, trees produce reaction wood in a radial direction when the stem or branch is off-vertical and experiences increased gravitational forces. Reaction wood is formed at the bottom side of branches and leaning stems in softwoods (compression wood), and at the upper side in hardwoods (tension wood) (e.g. Wilson and Archer, 1977).

It usually consists of wood with a higher density, thicker cell walls and a different cell wall ultra-structure than normal wood.

8.2.3 Ecotypes

Vascular pattern varies greatly among angiosperms in different habitats. Baas and coworkers (2004) present a classification on the basis of vessel size and distribution (Figure 8.3). The basic advantage of a large vessel size (width and length are correlated) is that water transport efficiency increases with the fourth power of vessel radius. There are, however, disadvantages (tradeoffs) for the 'safety' of water transport. Large vessels have larger pit membrane pores that increase the risks of embolism (Sperry and Tyree, 1988). Wider vessels are also more sensitive to implosion, particularly when they have thin cell walls (Hacke *et al*., 2001). The safety risks increase when wide vessels are exposed to strong, negative water potentials, such as in trees growing under dry conditions (Carlquist, 1985), or in tall trees (Koch *et al*., 2004; Box 1). In addition, wide vessels may contain more air bubbles after freezing, and thus face greater risks of embolism after thawing (Sperry and

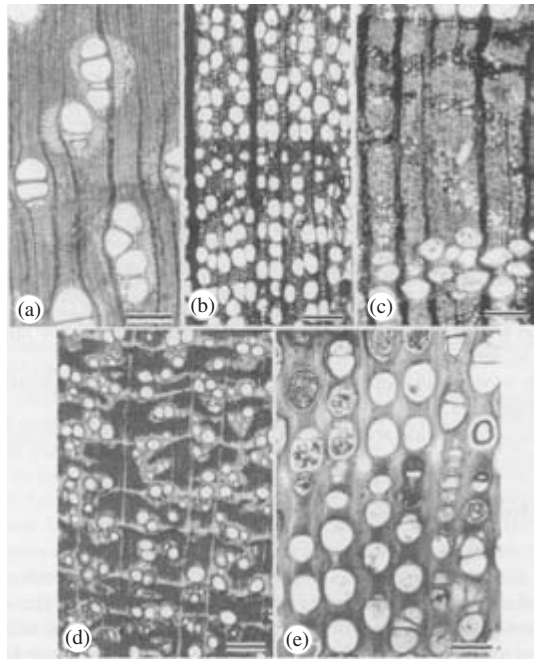

Figure 8.3 Different wood types. (a) Diffuse-porous wood with few wide vessels. (b) Diffuse-porous wood with many narrow vessels. (c) Ring-porous wood. (d) Diffuse-porous wood with different vessel size classes intermingled. (e) Liana-type wood; note the large number of wide vessels, not discussed in text [source: Baas *et al*., (2004)].

Sullivan, 1992). Even in conifers, with typically small tracheids, early wood with wide tracheids tends to be more sensitive to embolism than late wood (Wardrop and Davies, 1961). These mechanisms explain at least part of the differences in vascular architecture among different habitats.

Box 1 Maximum tree height

One key to the success of trees is their ability to grow to a great height, and thus win the struggle with neighboring plants for light (King, 1991a). In every forest, selection and competition cannot drive the height battle beyond certain limits. In some Australian hardwoods and American softwoods, height may surpass approximately 100 m, but not much more. Forests may differ in the maximum height they obtain, depending on the species and site characteristics (soil fertility and climate), and this maximum height is an indication of the potential of a site called site index by foresters. Several mechanisms may contribute to the finiteness of the height struggle.

When trees increase in height, they invest a greater proportion of biomass in wood, and a smaller proportion in leaves (Mäkelä, 1986). Since the woody biomass to leaf area ratio increases with tree size, trees face an ever-increasing respiration load with increasing tree size (Yoda, 1965). This hypothesis suggests that, when increasing wood construction and maintenance costs exceed the photosynthetic income, trees would be unable to maintain positive growth. Old stands are, however, characterized by rather low levels of maintenance respiration rates (5–12%; Ryan *et al*., 1995), and declining growth and growth-respiration costs (Ryan and Waring, 1992). These results suggest that these rates alone are not responsible for the maximum height. The respiration hypothesis is also rejected by a number of other observations: for example, widely spaced trees grow much more rapidly than trees of the same height in 'dense' competition with neighbors, and at maximum height, many trees continue to grow rapidly but do so by investing in diameter alone. Thus, the respiration hypothesis does not suffice to explain maximum tree height [see Ryan and Yoder (1997)].

In recent years, evidence on the hydraulic constraints contributing to the limitation in the maximum height of tree has been accumulating. The basic underlying mechanism is that trees transport water from roots to leaves by creating a sufficiently steep gradient in the water potential. The vapor pressure gradient in the leaves (stomata) creates a water potential gradient in the xylem columns, and thus pulls the water column from root, through the xylem vessels or tracheids, to the leaf. Strong binding forces among water molecules (cohesion) prevent the water column from breakage, as predicted by the cohesion–tension theory (Dixon and Joly, 1895). In tall trees, the total hydraulic resistance increases with the path length that the water must travel as the gravitational resistance increases (Koch *et al*., 2004). To some extent, trees compensate their tall stature by producing more conductive tissues (wider vessels; Pothier *et al*., 1989; West *et al*., 1999; Enquist, 2002), but still the whole tree resistance increases with tree height (Mencuccini and Grace, 1996; Koch *et al*., 2004). To overcome the gravitational forces, leaf water potentials are reduced to two-thirds of the minimum in the top leaves of the tallest trees on earth (Koch *et al*., 2004). Such tall trees transport water to the top leaves by further reducing the leaf water potential, but ultimately risk embolism when the water potential gradient becomes too steep. Tall trees reduce the risk for embolism by closing the stomata in the afternoon (Tyree and Sperry, 1988; Yoder, 1994), but consequently face reduced photosynthesis (Fredericksen *et al*., 1996; Ryan and Yoder, 1997). Tall trees also relax the pull on water column by producing less leaf area for a given sapwood area (Margolis *et al*., 1995; McDowell *et al*., 2002). The lower leaf area to

sapwood area ratio will result in a lower net photosynthesis at the whole tree level. In addition, the positive cell turgor pressure is reduced in top-leaves by the gravitational component of the leaf water potential, and this limits leaf expansion and branch extension growth (Woodruff *et al*., 2004). Strikingly, the top leaves in tall trees show 'desert properties', as they are extremely thick (highest leaf mass per area; Koch *et al*., 2004). These different 'hydraulic' phenomena may contribute to 'hydraulic death' (Midgley, 2003) above a certain stature, and hydraulic theory predicts a maximum height of approximately 120–130 m (Koch *et al*., 2004; Woodward, 2004).

In most forests, however, the majority of trees do not reach the biophysical maximum of approximately 130 m and, moreover, different tree species typically have maximum heights ranging from a few meters up to the maximum canopy height (e.g. Sterck *et al*., 2001; Poorter *et al*., 2003). Becker and coworkers (2000) suggest that in most species, genetic factors and their interaction with tree size and environment, limit the maximum height of trees, rather than physical constraints. Additionally, when trees reproduce, flowers and fruits may drain approximately 30% of the available resources (Kozlowski and Keller, 1966), and vegetative growth is often decreased substantially. So far, the theoretical and empirical evidence is too limited to understand why some tree species grow much taller than others in the same environment.

In the warm, humid environment of lowland tropical rain forests, trees most commonly have wide vessels (>200 μm) that are equally distributed over wood cross-sections (Baas *et al*., 2004), and have low incidence of scalariform perforation plates (Baas, 1982). These trees are thus able to maintain high transpiration levels, and they face little safety risks in the given humid conditions.

Cooler environments [higher latitude (subtropical, temperate) or high altitude] are dominated by two wood types. The diffuse-porous species have short, narrow vessels, have their vessels densely packed and can be deciduous or evergreen. The ring-porous species have very wide early wood vessels, and narrow late wood vessels, and are always deciduous. In ring-porous trees, the wide early wood vessels typically function for only 1 year, while their late wood vessels function for longer. In diffuse-porous species, all vessels may function for more than 1 year. In ring-porous species, bud break and vascular differentiation starts later in spring than in diffuse-porous species, probably because they are most sensitive to freezing due to wide early wood vessels. Once ring-porous species have produced leaves, they are very efficient in water transport. For example, in the ring-porous tree *Ulmus americana*, early wood accounted for more than 95% of water transport (Ellmore and Ewers, 1985).

In warm, xeric environments, three different wood types are encountered. Trees frequently have narrow, densely packed vessels that provide inefficient water transport, but are relatively safe under dry conditions. A second group has short, narrow vessels and long, wide vessels mixed throughout the wood. This type, thus, divides efficient vs. safe water transport between the two vessel types. A third group has only long, wide vessels, such as in moist tropical forest. Unlike most moist tropical forest trees, however, trees of this third 'warm and xeric' group can, if necessary,

access deep subterranean water and thus reduce the risks of drought-driven embolism (Baas, 1986).

Conifers do not show such marked patterns. They dominate only in temperate/boreal zones, or at high altitudes, and their xylem consist mainly of tracheids that vary relatively little in size.

8.3 Mechanisms and constraints

Most gymnosperm and dicot angiosperm trees grow from structurally simple seedlings to complex adult trees. The production of branched structures depends on the potential, position, timing and extension of apical and axillary meristems. The changes in the structure during a tree's life are controlled by particular processes (apical dominance, apical control) and constraints (carbon allocation, physical/mechanical stability).

8.3.1 Apical dominance

Apical dominance refers to the physiological responses that enable the apex of a leader shoot to control the activation of axillary meristems, thus leading to more or less excessive branching. Cline (1997) distinguished four phases in apical dominance (Figure 8.4). In the first phase, lateral meristem (or bud) primordia are formed by the apical meristem, and are often not more than a tiny part of the apical bud. In the second phase, the apical bud elongates and produces a new axis, while the new lateral buds along that axis remain dormant. The classic apical dominance model suggests that auxin produced by young leaves and expanding apical bud (Aloni *et al*., 2003) is transported basipetally and inhibits the release of lateral buds through interactions with other signals (Sachs, 1991). When the apex is decapitated and auxin production fails, one or more lateral buds are released and elongate into a new shoot. In the third phase, the apical dominance is released. In temperate hardwoods, lateral buds usually break 1 year after apical growth. Cytokinins – produced by the roots, possibly after induction by auxin arriving from above – may induce this release of apical dominance (e.g. Sachs, 1991). In the fourth phase, the lateral

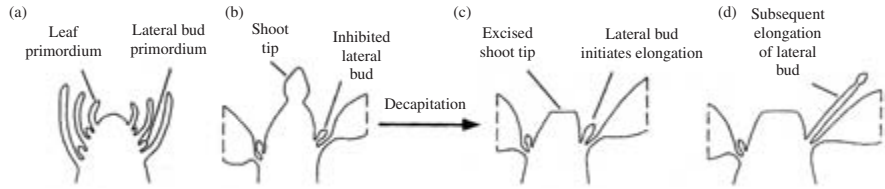

Figure 8.4 The four stages of apical dominance. (a) lateral bud formation; (b) imposition of inhibition on lateral bud growth; (c) release of apical dominance; (d) branch shoot development [adapted from Cline (1997)].

shoot starts to produce its own auxin, which may enhance its elongation (Thimann and Skoog, 1934) and inhibit outgrowth of its own newly produced buds. The shoots that are thus produced after a period of dormancy are described as proleptic (Hallé *et al*., 1978). In contrast, sylleptic shoots, common in tropical species (Hallé *et al*., 1978) and a few temperate species (Wheat, 1980), activate the apical and lateral meristems simultaneously (see Section 8.4.1).

8.3.2 Apical control

When lateral shoots develop (phase four), the growth and orientation of the lateral branches are further controlled by the leader shoot in a process called *apical control* (Brown *et al*., 1967; Wilson, 2000; Figure 8.5). Apical control differs from apical dominance. For example, most pines have one dominant vertical leader shoot and distinct lateral branches. These latter laterals grow shorter, thinner and more horizontal than the leader shoot. Such species have strong apical control, but, at the same time, they have weak apical dominance as laterals often grow out in the same season as the leader. Conversely, many hardwoods (e.g. oak, beech) are characterized by weak apical control and strong apical dominance. They have laterals that are often suppressed for 1 year or more (strong apical dominance), but often have no clear leader shoot and form a more round crown (weak control). In Wilson's words (Wilson, 2000) 'the basic question for apical dominance is what triggers the start of growth, and for apical control why some lateral shoots stop growing sooner than others'. Strikingly, both processes may relate to fluxes and gradients of the same signal, that is, auxin.

Apical control is the influence on growth of lateral branches by the main shoot and its apex. When a distal shoot is removed, this results in accelerated growth of a more proximal lateral shoot, which in addition may bend in the (vertical) direction of the former distal shoot (Wilson, 2000; Figure 8.5). Experimental evidence suggests that polar transport of auxin in the distal, dominant shoot causes the reduced growth of more proximal lateral shoots. In a number of girdling experiments on *Pinus strobus*, Wilson and coworkers (Wilson and Archer 1981; Wilson, 1981, 1986) showed that the auxin moving downward from the stem and branches increases the cambial activity and competitive carbon sink strength of the stem relative to its lateral branches. These mechanisms agree with observations that lateral shoots export carbohydrates to the leader shoot, but not vice versa (Sprugel *et al*., 1991). Only after a period of whole tree dormancy, that is, winter or dry season, is carbon allocated from roots and/or main stems to branches. In the latter case, bud numbers and sizes strongly correspond with the carbon sink strength of branches (Sprugel *et al*., 1991).

8.3.3 Leaf vs. wood allocation

Da Vinci observed that cross-sectional areas at various branch orders sumup to the same value (Richter, 1970; Zimmermann, 1983; Figure 8.6). This basic observation

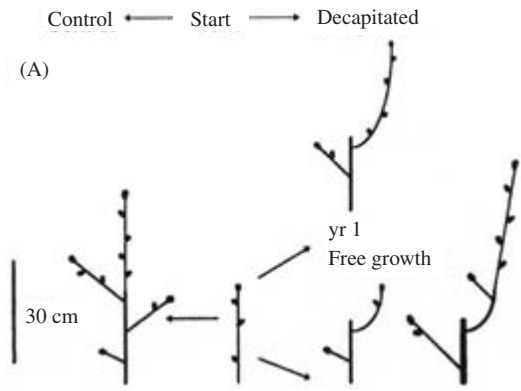

Figure 8.5 Apical control and release of apical control shown by removing the apex [adapted from Wilson (2000)].

served as a model to describe two phenomena in trees. First, the observation was refined to the ratio of cross-sectional area of sapwood (in main stem or branch) and the leaf area distal to the cross-section (Huber, 1928; Shinozaki *et al*., 1964a,b). The initial idea was that the ratio is constant, and that it reflects a functional balance between water transport in the sapwood and transpiration by the leaves. This view agrees with the concept that every leaf is connected to a woody pipe that connects with the roots, and that this wood–leaf continuum acts a physiological unit (Watson and Casper, 1984; Franco, 1985; Sachs and Novopolanski, 1995; Pertunnen *et al*., 1996; Smith *et al*., 1997). There are at least two reasons to believe that the idea of a constant 'functional balance ratio' may not hold. First, xylem conduits become wider in the downward direction (Tyree and Alexander, 1993; see Section 8.2.1) and thus more efficient at water transport. Strikingly, theoretical models suggest that such tapering in xylem conduits results from selection for minimizing the hydraulic resistance over the whole xylem conduit (Aloni, 1987; West *et al*., 1999; Enquist, 2002). Second, the leaf area to sapwood area ratio becomes smaller when trees increase in height (McDowell *et al*., 2002; see Box 1). These observations thus violate the long-held idea that species have a constant 'functional' leaf area to sapwood area ratio.

The production of new shoots with leaves is accompanied by production of a new shell of wood around the branches and stem and between the new shoot and the roots. In deciduous trees of seasonal climates, this growth pattern is manifested by the appearance of an annual leaf cohort, and an associated outer growth ring. The total area of a leaf cohort is almost linearly related with the cross-sectional area of this outer ring of newly produced wood (Rogers and Hinkley, 1979; Bartelink, 1997). These results suggest that Da Vinci's observation (Richter, 1970) holds better when comparing xylem area (heartwood and sapwood) at various branch orders, and shows how trees may allocate a greater proportion of available carbon to wood when they grow taller (e.g. Mäkelä, 1986; Pertunnen *et al*., 1996).

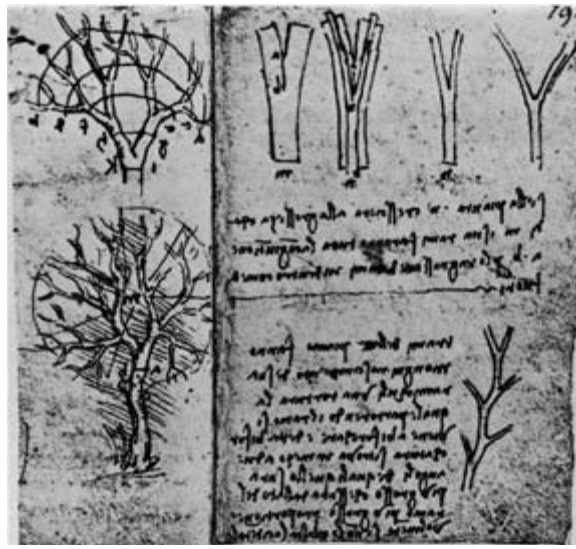

Figure 8.6 Tree design by Leonardo Da Vinci, suggesting that trees preserve summed branch cross-sectional areas over various branch orders [source: Richter (1970)].

8.3.4 Stability

Because of their large sizes, trees face huge mechanical stresses due to self-load and wind forces. Early studies showed that the largest trees have mechanical safety margins far beyond the predicted minimum, with diameters approximately four times that needed to support their own weight (McMahon, 1973). The situation, however, is completely different in the forest understorey, where trees only survive when they allocate more to leaves at the costs of wood (King, 1994; Sterck and Bongers, 2001). In such conditions, the investments in radial stem growth are marginal and mechanical safety margins are just enough to support their own weight (Sterck and Bongers, 1998). In more open conditions, investments in radial stem growth are accelerated (Bormann, 1965; Bongers and Sterck, 1998), increasing strength and stability (King, 1990; Bongers and Sterck, 1998). Selection is expected to secure stability at the lowest costs for wood (Givnish, 1986). Physical models predict that trees should produce their wood such that they distribute mechanical stresses equally over the whole woody body (Niklas, 1992).

Various physical models have been used to calculate the mechanical stresses in whole trees. The frequently applied elastic-stability model calculates the stem length that resists buckling from self-loading ($L \sim E/\rho \cdot D^{2/3}$) [where L is length, E is elastic modulus, ρ is wood density and D is tree diameter; McMahon (1973)]. The constant-stress-model calculates the stem diameter needed to resist wind pressures that operate on the crown [$D \sim (A \cdot L)^{1/3}$, where A is silhouette crown area; Niklas, 1992; Sterck and Bongers, 1998]. In line with physics, branches or branch units can be considered

cantilever beams and, accordingly, branch diameter growth is predicted on the basis of branch extension (Morgan and Cannell, 1987). When branches are elastically similar (the same arc per unit branch length), the branch diameter is proportional to the branch length (3:2) (Kronauer and McMahon, 1976). Wood production thus increases steeply, and non-linearly, with increasing branch extension, and the branch costs are very sensitive to branch angles and deflection (Cannell *et al*., 1988). These studies suggest that trees can grow more efficiently by producing slender crowns (see also Leopold, 1971), but such slender trees face greater risks of shading among leaves, and also that mechanical limits may constrain crown form and dynamics, but not the ultimate maximum tree height (see also Box 1).

8.4 Inter-specific patterns

8.4.1 Architectural tree models

Hallé and Oldeman (1970) showed that tree species conform to a limited number of developmental criteria. With these criteria, they described how trees develop from a simple pole seedling to a simple adult tree (e.g. palm species), or to more complex branched structures, such as in many conifers and angiosperms. The different developmental patterns ('architectural tree models') have a genetic basis, and are revealed most clearly and function best in a non-stressed environment. In a resource-limited environment, phenotypic plasticity responses may mask the inherited branching pattern, but, of course, plastic responses also have a genetic basis. In the architectural tree models, the apical meristems determine the development of the apical shoot and, indirectly, the development of axillary shoots. A variety of branch complexes can develop. Axes of different order in such branch complexes can be described by a number of criteria (Figure 8.7): (i) each axis either results from one apex (monopodium), or from several apices (sympodium); (ii) they develop directly after meristem initiation (sylleptic shoots, no apical dominance), or after a period of bud dormancy (proleptic shoots, apical dominance); (iii) they tend to grow vertically and to place leaves and axillary buds in a spiral (orthotropic, inferior apical control), or tend to grow horizontally and place leaves and axillary buds in a horizontal plane (plagiotropic, strong apical control); (iv) they continue to grow apically (and may produce flower by axillary meristems), or cease apical growth after apex death or flower production. Branch complexes may consist of different axis categories. Using these criteria, Hallé and coworkers (1978) distinguished more than 20 different patterns of tree development. These criteria were, in some cases, refined for the purpose of detailed specific descriptions (Edelin, 1977, 1984; Drenou, 1994; Loubry, 1994). In line with these latter studies, the term architectural unit was introduced to describe the developmental pattern of a species in more detail than the architectural tree model.

Thus, in an unstressed environment, trees develop in a predictable way and, at a certain stage, exhibit the whole hierarchy of architectural axes as described by the

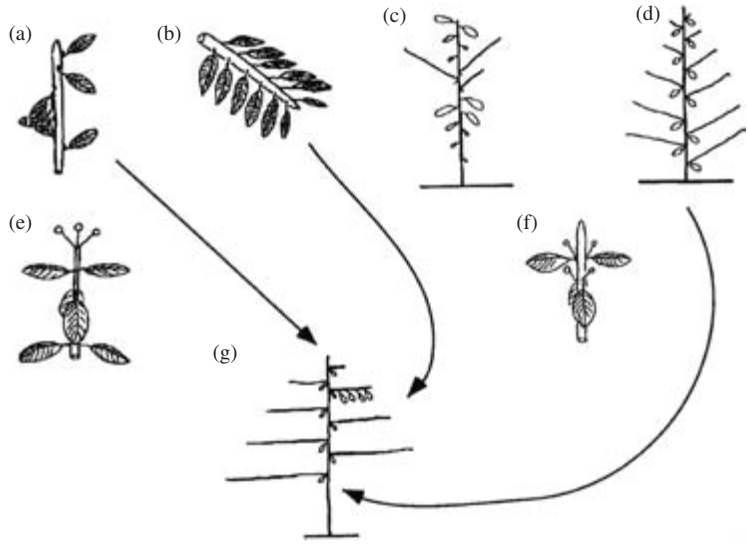

Figure 8.7 Several criteria in architectural analysis jointly determine architectural tree models. Here, an example is given for an architectural tree model – the model of Roux – combining (a), (b), and (d) (see arrows). (a) Orthotropic axis, vertical orientation, spiral phyllotaxis. (b) Plagiotropic axis, horizontal orientation, distichous phyllotaxis (leaves in two rows). (c) Rhythmic growth results in leaves of different size, and in tiers of branches. (d) Continuous growth results in leaves of similar size, and homogeneous distribution of branches. (e) Determinate axis, with apex turning into flower. (f) Indeterminate axis, with theoretically indeterminate growth. (g) The architectural tree model, a combination of the various criteria [source: Vester (1997)].

architectural unit. The basic development according the architectural unit can start from more than one meristem in the same tree. This process called reiteration (Oldeman, 1974) can occur under various conditions (Figure 8.8). (i) Most obviously, reiteration occurs when trees are damaged. When a stem is damaged by fire, wind or cutting, one or more dormant (epicormic) buds are released, or adventitious buds develop, and gradually develop into new architectural units. Such new reiterations, or sprouts, may originate from the base of the trunk (such as in coppice systems), from underground stems or from the roots (Del Tredici, 2001). When crown parts are pruned, dormant buds usually produce the same hierarchy of axis as that of the cut branch, and, thus, reestablish the former crown shape (e.g. Zeng, 2001). Coppice and pruning systems have a long history for many angiosperm trees, but are rare for conifers that often lack the reiterative capacity (see Del Tredici (2001) for exceptions). (ii) Trees that are suddenly exposed to new, open, conditions may also release dormant buds that develop into new architectural units. This happens when trees are liberated from shading neighbors, or when trees fall over and their architecture does not ‘fit’ with the environment. In both cases, reiteration enables

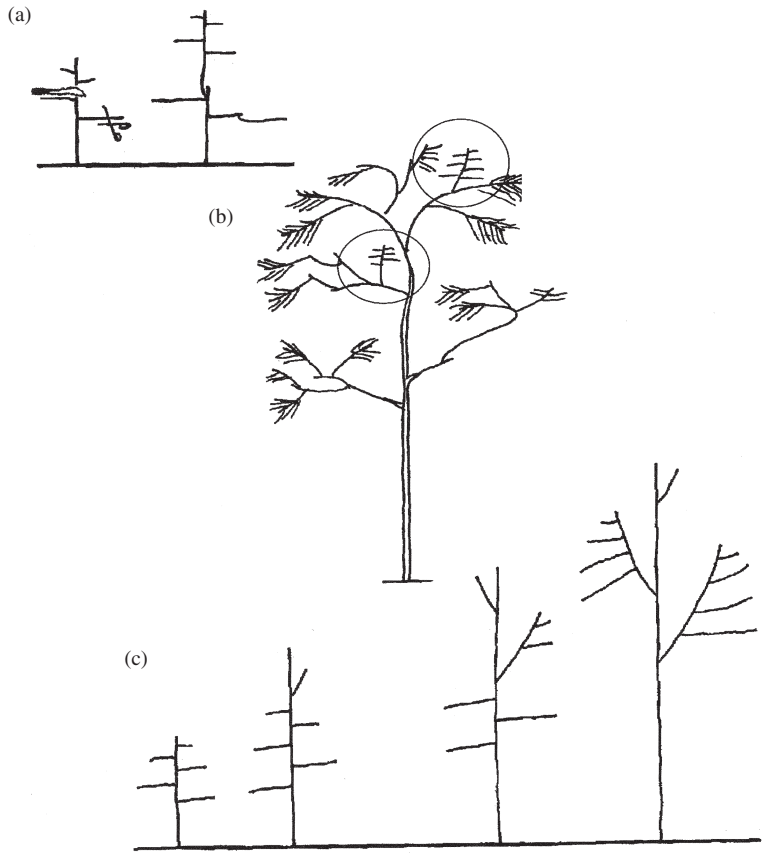

Figure 8.8 The three types of reiteration. (a) Reiteration that reestablishes the crown after branch removal. (b) Reiteration that results from new, favorable, conditions, also named 'lichtreiser' in older German literature. (c) Reiteration through intercalation of branch orders, part of the ontogenetic pattern in many species [adapted from Edelin (1984) and Vester (1997)].

trees to re-position their crown in a new environment. (iii) Edelin (1984) suggested that trees may develop multiple architectural units as part of the 'normal' inherent ontogenetic trajectory, and, thus, not in response to damage or changing environmental conditions [see also Vester (1997)].

Trees do not die from one day to the other, but gradually transform from adult (fully reproductive) to senescing trees. In adult trees, apical control is relatively low; the number of architectural units increases while the sizes of these units decrease. When trees are fully reproductive, often only parts of architectural units are left (Barthélémy, 1988). Later, apices may frequently die and break, and even whole branches or architectural units may break; then, more proximal dormant buds produce new, relatively small architectural units (Drenou, 1994). Where the

number of apices was increasing in earlier growth phases (Sterck *et al*., 2003a), this number now gradually decreases, and, ultimately, the tree will die (Drenou, 1994) (see Box 1 for a more functional explanation of tree senescence).

Strikingly, architectural tree models do not distinguish differences in light interception among different tree forms. Fisher (1986) noted that trees with different architectural models may show the same crown shape and leaf distribution, whereas trees conforming to the same model may differ greatly in crown shape and leaf distribution. The problem is that many quantitative aspects of tree development have been neglected, such as the timing, position, orientation and extension of buds. Most importantly, trees appear to be extremely plastic in many of their developmental decisions/responses, and the cumulative effects of such responses may result in contrasting tree shapes and leaf distributions, even among trees of the same species. Conversely, trees of different species, and with contrasting architectural tree models, may exhibit very similar leaf distributions and photosynthetic performances under similar understorey light conditions (Valladares *et al*., 2000, 2002). Apparently, the architectural tree models show different solutions to the same problem: how to grow to greater size, to intercept light effectively and/or to win the height struggle with neighboring plants.

8.4.2 Tree dimensions

Inter-specific variation in tree shape may reflect more ecological information than the architectural models. For example, higher latitudes are dominated by conifers with deep crowns, that probably improve light interception at low sun angles (Kuuluvainen, 1992). Tropical savannas, on the other hand, are dominated by umbrella shaped acacias, probably improving efficient light interception of a more vertical light source. However, trees of tropical moist forests show huge variation in crown shape, ranging from very flat to very deep (e.g. Sterck *et al*., 2001). The situation appears to be complex. First, light interception is controlled not only by crown shape, but also by foliage shape and distribution (Chen, 1994; Valadares, 1999). Second, in tropical forests, incident light from the side often exceeds that from above (Oberbauer *et al*., 1988). Third, side light levels are particularly high on slopes (Ackerly and Bazzaz, 1995; Ishii and Higashi, 1997). Under such conditions, the question of how crown form (and leaf distribution) would contribute to better performance remains a matter of conjecture.

Horn (1971) was one of the first to hypothesize on the role of tree shape for successional status of trees. He argued that trees balance between rapid height expansion to reach more favorable light conditions, and crown width expansion to spread leaves and occupy space while avoiding self-shading among leaves. However, spreading of leaves is limited by additional costs for wood needed to support a wider crown (Leopold, 1971). Horn's concept seems to work reasonably well for temperate forest situations in Northern America (Horn, 1971), and for saplings of tropical rain forests (Shukla and Ramakrishnan, 1986; Kohyama and Hotta, 1990), but not for bigger-sized individuals of tropical rain forests.

The species rich communities of tropical rain forests can be used for powerful multi-species comparisons. Apart from successional status, tree species strongly vary in adult stature (e.g. King 1991a; see also Box 1). In the forest understorey, trees of species differing in adult stature may compete with one another, most obviously for light. Juvenile trees of tall-stature-species produce slenderer stems (high height/diameter ratio) and smaller crowns than similar sized individuals of shorter-stature-species (King, 1991a; Sterck *et al*., 2001; Poorter *et al*., 2003). Everything else being equal, tall-stature-tree juveniles grow more efficiently (at lower wood costs) in height than short-stature juveniles, while they maintain similar mechanical safety margins as the shorter species (Sterck *et al*., 2001). Trees of species differing in adult stature occupy different vertical positions when they are reproductive. As such, differential adult stature may contribute to the coexistence of trees in temperate and tropical forests (Iwasa *et al*., 1984; Terborgh, 1985, 1992; Poorter *et al*., 2003).

Trees differing in light requirements also differ in dimensions. Early pioneer species grow most rapidly in height, and, typically, die at a relatively short stature (Finegan, 1996). Long-lived pioneers grow slower, but may become as tall as the tallest shade tolerant species. Across the large group of shade-tolerant species exists the whole range of possible maximum heights – from understorey shrubs to the tallest canopy species. When trees of similar size are compared, the species with greater light demands (pioneers) produce more slender stems than species with greater shade tolerance. In primary forests, the pioneers may develop the same crown size as neighboring shade tolerant trees (Poorter *et al*., 2003), and enable them to increase carbon acquisition in a canopy gap, to win the height struggle and to start reproduction more rapidly, but this is at the cost of greater mechanical risks. In secondary forests, trees often occur at extremely high densities and, under those space limited conditions, pioneers then produce rather slender crowns (Montgomery and Chazdon, 2001; Sterck *et al*., 2003b).

These comparative studies have limits as they focus only on the role of light. Other factors are expected to affect crown shape as well, such as the water transport–transpiration system (Box 1), mechanical stability (e.g. snow loads) and future reproductive capacity (Farnsworth and Niklas, 1995; Valladares, 1999). Because different species occupy different light environments (Bongers and Sterck, 1998; Poorter *et al*., 2005), inter-specific differences may result from an inherited ‘average trait’, as well as from phenotypic plasticity. Therefore, most of the ideas presented remain hypothetical and need further testing by studies that disentangle the effects of different factors.

8.5 Intra-specific patterns

Intra-specific patterns are most clearly observed in mono-species stands. In such stands, trees of the same species occupy different positions, that is, either dominant (no close neighbors), co-dominant (with close neighbors, but not overtopped),

intermediate (overtopped, but not fully yet), to suppressed positions (fully overtopped; Smith *et al*., 1997). In natural forests, trees may typically alternate periods of dominance with periods of suppression, and vice versa. Suppressed trees, typically, have small crowns and slender cylindrical stems. Dominant trees have big crown and a thick tapering stem. These patterns suggest that trees in different dominance classes differ not only in growth rate but also in allocation: suppressed trees pay the maintenance costs [for details, see Amoth (2000)] and just replace leaves (plus fine roots and root hairs), but invest little in crown expansion or radial stem growth. Dominant trees, however, have sufficient resources to grow quicker in height, and to produce large, heavily branched crowns, and thicker and tapered stems and branches (Bormann, 1965; King, 1990; Bongers and Sterck, 1998). These observations show that trees are very plastic in their response to light and space.

An extensive field study on a shade tolerant tree species of a moist tropical forest showed that when juvenile 1–20-m tall trees were exposed to high light they (i) released lateral buds and thus ramified more quickly (Sterck *et al*., 2003a), (ii) favored the extension/radial growth of the leader shoot over the growth of (existent and new) lateral shoots (Sterck, 1999), (iii) produced shoots with more leaves and with longer internodes (Poorter, 2001; Sterck and Bongers, 2001), thus, spacing leaves at greater distances [see also King (1991b)], and (iv) invested more in radial stem growth (Bongers and Sterck, 1998). From these results, it may be hypothesized how juvenile trees obtain their dominant habit (big, deep crown, thick stem) at high light. First, apical dominance is released as happens in other smaller plants under high light (Hutchings and de Kroon, 1994). Second, apical control is enhanced. This accords with studies on temperate forest trees [Sprugel (2002), see within-tree patterns]. Third, allocation to wood was favored over allocation to leaves; this agrees with other studies (Bormann, 1965; King, 1990; Bongers and Sterck, 1998). Conversely, trees exhibit a suppressed habit in shade, producing fewer and smaller shoots with fewer leaves, and stems that are only just capable of resisting the mechanical load (Sterck and Bongers, 1998). When trees manage to replace their leaves in time, they may survive in persisting shaded conditions (King, 1994; Sterck *et al*., 2003a).

8.6 Within-tree patterns

When distal shoots are removed, the main source of auxin is removed, releasing inhibition of lateral buds and leading to growth of lateral branches. Consequently, trees release lateral buds (apical dominance release) or, when lateral shoots are already present, the most distal lateral shoots gradually bend toward a more vertical direction (apical control release). Angiosperm hardwoods bend branches by producing tension wood (Wilson and Archer, 1977), whereas coniferous softwoods do so by producing compression wood (Timell, 1986; Mattheck, 1991; Wilson, 2000). These responses enable trees to recover from damage and, ultimately, reestablish their crown as before.

Trees of a heterogeneous light environment may have some shoots in high-light conditions, and other shoots in low-light conditions. While high-light shoots release apical dominance resulting in early outgrowth of lateral buds, the low-light shoots maintain apical dominance and inhibit outgrowth of laterals (Sachs, 1991). These ideas agree with a number of observations on trees grow at forest edges. For example, better-lit shoots branched more rapidly than shaded shoots in *Pinus sylvestris* (Stoll and Schmid, 1998) and in *Betula pendula* (Jones, 1985). Better-lit shoots also grew more rapidly than shaded shoots, suggesting that they benefit from their more favorable light conditions (Stoll and Schmid, 1998). Trees at forest edges are thus said to 'forage' for light, and develop a typical asymmetric habit with most branches and leaves at the more exposed site.

Trees that grow in dense stands, but manage to keep their upper meristems fully exposed, face a sharp decrease in light with decreasing height in the crown. These trees may drop their lower branches even when such branches are still potentially productive. In contrast, trees of the same size but in full shade keep their heavily shaded lower branches alive (Sprugel, 2002). One explanation may be that all shoots on shaded trees produce auxin at low rates. This would result in low competitive sink strength of the leader shoot and limited apical control (O'Connel and Kelty, 1994). In trees with the leader shoot fully exposed, the leader becomes a strong competitive sink, resulting in early death of shaded branches perhaps via higher auxin production (Sprugel, 2002). These more exposed trees may thus produce branch-free stems more rapidly than do fully shaded trees.

Several studies point to auxin as a key trigger in these growth patterns. First, Aloni (1987) predicted high auxin concentrations at branch junctions, where two sources of auxin join. High auxin concentrations at branch junctions would result in numerous small vessels (compared to controls with tapering vessels, section vascular differentiation), thus maintaining low conductivities at branch junctions (Zimmermann, 1978; Aloni, 1987; Tyree, 1988; Aloni *et al*., 1997). Consequently, the dominant shoot or stem, will be the better competitor for water and nutrients, and tend to grow more rapidly than more proximate, lateral shoots, particularly during drought (Aloni *et al*., 1997). These latter properties of the vascular system were expected to contribute the relative autonomous behavior of branches (Watson and Casper, 1984). Second, auxin gradients determine the growth-sink gradients within a tree (see apical control), and moreover, they orientate the new vascular strands to the greater sinks (Kramer and Bozlowksi, 2004). The secondary re-orientation of the vascular system toward better-lit shoots in herbs (Sachs and Novopolanski, 1995) does not occur in the wood of trees (e.g. Kramer and Bozlowksi, 2004). Auxin appears to integrate growth among shoots at different positions, or in different light environments. Such an integrated organization of different shoots allows a tree to allocate more resources to better lit shoots, and thus to respond optimally to a heterogeneous, unpredictable light environment (Snow, 1931; Novoplansky *et al*., 1989; Sachs and Hassidim, 1996). Thus, although competition among shoots would be an advantage for some, but a disadvantage for others, the mechanism may contribute to the overall performance of the whole tree (Tuomi and Vuorisalo, 1989; Vuorisalo and Hutchings, 1996).

8.7 Applications in forestry

Foresters anticipate within-tree and between-tree responses by thinning stands, that is, taking away suppressed, intermediate and/or codominants to free the most successful, most dominant, trees (Smith *et al*., 1977), or by liberating suppressed trees with commercial value by removing dominant trees (de Graaf 1986). The effects of stand density are nicely illustrated by profile drawings of trees along transects of increasing density (Figure 8.9; Houtzagers and Schmidt, 1994). Moving from very dense to intermediate densities, the crown size, height growth, and radial branch/stem growth increase, suggesting more effective resource acquisition, and the lower branches are still self-pruned (see apical control, within-tree variation). At very low densities, branches are not self-pruned; various branches compete with the leader (loss of apical control) and a big crown is produced, but height growth is slower than at intermediate densities. Obviously, a forester will aim at the intermediate situation with maximum height growth rates and effective self-pruning, thus reducing knots that reduce the wood quality.

Trees of different species may differ greatly in inherited branching patterns (see Section 8.4.1) and, in a quantitative sense, in their plastic responses to thinning. Experienced foresters know these properties in the major timber species in their forests. Simple criteria for tree shape may then help foresters to tune thinning and liberation operations to their goals. Two such criteria are the live crown ratio – the ratio of the crown length to the tree height – and the height/diameter ratio (Smith *et al*., 1997). The live crown ratio indicates the relative length of the branch-free stem, and the size of the crown. When too small, thinning may be needed to improve future growth, development and architecture. Low live crown rate is often associated with a high height/diameter ratio. When too high, this ratio may indicate that trees become unstable, and that thinning is needed. Since species differ in so many properties, these criteria are evaluated separately for each species, especially when the species are well described, such as in temperate forests. In lowland tropical forests, liberation operations are often applied, but usually the different species are exposed to the same treatment, because the species have been little studied and because these forests are complex in species composition and structure.

There are several other ways in which trees are manipulated. One of the oldest, and most widespread silvicultural systems is coppicing. In its most simple form, all trees are cut after a rotation cycle, and new trees develop as reiterations (also named epicormic branches) from buds at the stem base (del Tredici, 2001). In some cases, a stem part is left behind and reiteration occurs from buds at the knot of the stem (coppicing). These buds originate from formerly inhibited lateral buds that are still connected with the pith. Alternatively, cells may differentiate into new buds, named adventitious buds. In seasonal climates, trees are usually cut in the dormant (cold or dry) season, because new reiterations develop rapidly by using the high carbon reserves in the roots (Kramer and Kozlowski, 1979). A more special case is lopping, where only top shoots are removed and new reiterations reestablish an approximation

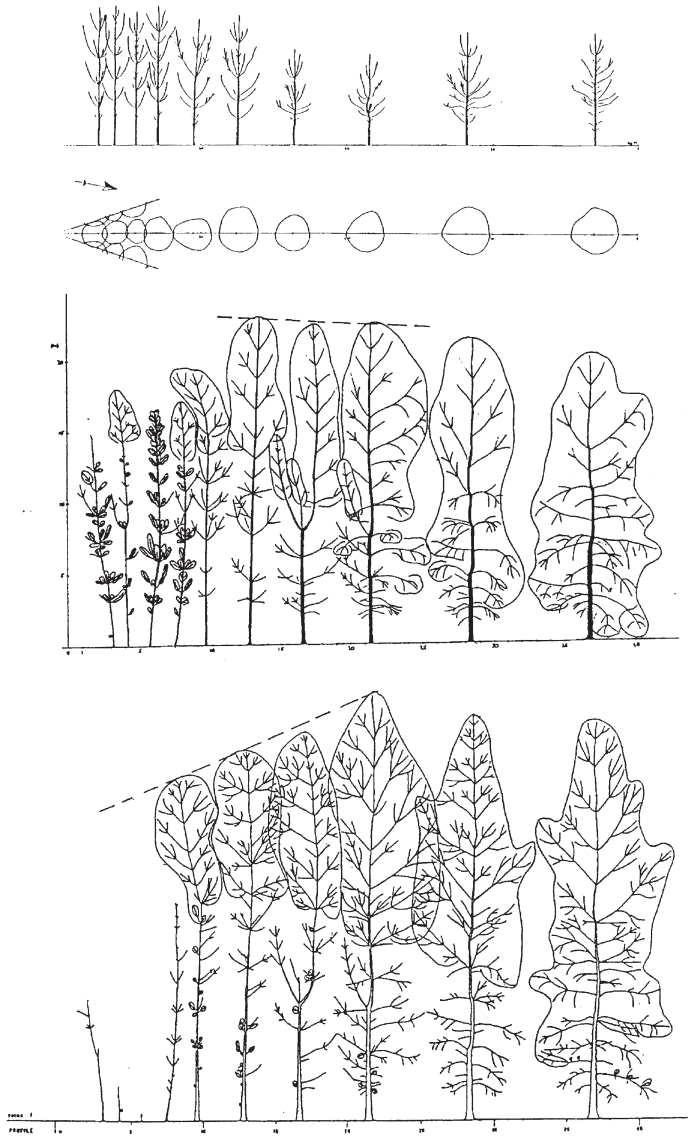

Figure 8.9 Effect of stand density on tree dimensions of poplar trees. Note that tree height reaches a maximum at intermediate density, and that tree diameter increases from high to low density. Crown depth strongly increases at the highest densities [source: Houtzagers and Schmidt (1994)].

of the old crown form. Such treatments mimic removal of distal shoots (see Section 8.6), and thus induce the release of apical dominance and control, so that trees produce a new branch (reiteration) that reestablishes the former structure of the tree. Such reiterations may develop relatively quickly, as pruned trees allocate more of their resources to leaves by re-using old sapwood, thus accelerating the acquisition of new resources (Zeng, 2001).

8.8 Conclusions

(1) Apical dominance, apical control and allocation patterns can explain patterns in woody architecture among different woody species, between different trees of the same species and among shoots in the same tree (Table 8.1). In particular, within-tree and intra-specific patterns appear largely predictable, whereas underlying causes of inter-specific difference patterns remain unresolved.

(2) Foresters manipulate trees by modifying the light environment, for example, through thinning and liberation. Physiological and ecological studies suggest that trees of different species have qualitatively similar responses to such manipulations. In dense and/or shaded conditions, trees grow slowly, particularly in stem diameter, inhibit outgrowth of lateral buds (apical dominance) but show little apical control. Trees in partial shade with their crown tops fully exposed release the apical dominance of the top. However, they have stronger apical control and thus produce longer crowns, but may 'self-prune' the lowest branches. Fully exposed trees grow rapidly, particularly in stem diameter, release apical dominance, lose apical control, keep the lower branches alive and thus produce big, more round crowns, often with multiple equivalent leader shoots (at least in hardwoods). The driving mechanism behind the patterns appears to be auxin production by the growing shoots and leaves and, in particular, enhanced auxin production (and basipetal transport) when these shoots and leaves are exposed to high light.

(3) Foresters, farmers and horticulturalists manipulate trees by pruning branches, or coppicing trees. Trees respond minimally after the pruning of shaded branches at the crown bottom, because such branches add scarcely any resources (nor probably hormonal fluxes) to the rest of the tree body. After pruning dominant and/or well-lit branches, inhibitory influences from auxin production in leaves and shoot tips over lower buds or shoots are relieved, lateral shoots start to develop and they take over the role of the former dominant shoot. A new branch hierarchy develops, usually conforming to the preexisting one.

(4) When foresters aim for tall trees, the best solution seems to be to keep minimal space between adjacent crowns in horizontal direction, while exposing the crown tops vertically to the open sky. This obviously results in steep light gradients from top to bottom through the crown, which

Table 8.1 Summary of architectural processes observed at three organizational levels, and in their forestry applications. '+' indicates that a process strengthens with a particular trait, '−' a decrease, and '?' shows that it is unclear

With increase in	Processes		
	Apical dominance	Apical control	Allocation to wood
Species			
Shade-tolerance	?	?	?
Adult stature	?	+	?
Individuals			
Age	−	−	+
Dominance	−	−	+
Gap size	−	+/−*	+
Branches/shoots			
Light level	−	+/−*	+
Application			
Thinning	−	+/−*	+
Pruning	−	−	−

* Dependence on gap size (i.e. light range) considered.

differentiates signal fluxes and resource acquisition. Upper branches become much stronger competitors for resources than lower branches, and this leads to stimulation of vertical expansion. With thinning operations, foresters may often search for a treatment that maintains and even enhances vertical expansion.

(5) Comparative studies suggest that trees in different biomes develop different wood properties (e.g. conduit sizes, perforation plate type) according to different climatic factors (temperature, water). Auxin gradients and the cambial responses to such gradients, may provide the key to the differentiation between ring-porous and diffuse-porous trees in temperate forests. The role of such mechanisms behind the differentiation of other wood types is not yet well understood.

(6) Inherited branching patterns ('architectural tree models') are little understood in terms of the underlying physiology (especially hormonal control), and from an ecological or evolutionary perspective.

(7) Besides their structural complexity, trees are characterized by the large sizes they can achieve as adults. There is an ongoing debate about the actual mechanisms that set a maximum height limit for trees. Although hydraulic limitations control the height of the tallest trees in an ecosystem, multiple other factors are likely to contribute to maximum height limits in shorter tree species.

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9 Plant architecture modelling
Virtual plants and complex systems

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9.1 Introduction

The understanding and control of the development of plants have always been of central importance in human activities. Numerous societal challenges involve monitoring or predicting vegetation state, yield or growth. Today, beyond traditional agronomic approaches, which attempt to characterize biomass production as a function of different treatments, the new interest expressed by society in ecology, sustainable agronomy and environmental changes calls for a better understanding of plant functioning and development. The associated scientific problems are complex and need a new multi-disciplinary scientific approach in which not only the production of plants is controlled but also the way in which plants elaborate this production during their lifetimes.

To investigate these issues, researchers are developing models of plant functioning and development and corresponding simulation tools. Over the last decade, research in plant modelling has benefited from the increase in computer power and the development of corresponding new methods of mathematics and computer science. This, in turn, has fostered the development of 3-D computational models of plants, called *virtual plants* (Room *et al*., 1996; Prusinkiewicz, 2004). The new challenge is to understand, through the use of 3-D representations, the importance of taking into account the *spatialization* of processes in plant morphogenesis. This challenge has several aspects:

- *Nature of plant spatial representation*. What spatial representation of plant should be used? At which scale? What are the actual means of digitizing plant geometry or to map their structure? How can we deal with their apparent complexity, and with what tools?
- *Plant structure as an interface*. The surfaces of plant organs are the sites of a number of exchanges between plants and their environment (light, air, water, soil, contact, rain, insects, disease propagation, etc.). Research questions relate to how the complex geometry of plants interacts with the various processes of the environment.
- *Plant as a network*. The plant structure provides the support for different forms of fluxes (water, sugars) and signals (via hormones) that control the plant functioning and growth and are highly sensitive to environmental changes.

- *Plant as a developing organism*. In turn, the growth of the plant continuously modifies this network and its occupation of space. This dynamic feedback between structure and function is probably a key issue in the understanding of plant development which necessitates further theoretical developments.

In this chapter, our goal is to sketch how current research in plant architecture modelling addresses these different problems. We shall give the reader basic notions about the main modelling approaches and formalisms used in these different areas of plant research. Section 9.2 briefly recalls the biological and botanical notions that underlie concepts of plant architecture today. Section 9.3 studies the question of measuring, representing and analyzing plant architecture. Models that make use of 3-D plant structure are then considered. In Section 9.4, we consider models of plants at the typical timescale of an hour, where the macroscopic structure of the plant can be considered as a constant. At this timescale, the physiological processes that occur within the plant do not significantly affect its structure, and reciprocally, they are not affected by structural changes. Finally, in Section 9.5 we consider timescales of the order of a growth cycle (e.g. 1 year), where models of plant morphogenesis can be developed, and where function and form interact.

9.2 Nature of plant architecture: basic concepts

9.2.1 Meristem activity and phyllotaxy

The shoot apex (SA) has a key role in producing the different tissues and organs which constitute the whole plant. The cellular processes that are involved in this formation of tissues and organs have been widely studied in recent decades, at the biochemical, physiological, biophysical, molecular and genetic levels (Lyndon, 1998; Bowman and Eshed, 2000; Nougarède, 2001).

The SA is the major site of cell divisions that provides a source of cells for shoot and root growth. Its organogenetic activity depends on either the divisions of a single apical meristematic cell as in the case of ferns, or the divisions of a discrete set of meristematic cells as in the case of higher plants (Lyndon, 1998). In the latter case, one to three layers of meristematic cells are usually observed (named L1, L2 and L3) but other terms and organization have been considered (in particular a tunica-corpus organization) [see Lyndon (1998) and Nougarède (2001) for reviews]. In the lateral and medullary zones of the SA, cell fate has been shown to depend on cell position. Two main hypotheses have been proposed to account for this positional information: (i) gradients of phytohormones, especially indole 3-acetic acid (IAA) which originates largely from leaf primordia (Reinhardt *et al*., 2000) and (ii) mechanical constraints (Green, 1999) could both be involved.

At a macroscopic scale, plant growth can be depicted as the result of two growth processes. First, the apical growth process gives the plant the ability to develop in one direction. Second, shoot meristems can give rise to additional organized zones of cell proliferation (always associated with corresponding leaves), called axillary

or lateral meristems. This second process defines branching. Plants make branching structures if the meristems located at leaf axils enter an apical growth process. Using the branching process, plants can develop shoots in more than one direction. The overall growth process is thus the combination of both the apical growth process and the branching process.

At the organ scale, growth is a fundamentally repetitive process which creates various forms of patterns repeated as 'modules' throughout the plant structure (Harper *et al*., 1986; Barthélémy, 1991). Many authors have been fascinated by the regularity of leaf positioning and the so-called 'mystery of phyllotaxy' [see e.g. Thompson (1961), Jean (1983, 1995), Douady and Couder, (1996) for reviews]. Indeed, leaf primordia emerge according to a rhythmic and regular pattern which is specific to a set of axes within a given species even though, in some cases, apices can change their phyllotaxy during their life (see Chapter 2). Phyllotaxy has thus been defined as the overall arrangement of leaves along an axis. It is frequently expressed simply as an angle between two successive leaves or by an index corresponding to a fraction of 360° between two successive leaves. Different patterns of leaf positioning were thus described, the corresponding terminology depending on both the number of leaves per node and the phyllotaxy angle [see Bell (1991) for a review of the standard terminology of phyllotaxy]. Since in higher plants the lateral meristems are located at the leaf axil, phyllotaxy can be considered as a main determinant of the plant space colonization strategy.

9.2.2 Differentiation of axes

This concept has been introduced by Hallé *et al*. (1978) who combined the phyllotactic organization of primordia to other morphological criteria in defining the concept of 'axis differentiation'. Five main criteria were considered:

(1) the growth direction associated with phyllotaxy allows to distinguish plagiotropic axes from orthotropic axes. Plagiotropic axes are characterized by a horizontal to oblique growth direction with alternate or distichous phyllotaxy and planar symmetry while orthotropic axes combine a vertical growth direction with a spiral phyllotaxy and an axial symmetry;
(2) the growth rhythm which can be either continuous or cyclic;
(3) the branching mode (monopodial vs. sympodial), position (acrotonic vs. basitonic) and dynamics (immediate vs. delayed);
(4) the presence of sexual differentiation of the meristems;
(5) branching polymorphism which differentiates short (or brachyblastic) from long shoots (mesoblastic or auxiblastic).

The combination of these criteria led to a series of defined architectural models which correspond to large categories of plants and were dedicated to famous botanists. The first two categories separates monoaxial and polyaxial plants. The following categories separate plants built (i) with equivalent (i.e. non-differentiated) axes, (ii) with differentiated axes, and (iii) with mixed axes. In monoaxial plants,

subcategories take into account the position of sexuality, either terminal (Holtum model) or lateral (Corner model). In the category of plants with equivalent axes, that is, resulting from repetitions of orthotropic and similar axes, different models were considered according to (i) the location of lateral branching, either basitonic (Tomlinson model) or acrotonic (Shoute model), and (ii) the position of flowering (Chamberlain and Leuwenberg models).

In the category of plants with differentiated axes, more models were considered which cannot all be detailed here. Two widely represented models correspond to the Rauh model composed of orthotropic axes, with rhythmic growth and branching and the Massart model with a monopodial and rhythmically growing trunk bearing plagiotropic branches.

The last category corresponds to plants whose axes change their morphological differentiation state during growth (mixed axes). This change can result from different growth directions and phyllotaxy during primary growth (Mangenot model) or from changes in secondary growth. This can be illustrated by the Champagnat and Troll models which are characterized, respectively, by orthotropic axes secondarily becoming bending axes, and by plagiotropic axes secondarily becoming erect axes.

9.2.3 Architectural gradients

In parallel, other concepts emerged from the analysis of plants, in particular at more detailed scales than axes, and from the observation of the repetitive nature of tree building which results from repetitions of similar organs or sets of organs (metamers or phytomers) (White, 1979). However, repetition is not sufficient to account for the existence of specific architectural patterns which involve the organization of organ topology, not only by chance but according to particular rules. Different concepts have been proposed to explain these specific organizations: 'morphogenetic programme' and internal correlation (Nozeran, 1984), 'age state' (Gatsuk *et al*., 1980) or 'physiological age' of meristems (Barthélémy *et al*., 1997). All these concepts state that bud fate changes according to position within the tree structure and during plant development. Even though these changes in bud fate are specific to each species and lead to the differentiation of axes, general rules have been highlighted and summarized in a Rauh model as follows by Barthélémy *et al*. (1997):

(1) an increase in shoot length and in axillary shoot development during an initial period (observed in seedlings and called 'establishment growth');
(2) a period of stability during which specific gradients can be observed (such as acrotony);
(3) a progressive decrease in shoot length and in axillary shoot development towards the final development stage or senescence.

During the branching process, lateral axes are usually included in the undergoing gradients and are therefore less developed than their bearer. However, sometimes, a lateral bud produces an axis comparable to, or even more vigorous than, the

bearing axis. Such an axis, subsequently, develops into a dominant branching system which repeats the tree structure totally or partially. These particular repetitions have been named 'reiterations' by Oldeman (1974) who interpreted their development as an unpredictable phenomenon reflecting adaptation of the tree to changes in the environmental conditions. Like branching, reiteration process can be immediate or delayed after a dormant period of the bud.

From a quantitative point of view, all these gradients have been demonstrated using methods proposed to represent and analyse plant architecture. These methods are detailed below and illustrated by examples of architectural databases to show the remarkable variation that exists in positional information of plants.

9.3 Representing and analysing plant architecture

9.3.1 Representing plants as graphs

On the basis of morphological studies, plants appear as intricate structures due to the existence of many sub-structures at various levels of detail, called modules (Harper *et al*., 1986; Barthélémy, 1991; Room *et al*., 1994; Godin and Caraglio, 1998). For a given type of module, the plant can be split up into a set of modules. This defines a particular plant modularity.

A plant modularity is characterized by the type of modules considered and their adjacency within the plant. Formally, this information can be represented by a directed tree graph, where vertices represent botanical entities and edges the adjacency between these entities (Figure 9.1). Edges are always directed from the oldest entities to the youngest ones. Given an edge (a,b), we say that a is a father of b and b is a son of a. Directed graphs representing plants have tree-like structures: every vertex, except one called the root, has exactly one father vertex. Moreover, in order to identify the different axes of a given plant, two types of connections are distinguished: an entity can either precede (type '$<$') or bear (type '$+$') another entity (Figure 9.1). In order to describe different characteristics of plant entities, vertices can bear attributes, for example, length, diameter, spatial location, leaf area, number of flowers, type of branched entities, etc. A special kind of tree graph, called an axial tree (Prusinkiewicz and Lindenmayer, 1990), in which a main path is identified from the tree root to a particular leaf vertex, is frequently used in simulation of plant growth.

Many 'modularities' can exist on a single individual. Classical modularities are associated with modules like metamers, shoots, axes, branching systems and reiterated complexes. For a single plant, there is thus the theoretical possibility of finding numerous types of modularity, each one corresponding to a particular topological interpretation of the plant. The set of these topological structures defined at every scale and their relationships characterize the overall topological structure of the plant, that is, its multi-scale topological structure.

The existence of several modularities on the same plant is illustrated in Figure 9.2. For this plant, the number of natural modularities stemming from

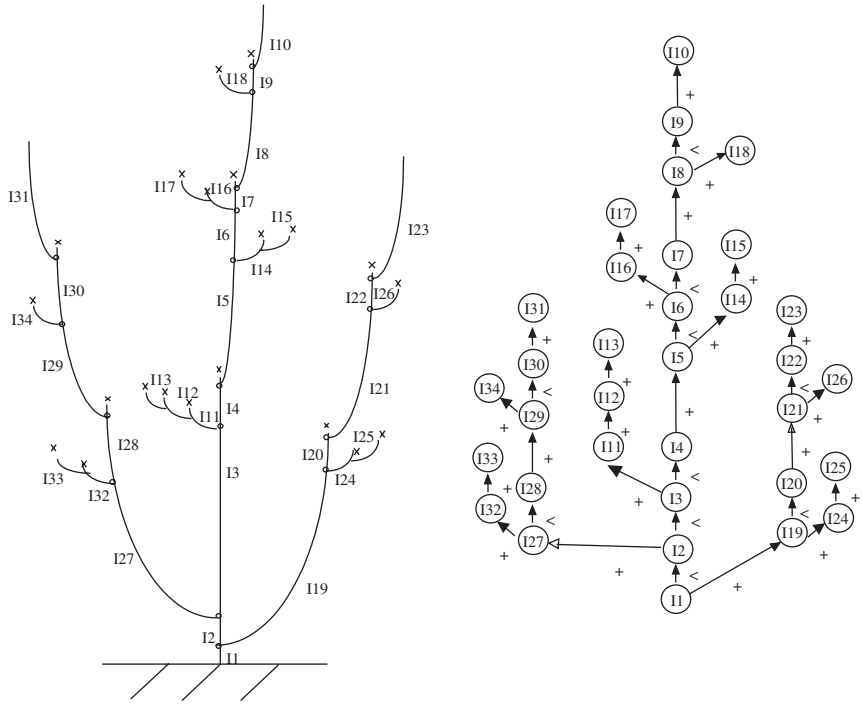

Figure 9.1 Internode modularity organization of a theoretical sympodial plant (left) represented as a tree graph (right) where x = terminated shoot, o = node and I*n* = internode number *n*. Internodes are represented by vertices (circles) and adjacency between two internodes is represented by an arrow, labelled by a '<' if they are both in the same axis, and '+' otherwise.

biological markers is relatively high. The highest scale corresponds to the description of the topological structure in terms of internodes (I). At a lower scale, each plant meristem builds up a series of metamers and dies. Corresponding portions of the axes are called modules (M). At a more macroscopic scale, the sympodial development of the plant forms branching sympodes (S). The plant can thus be represented by a specific tree-like topological structure for each possible scale. The set of these topological structures defined at every scale and their relations characterize the overall multi-scale topological structure of the plant.

To formally represent the multi-scale structure of plants, extensions of tree graphs, called multi-scale tree graphs (MTGs) have been introduced (Godin and Caraglio, 1998). An MTG integrates in a homogeneous framework the different tree graphs corresponding to plant descriptions at different scales (Figure 9.2). Each scale corresponds to a modular structure which can be formally represented by a tree graph. Entities at one scale are decomposed into entities at finer scales. If an entity a is composed of n entities $x_1, x_2, \ldots, x_n$, for every i in $[1, n]$, a is called

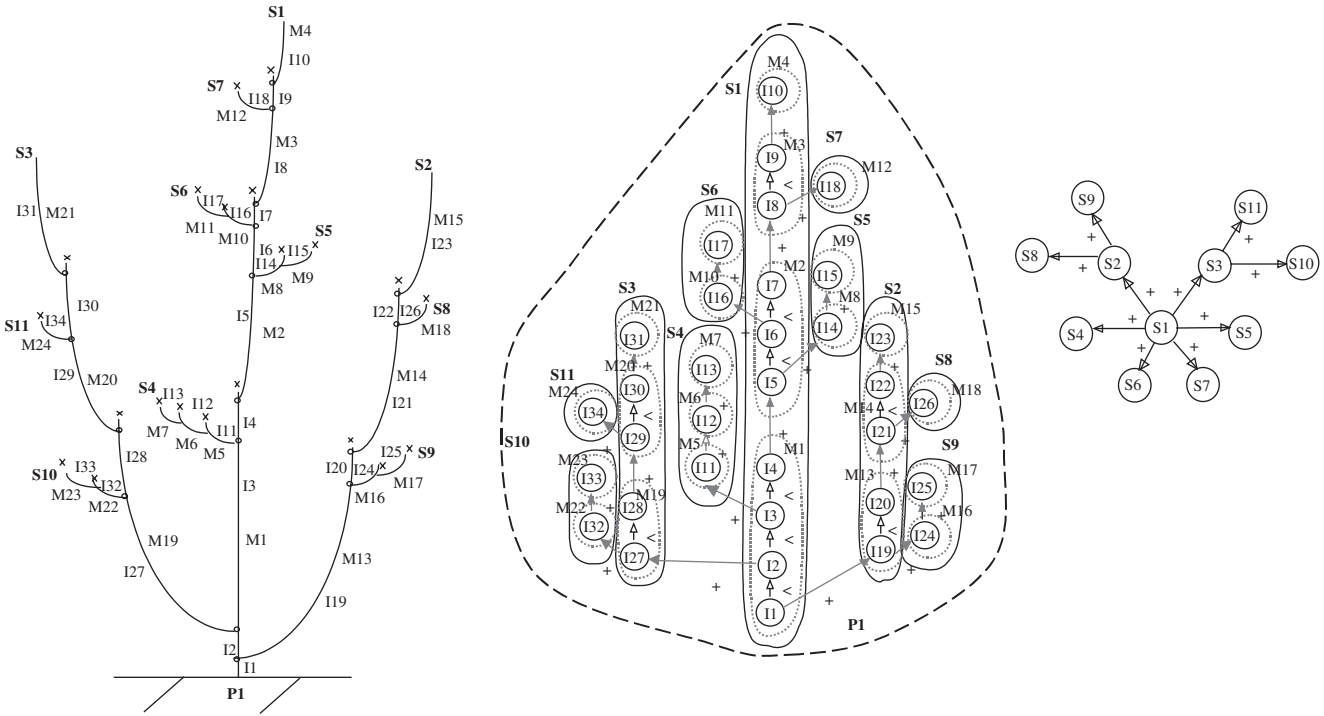

Figure 9.2 Several modularities on the same plant (left) are represented by nested tree graphs (middle), called multi-scale tree graphs. At one scale (i.e. for a given component type), the plant structure is a tree graph (right). Codes: Sn = branching system number; Mn = module number.

the complex of x_i, and x_i is a component of a. The complex of any entity x_i is denoted $\pi(x_i)$; if the scale of a is defined by the integer s, then for every i in [1, n] the scale of x_i is $s + 1$. For instance, internodes can be grouped into growth units (GUs), leading to a more macroscopic description of the plant topology. The most macroscopic scale s_0 consists of a single vertex, representing the entire plant, and by convention has value 0. In order to maintain coherence between the different tree graph representations of a same individual, MTGs must respect the following consistency constraint: if there exists an edge (x, y) in the tree graph representing the plant structure at scale $s + 1$ and if the complexes of x and y are different, then there necessarily exists a corresponding edge ($\pi(x)$, $\pi(y)$) between these complexes in the tree graph representing the plant at scale s (Figure 9.2, right). This expresses that the connection between two macroentities results from the connection between two of their components.

9.3.2 Coding plant architecture

Any tree graph can be encoded by a string of characters using brackets. Assume that tree nodes have (local) identifiers. Starting from a node a, if a has only one descendant (say b) in the tree, then the tree description consists of string ab. If the node has more than one descendant, say b, c and d, brackets are used to mark this bifurcation point: $a[b][c][d]$. If the order of the descendants of a is meaningful, it can be represented by the order of the bracketed expressions in the string (total order). If the order is not meaningful, neither is the order of the bracketed expressions (note that this strategy cannot account for partial ordering of siblings).

For the tree depicted in Figure 9.1, the string coding would describe the main axis (internodes `I1` to `I4`) with the code of its branches between square brackets []:

```
I1[I19 ... ]I2[I27 ... ]I3[I11 ... ]I4[I5 ... ]
```

The coding procedure is defined recursively on branches, which gives rise to the following complete code:

```
I1[I19[I24[I25]]I20[I21[I26]I22[I23]]]I2[I27[I32[I33]]I28[I29
[I34]I30[I31]]]I3[I11[I12[I13]]]I4[I5[I14[I15]]I6[I16[I17]]I7
[I8[I18]I9I10]]
```

In annotated tree graphs, entities may bear different attributes. For example, if the trunk entities have a measured diameter and length, the preceding code may be changed by adding the corresponding attributes after each entity:

```
I1(10.5,18)[I19[I24[I25]]I20[I21[I26]I22[I23]]]I2(9.2,20)
[I27[I32[I33]]I28[I29[I34]I30[I31]]]I3(8,18)[I11[I12[I13]]]
I4(6,15)[I5[I14[I15]]I6[I16[I17]]I7[I8[I18]I9I10]]
```

This strategy is the backbone of several notations that have been developed by different groups for encoding plant architectures at a given level of description, represented by annotated tree graphs, using slightly different notational and bracketing

conventions (e.g. Bourland and Watson, 1990; Prusinkiewicz and Lindenmayer, 1990; Godin *et al*., 1997; Hanan and Room, 1997).

This coding strategy can be extended to encode plants at several levels of description. This extension uses the following property of multi-scale graphs: every multi-scale tree graph can be transformed into an annotated tree graph without loss of information (Godin, 2003). The basic idea is to encode the plant at the most microscopic level, as previously described, and to insert new codes each time the frontier of a new macroscopic entity is crossed. Assuming that the decomposition marker is denoted by /, the multi-scale organization of the plant depicted in Figure 9.2 can be encoded as:

```
/P1/S1/M1/I1[S2/M13/I19[S9/M16/I24[M17/I25]]I20[M14/I21[S8/M18/
I26]I22[M15/I23]]]I2[S3/M19/I27[S10/M22/I32[M23/I33]]I28
[M20/I29[S11/M24/I34]I30[M21/I31]]]I3[S4/M5/I11[M6/I12[M7/
I13]]]I4[M2/I5[S5/M8/I14[M9/I15]]I6[S6/M10/I16[M11/I17]]I7
[M3/I8[S7/M12/I18]I9[M4/I10]]
```

To facilitate human reading/writing of such codes, equivalent notations have been designed that do not use brackets and make the code more legible. The detailed encoding strategy and notations are depicted in Godin *et al*. (1997) and Godin (2003).

9.3.3 3-D Digitizing

The architectural description of plants in the field may also require recording of the spatial location of the vegetation entities making the plant. Two classes of methods have been proposed to measure the 3-D coordinates of plant organs: contact and non-contact digitizers (Moulia and Sinoquet, 1993).

In the contact method, a pointer is set on the plant point to be recorded and the method computes the coordinates of the pointer. Several devices have been proposed. First, mechanical devices allow one to compute the pointer coordinates from length and angle measurements: articulated arms (Lang, 1973) where rotation angles are recorded from potentiometer resistance values was the first 3-D plant digitizer; later Takenaka *et al*. built a device called a Pocometer where a string is tied between a fixed point and the point to be recorded, and the length and the orientation angles of the string are recorded (Takenaka *et al*., 1998). Second, a range of 3-D digitizers are based on a triangulation method: the coordinates of the measured point are derived from the distance to three fixed points, the coordinates of which are known. Several methods allow measurement of the three distances – tapes (Bland, 1989; Godin and Costes, 1997) and sonic digitizers for example, SACDAC (see Sinoquet *et al*., 1991). In the latter, the pointer is an ultrasound microphone, the three fixed points are ultrasound receivers and the distances are computed from the time elapsed between sound emission and reception assuming constant sound speed in the air. Third, magnetic digitizers record the spatial coordinates and the orientation angles of the pointer. The device generates a magnetic field around

the plant, and the pointer includes magnetic coils where currents are induced according to location and orientation with regard to the magnetic field lines.

Articulated arms and string methods are inconvenient because the device placement in the canopy may disturb canopy structure or because some points may not be reached by the pointer due to trunk and branch distribution in the canopy space. Sonic methods have been applied to 3-D plant description (Sinoquet *et al*., 1991; Room *et al*., 1996). In our experience, sonic digitizing should be used only in the laboratory, mainly because sound speed is very sensitive to wind fluctuations occurring in the field. Moreover, the active volume is rather small, that is, about 8 m^3, and spatial coordinates in the inner part of dense canopies cannot be recorded because vegetation elements between the pointer and sound receptors may disturb sound propagation. Among contact digitizers, magnetic devices are likely to be most suitable because the magnetic fields are insensitive to the presence of the plant, the active volume can be large (up to 80 m^3), orientation angles can be measured and accuracy is good: a few millimetres in small canopies (Rakocevic *et al*., 2000) to a few centimetres in an 8-m tall tree (Sinoquet and Rivet, 1997). This is probably the reason why magnetic digitizers have been intensively used in recent years on a large range of canopy structures: trees (Sinoquet and Rivet, 1997; Costes *et al*., 2003), annuals (Thanisawanyangkura *et al*., 1997) and forage crops (Sonohat *et al*., 2002). Magnetic devices, however, are sensitive to presence of metal in the active volume.

Software has been developed to aid 3-D plant structure acquisition: Floradig (Hanan and Wang, 2004) is able to drive both sonic (GP8–3D, SACDAC) and magnetic digitizers (Fastrak, Polhemus) while 3A (Adam *et al*., 1999) allows recording of plant topology according to AMAPmod coding and 3-D coordinates by driving a magnetic digitizer (Fastrak, Polhemus) (Figure 9.3).

Non-contact digitizers sample spatial coordinates of the vegetation surfaces. A range of methods use the same principle of triangulation: stereovision (Ivanov *et al*., 1995), laser triangulation (Walklate, 1989) and laser plane range finding (Kaminuma *et al*., 2004). Triangulation methods need the plant elements to be viewed from two directions so that they are suitable for light plants with a few leaves [e.g. *Arabidopsis* (Kaminuma *et al*., 2004)]. In case of maize, Ivanov *et al*. (1995) had to cut the upper layers of the canopy to measure spatial location of vegetation elements in the lower canopy. Laser telemetry could also be used to measure spatial coordinates in plant canopies (e.g. Sinoquet *et al*., 1993); in that case, the vegetation elements must be viewed from a single direction. Belowground investigation of root architecture has also been proposed by using NMR imaging (Southon and Jones, 1992) and X-ray tomography (Heeraman *et al*., 1997). Both techniques apply to small root systems, while tomography reconstructs the 3-D structure from 3-D images.

Contact methods are more tedious, as they need an operator moving the pointer onto the vegetative surfaces. However, the operator can simultaneously record additional information about the measured organs, including the identification of the plant components and topology. Conversely, automation of data acquisition in non-contacts methods is higher, but the devices cannot identify the nature of the target – for example, distinguish between soil and vegetation, identify plant organs

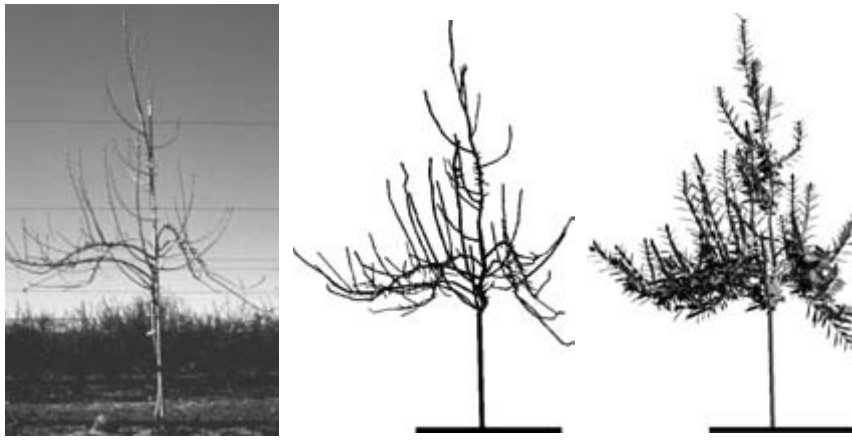

Figure 9.3 3-D digitizing of an apple tree (left) using the 3A software. 3-D reconstruction of the branching system (middle) and 3-D reconstruction of the leaves using allometric relationships between shoot length, number of leaves per shoot and leaf area and the AMAPmod software.

or record topology. Moreover, reconstructing the plant structure from a scatter diagram of spatial coordinates is a complex problem, which can be solved only for simple plants.

As 3-D digitizing methods remain tedious and time-consuming, more simplified methods should be developed, especially partial digitizing of plants combined with reconstruction procedures from allometric relationships and botanical rules. Other attempts at architecture reconstruction based on plant photographs, (Shlyakhter *et al*., 2001) are in progress. When reconstruction rules are used, the resulting 3-D plant architecture should be quantitatively assessed by comparing plant properties that are measured in the field and computed on the 3-D plant mock-up (Casella and Sinoquet, 2003).

9.3.4 Analysis of plant architecture databases

Different aspects of plant architecture can thus be measured in the field: topological measurements to a description of the plant organ adjacency, geometric measurements to a description of the organ shapes and spatial measurements correspond to a description of the distribution of the organs in 3-D space. A database containing positional information (i.e. either topological or spatial information) is called a plant architecture database (Godin, 2000). It is possible to extract data with varying degrees of structural complexity from architectural databases.

9.3.4.1 Looking for remarkable variations of positional information

In order to study repeated patterns and architectural gradients in the plant, different variables (e.g. length of branches, number of axillary productions, number of fruits,

diameters, size/shape of leaves, etc.) can be extracted and their variations analysed according to their positions within the plant structure. Depending on the application, the position can be characterized either by a topological variable (e.g., the rank of a node in its axis, the distance from the basis of the plant, etc.) or by a spatial variable (e.g., the coordinates of a node or a leaf in a global reference system).

An example of such a database exploration can be illustrated by a study carried out on two apple trees belonging to cv. 'Fuji', which were described over 6 successive years using both AMAPmod encoding strategy for topological description and a 3-D digitizing method for geometrical description (Costes *et al*., 2003). The analysis of topology compared all the axes as a function of their branching order and age. The results confirm the existence of within-tree morphological gradients and show that the decrease in growth was comparable in magnitude for all axes and GU, whatever their position (Figure 9.4).

Another example, using a spatial variable is illustrated in Figure 9.5, where a 20-year-old walnut tree was digitized in 3-D. Computation of foliage densities in different regions of spaces were analysed for later application in light simulations in isolated tree canopies (Sinoquet *et al*., 1997; see Section 9.4.1).

9.3.4.2 Analysing spatial or temporal series

The study of variables along an axis or throughout time can be automated by extracting corresponding (spatial or temporal) series and using adequate algorithmic or statistical tools to analyse them. Such techniques have been used extensively to analyse different types of branching patterns on different species or genotypes.

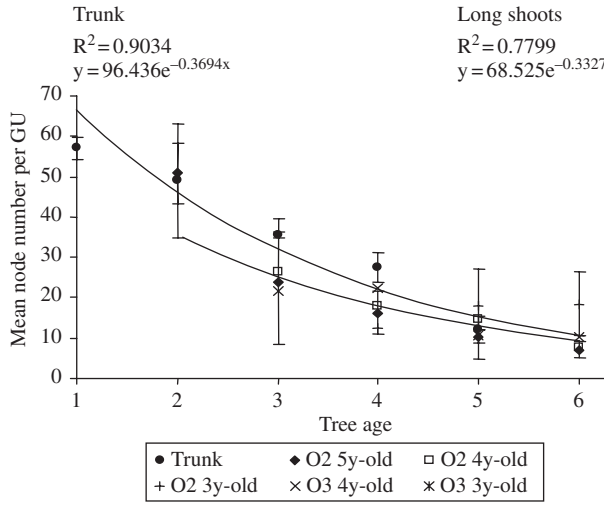

Figure 9.4 Decrease in the mean number of nodes per growth unit (GU) along the trunk and long shoots of 'Fuji' apple, according to their branching order and insertion rank (Costes *et al*., 2003).

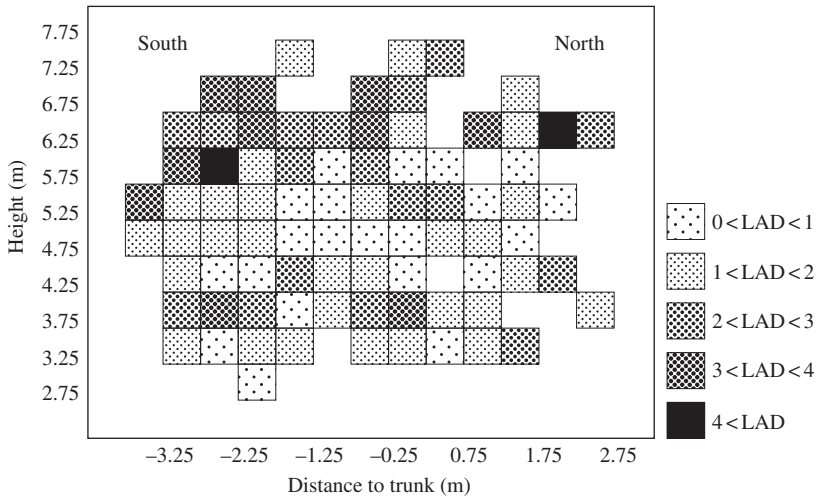

Figure 9.5 Spatial information. Spatial distribution of leaf area density (LAD, square metre leaf area per unit volume, $m^2\,m^{-3}$) in a North–South vertical plane of a digitized 20-year-old walnut tree crown (Sinoquet *et al*., 1997).

Qualitatively, branching can be more or less regular or clustered along the main stem. A preliminary stochastic modelling of branching patterns was initially carried out by de Reffye (1982) on coffee plant, where it was recognized that the probability of occurrence of a branch at a node depends on the presence or absence of a branch at the preceding node. Such dependency on the past reveals a kind of memory in the underlying biological process. Guédon *et al*. investigated these dependencies in depth and formalized them using Markov chains (Guédon *et al*., 2001). In particular, they introduced the idea that branching systems can be characterized at macroscopic levels by successions of zones with particular branching patterns; see Guédon *et al*. (2003) for a review. Let us describe key aspects of these approaches.

Formally, a stochastic process is a series of random variables X_t, where X_t denotes the observed variable (e.g. presence of a branch) at index t (node t). Each variable can take a value, called a state, in some discrete set. The probability $P(X_t = s_t, X_{t-1} = s_{t-1}, \ldots, X_1 = s_1)$ of jointly observing a series of t states ($s_1, \ldots, s_{t-1}, s_t$) can be recursively expressed as:

$$P(X_t = s_t, X_{t-1} = s_{t-1}, \ldots, X_1 = s_1) = P(X_t = s_t \mid X_{t-1} = s_{t-1}, \ldots, X_1 = s_1)$$
$$P(X_{t-1} = s_{t-1}, \ldots, X_1 = s_1)$$

If $P(X_t = s_t \mid X_{t-1} = s_{t-1}, \ldots, X_1 = s_1)$ simplifies in $P(X_t = s_t \mid X_{t-1} = s_{t-1}, \ldots, X_{t-n} = s_{t-n})$, the stochastic process is called an nth order Markov chain (i.e. with memory length n). Thus, for a first-order Markov chain, we have:

$$P(X_t = s_t, X_{t-1} = s_{t-1}, \ldots, X_1 = s_1) = P(X_t = s_t \mid X_{t-1} = s_{t-1})$$
$$P(X_{t-1} = s_{t-1} \mid X_{t-2} = s_{t-2}) \ldots P(X_2 = s_2 \mid X_1 = s_1) P(X_1)$$

If the transition probabilities between two states of the observed variable are independent of index t, the process is said to be homogeneous and $P(X_t = i \mid X_{t-1} = j) = p_{ij}$.

Markov models can be used to characterize different types of biological series using only a few parameters. Let us consider, for example, the simple two-states Markov model depicted in Figure 9.6a. State 1 corresponds to a branching node while state 2 corresponds to an empty node. For given values of transition probabilities (p_{12}, p_{21}), a series of nodes can be produced, node after node, with the corresponding model. In the generation process, $t = 1, 2, 3, \ldots$, if the last node t is in state s ($s = 1, 2$); the state (type) of the next node is chosen according to the current state s and to its transition probability, that is, the next node will be in the same state s with probability p_{ss} and in the other state with probability $(1 - p_{ss})$. Figures 9.6b–c show different series of nodes produced for different sets of transition probabilities.

While the use of a simple branching frequency may not be sufficient to discriminate between different types of branching habits (Figures 9.6c and d), the use of such a model 'with memory' allows to account for more structural differences between branching habits. For example, consider the two models that were used to generate stems in Figures 9.6c and d, respectively, with parameters $p_{11} = 0.25$, $p_{22} = 0.75$ and $p_{11} = 0.85$, $p_{22} = 0.95$. The probability p_b of a branching node can be theoretically computed from the model parameters:

$$p_b = \frac{1 - p_{22}}{(1 - p_{11}) + (1 - p_{22})}$$

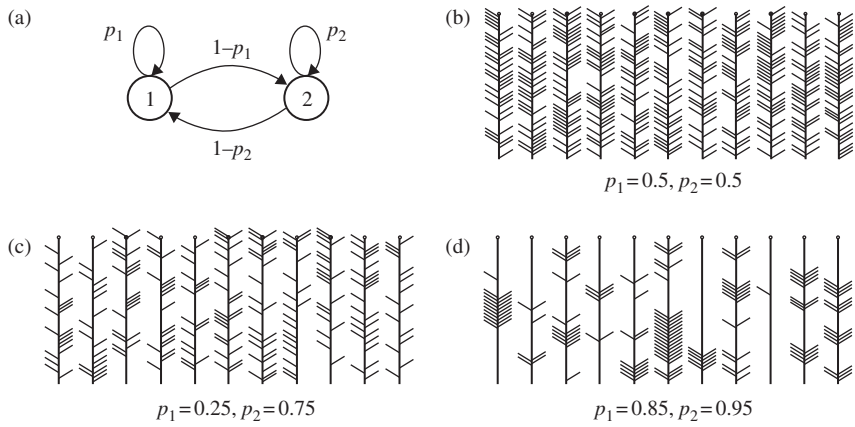

Figure 9.6 Modelling branching habits with Markov models. (a) A two-state Markov model, which consists of a graph whose vertices represent the branching state of stem nodes and p_{ii} is the probability of staying in state i. (b) Memoryless branching process (zero-order Markov chains); $p_{11} = p_{11} = p_{21} = 0.5$, $p_{22} = p_{12} = 0.5$ ($p_b = 0.5$). (c) $p_{11} = p_{21} = 0.25$, $p_{22} = p_{12} = 0.75$ ($p_b = 0.25$). First-order Markov chains allow for clustered branching with (d) $p_{11} = 0.85$, $p_{22} = 0.95$ ($p_b = 0.25$).

and has, in both cases, the value $p_b = 0.25$. However, we can observe that the branching patterns are quite different. A rather uniform branching pattern along the main stem corresponds to a zero-order Markov chain (no memory) (Figure 9.6c) while a clustered branching pattern, that is, alternate series of branching and empty nodes (Figure 9.6d) is characterized with a different set of transition probabilities corresponding to a first-order Markov chain.

Such simple Markov models have intrinsic properties that may or may not be satisfied by the biological data. Many variants of Markov models have thus been introduced to adapt to particular structural features of observed data. For instance, the number of times the process stays in a given state consecutively has a geometrically decreasing distribution (Figure 9.7a). Indeed, in a homogeneous first-order Markov chain, the probability $p_s(n)$ of staying exactly n times in state s is:

$$p_s(n) = p_s^{n-1}(1 - p_s)$$

This property of Markov chains may be inadequate for modelling some actual data, for which the length of these zones may be distributed in a non-geometric way. One way to overcome this limitation is to replace the implicit geometric state-occupancy distribution with an explicit distribution chosen in a suitable parametric family. The resulting model is known as a semi-Markov model (Figure 9.7b).

Another major extension of the Markovian approach comes from the need to describe more than one variable at each index of the series (e.g. at each node of a stem). Let us assume, for instance, that we are interested in the simultaneous occurrence, at each node, of the axillary branch type and the numbers of flowers. Each variable can possibly take several values and the number of potential states of the observation at one node results from the combinatorial arrangement of all these values, leading to a large number (N) of states. The corresponding number of model parameters (of the order of N^2) would be incompatible with the building of a good

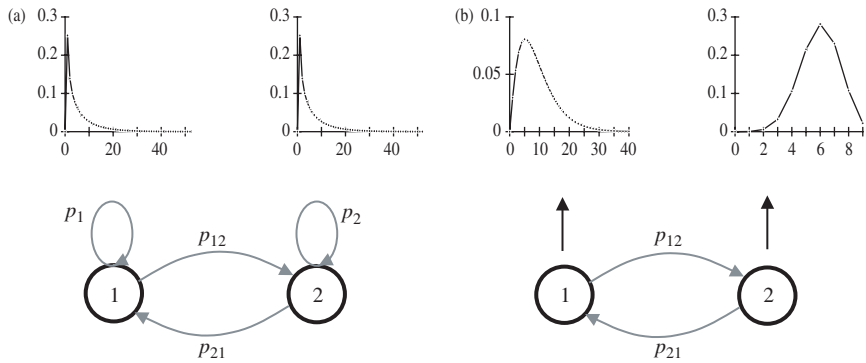

Figure 9.7 (a) Markov chain with implicit geometric state occupancy distributions. (b) Semi-Markov chain. The implicit state occupancy distributions are replaced with explicit distributions.

statistical model. To alleviate this combinatory problem, a new hypothesis may be introduced: each series of observed values is supposed to result from a succession of 'hidden' states, not directly observable in the data, in which the actually observed data are produced according to distributions that only depend on this state. States are thus abstractions of 'zones' which express remarkable combinations of the observed variables (Figure 9.8). The resulting so-called hidden Markov chain characterizes this succession of zones hidden in the observed data [see Ephraim and Merhav (2002) for a mathematical tutorial on hidden Markov chains; Durbin *et al*. (1998) and Guédon *et al*. (2003) for an introduction to hidden Markov models in biology; and Costes and Guédon (2002), Seleznyova *et al*. (2002) and Heuret *et al*. (2003) for applications to plant architecture analysis].

The analysis of biological sequences extracted from plant architecture and corresponding methods has been reviewed by Guédon *et al*. (2001). Beyond sequences, new methods are being investigated to directly analyse tree-organized data. Such methods will enable the identification of repeated branching patterns in trees (Ferraro *et al*., 2004); to find marked transitions between stationary zones (Durand *et al*., 2004), to compare the topology of branching structures (Ferraro and Godin, 2000) and to give the mathematical background to quantify key botanical notions applying to tree structures (reiteration, basis effect, architectural gradient, physiological age; see Section 9.2).

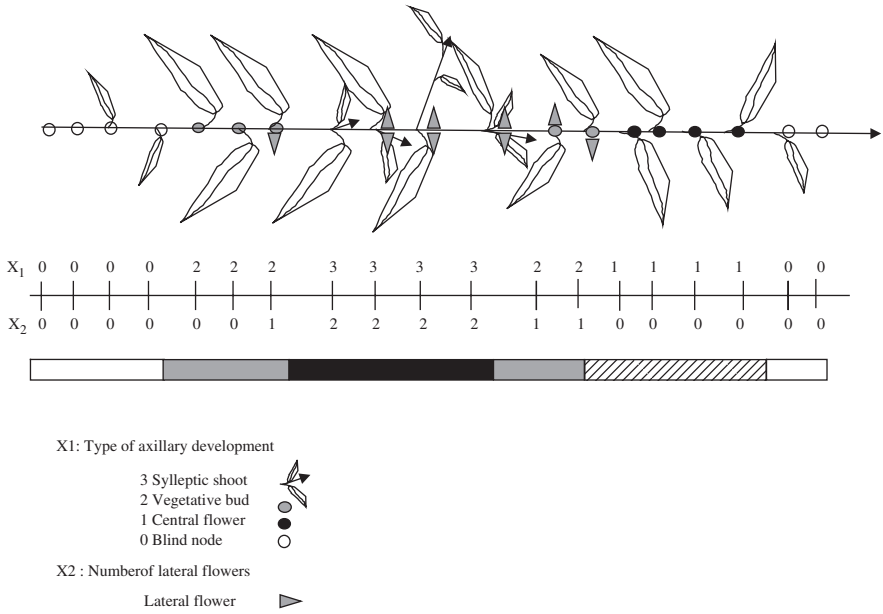

Figure 9.8 Schematic representation of a 1-year-old peach shoot as a sequence of two variables. At each node, X_1 represents the axillary bud fate and X_2 the number of associated flowers (Fournier *et al*., 1998).

9.3.4.3 The fractal nature of plants

The previous section illustrated the analysis of macroscopic topological patterns in plants. We now consider the analysis of plant geometry. In the last two decades, researchers have been fascinated by the possibility of generating complex tree-like forms with very simple and compact procedures [e.g. Smith, 1984; Prusinkiewicz and Hanan, 1989; Newman *et al*., 1997]. A 'fractal plant', for example, can be generated by the following geometric procedure: an initial (arbitrary) macroscopic form A is chosen (Figure 9.9, first form), and contracted by a scale ratio s ($s > 1$) (see legend for Figure 9.9). Then, n duplicates of the resulting form are positioned at different points in space with different orientations, mimicking the leafy zone organization of a theoretical plant (Figure 9.9, second form). The form resulting from the aggregation of the n duplicated initial objects then defines a new initial form to which the same series of transformations (contraction and duplications) can be applied (Figure 9.9, third form). Such a set of transformations is known as an iterated function system, IFS (Barnsley, 1988). It can be shown that the recursive application of an IFS to an initial object converges to a form (attractor, Figure 9.9, last form) that has, in general, fractal properties (irregular, with possibly a non-integer dimension). If the duplications of the IFS do not overlap, the theoretical fractal dimension D_T of the IFS attractor is (Falconer, 1997):

$$D_T = \frac{\mathrm{Ln}\, n}{\mathrm{Ln}\, s}$$

Fractal dimension expresses roughly the (constant) rate at which new geometrical details appear as one zooms into an object. In this respect, the fractal dimension is a measure of its geometric irregularity. Several estimators have been developed to compute this characteristic from raw data. Among them, the most widely used are the box counting, the mass dimension and the two-surface methods. These estimators were usually applied on 2-D images of plants canopies, leading to first estimations of canopy dimensions (Eshel, 1998; Alados *et al*., 1999). Recent studies have considered the analysis of fractal dimension of plants in 3-D, of either the wood (Oppelt *et al*., 2000) or the leaves (Godin *et al*., 2004b), using an adaptation of the

Figure 9.9 Series of iterations that generate a self-similar plant-like form from an iterated function system (IFS) consisting of nine duplications of an initial object (a tapered ellipsoid) contracted by a factor $s = 3.5$ (theoretical fractal dimension $D_T = 1.754$). This IFS has been generated with the *PlantGL* library (Boudon *et al*., 2001).

box-counting method for 3-D objects. This method has been extensively used to estimate fractal dimension of objects embedded in the plane. Its adaptation to 3-D analysis consists of building a 3-D grid dividing space in voxels of size δ (volume δ^3) and counting the number $N(\delta)$ of grid voxels intercepted by the studied object at scale δ, Figure 9.10. The estimator of the fractal dimension, D_b, of the object is defined as:

$$D_b = \lim_{\delta \to 0} \frac{\text{Ln } N(\delta)}{\text{Ln } 1/\delta}$$

Figure 9.11 illustrates the variation of the number of intercepted grid elements as a function of the precision of the grid cell on the digitized plant shown in Figure 9.10.

Figure 9.10 Box method applied to a digitized leafy tree in 3-D. The geometry of the plant varies as a function of the scale δ (size of a grid element).

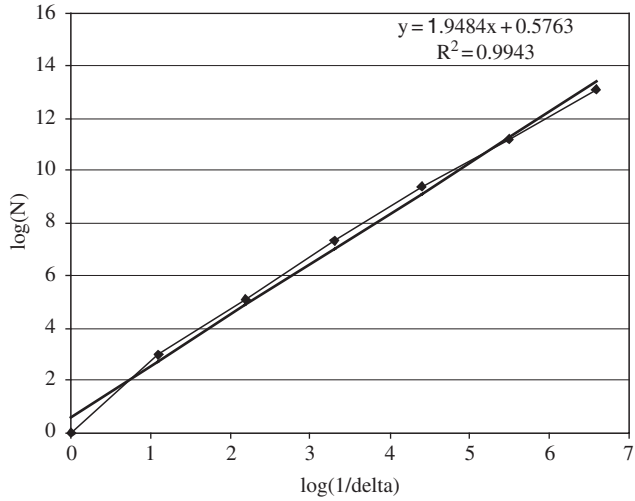

Figure 9.11 Estimation of the fractal dimension as a regression between the log values of the grid precision ($1/\delta$) and the number of intercepted grid elements (N).

Fractal dimension is only one means of characterizing the complexity of plant geometry. Other quantities can be used to analyse the irregular shape of a plant as a function of scale. For example, lacunarity characterizes the distribution of gaps in a particular structure (Mandelbrot, 1983). The variation of these distributions at different scales defines a complementary measure of the object complexity (Allain and Cloitre, 1991; Plotnick *et al*., 1996) and (Godin *et al*., 2004b) for an application to plant architecture analysis.

Fractal analysis emphasizes the dependence of plant geometry upon scale: plant geometry is not defined independently of an observation scale. The variation of geometry with the scale (as illustrated in Figure 9.10) characterizes an important aspect of the plant architecture complexity.

9.4 Modelling functions on static structures

In static structural–functional models, plant architecture is used as a model input and assumed not to change. Plants are represented as a collection of organs, which interact with each other through physical connections (i.e. plant topology) that allow them to internally exchange substances. Plant organs also interact with the environment as a function of their spatial distribution and functioning. This implies a spatial distribution of both the environmental conditions (i.e. the effect of the tree on microclimate) and the tree responses (i.e. at a local scale).

9.4.1 Models of plant–environment interaction

Interactions between plants and the environment have been extensively studied for their consequences on both the plant and the environment. From the point of view of plants growth and development processes are closely related to resource availability (light, water, carbon, nutrients and heat) and to environmental perception mechanisms (gravity, light quality, etc.). Organs of the same plant may be subject to contrasting environmental conditions, and this may result in differential responses which may have consequences on the growth and morphology of the whole tree. This is especially the case for light distribution within plant canopies, where functional consequences are numerous: short-term responses (e.g. photosynthesis, stomatal conductance, energy balance and organ temperature), delayed responses resulting from plant acclimation (e.g. nitrogen distribution and consequences on leaf assimilation ability; Le Roux *et al*., 1999) and plant morphology resulting from photomorphogenesis processes (Varlet-Grancher and Gautier, 1993). From the point of view of the environment, plants may act as modifiers of both soil properties and microclimate variables. This may be due simply to the presence of the plant (e.g. light interception, wind attenuation) or also due to plant functioning (e.g. increase of air humidity due to transpiration). Such effects of plants on microclimate have been used for environmental purposes such as estimation of carbon sequestration in a global change perspective (Dixon *et al*., 1994),

or fuel economy and pollutant capture in urban environments (Freer-Smith and Broadmeadow, 1997).

Interactions between plants and the aerial environment mainly concern the spatial heterogeneity of microclimate variables induced by the presence of the plant. The above-ground environment includes variables related to energy (radiation, heat, momentum characterized by vertical and horizontal wind speed) and gas (water vapour, CO_2 and other biogenic gases) content of the air. Heat and gas content of the air are called 'scalars' because they are characterized by a single variable, either temperature or gas concentration. The modification of microclimate is primarily due to the production and capture of energy and gases by the plant components.

With regard to plant architecture, light, wind and scalars are affected only by the spatial distribution of plant components (i.e. the geometrical component of plant architecture). In contrast, due to stemflow (flow down external plant surfaces), rainfall interception also depends on tree topology. For all resources, the modelling approach is primarily driven by the method of representing tree architecture: (i) as a collection of plant organs, where complete information about plant architecture (i.e. shape, size, location and orientation) is explicitly taken into account (Godin *et al*., 1999); (ii) as a turbid medium – that is, a vegetation 'gas' – where the spatial distribution of vegetation elements is described in terms of density functions (e.g. spatial distribution of leaf area density (LAD) [see Ross (1981)]. In the latter case, plants can be abstracted as a single (Norman and Welles, 1983) or a collection of geometrical shapes (Oker-Blom and Kellomäki, 1983), or as a matrix of 3-D cells (Sinoquet *et al*., 2001) filled with turbid medium.

9.4.1.1 Light capture

Most light models for plant canopies are based on the turbid medium analogy, that is, Beer's law which takes into account the amount of leaf area, and the leaf angle distribution with regard to the direction of incident radiation. The most common application of Beer's law is the computation of gap fraction, P_0, of a horizontally homogeneous canopy, that is, the fraction of transmitted radiation below the canopy in a given direction Ω or the fraction of sunlit ground area if direction Ω is the sun direction:

$$P_0 = \exp[-G_\Omega \cdot L / \sin(h)] \quad (1)$$

where G_Ω is the projection coefficient of leaf area on a plane perpendicular to direction Ω, which depends on leaf angle distribution (Ross 1981), L is the leaf area index ($m^2\ m^{-2}$) and h is the elevation angle of direction Ω.

Theoretical derivation of equation 1 assumes that (Nilson, 1971): (i) leaves are randomly located within the vegetation space, that is, the spatial location of one leaf does not depend on that of other leaves; (ii) LAD is uniformly distributed within the canopy volume. Real canopies, of course, deviate from these assumptions.

Leaf dispersion parameters have been proposed to overcome the Beer's law limitation of foliage randomness in the canopy volume. The most popular expression involves the introduction of an additional parameter μ_Ω in Beer's law (Chen, 1996; Kucharik *et al*., 1999):

$$P_0 = \exp[-G_\Omega \cdot \mu_\Omega \cdot L/\sin(h)] \quad (2)$$

Parameter μ_Ω is lesser than 1 in case of foliage clumping, that is, the general case in trees, where leaves or needles are clumped around the shoot axis. Unfortunately, parameter μ_Ω has not been explicitly related to canopy geometry parameters that can be measured in the field, although Foroutan-Pour *et al*. (2001) related leaf dispersion to the fractal dimension of images of the unleafy branching system and Niinemets *et al*. (2004) showed recently some correlations between parameter μ_Ω and petiole length. Departure from non-randomness is therefore the main limitation of the turbid medium approach.

As mentioned above, two general ways have been proposed to take into account the non-uniform spatial distribution of foliage in real canopies. First, the canopy can be described as a grid of 2-D (Cohen *et al*., 1987) or 3-D voxels (Myneni, 1991) resulting from a spatial discretization of the space occupied by the canopy. LAD in a voxel is assumed to be uniformly and randomly distributed, so that Beer's law is used at the voxel scale. Spatial variation of LAD is therefore accounted for by the inter-voxel differences in LAD. Second, the canopy can be divided into sub-canopy envelopes filled with uniform LAD. In forest applications, sub-canopies are mainly defined at tree scale, that is, tree geometry is described from a geometrical shape. Most models use simple shapes like ellipsoids or frustrums, for example, MAESTRO (Wang and Jarvis, 1990), although Cescatti (1997) proposed a more general eight-parameter tree shape model allowing for tree asymmetry and a large range of tree shapes. Multi-scale applications have also been proposed, where needles have been included in shoot envelopes, and shoots in whorls and crowns canopies (Norman and Jarvis, 1975; Oker-Blom and Kellomäki, 1983).

As a partial conclusion, the turbid medium analogy provides equations that can be used to compute radiation balance at canopy, plant and shoot scale (Sinoquet *et al*., 1991). The main limitation is the assumption of foliage randomness because real canopies show departure from it and some sources of non-randomness cannot readily be taken into account in the models with biologically sound parameters.

Instead, 3-D plant mock-ups allow one to compute light interception without the turbid medium assumptions of foliage randomness. In this approach, plant organs are explicitly described as a set of polygons, where organ shape, size, orientation and spatial coordinates can be taken into account. Light interception models based on 3-D plant mock-ups can be classified into three groups.

First, methods based on the polygon projection allow computing of incident light interception, that is, disregarding scattering processes. Polygons are projected on a plane made of pixels. Indeed, the plane area covered by plant organ projection corresponds to light interception by foliage in the view direction. Several computer

algorithms have been proposed to calculate projections, especially the z-buffer method (Ariès *et al*., 1993). Projected leaf area on a plane perpendicular to the incident direction can also be simply computed in a similar way by using plant image processing (Pearcy and Yang, 1996; Sinoquet *et al*., 1998). This requires generation of plant images from the 3-D information about plant structure, that is, by using graphics software (e.g. POV-Ray freeware that can be downloaded at www.povray.org) or OpenGL libraries (e.g. like in VegeSTAR software; Adam *et al*., 2002). Projection methods were first applied to small plants displaying a few leaves (e.g. cactus plants; Garcia de Cortazar *et al*., 1985) but present computers and software allow inclusion of several thousand polygons. Projection methods have been used to assess light capture efficiency at plant scale – namely identify differences between species, plasticity responses according to light availability and consequences for carbon gain (Pearcy *et al*., 2004) – and at shoot scale – for example, to assess light distribution in fruit trees (Willaume *et al*., 2004).

Second, methods based on Monte-Carlo ray-tracing allows computation of light–vegetation interactions, including scattering processes (Ross and Marshak, 1988; Chelle and Andrieu, 1998). The general principle is to send photons from the sky to the canopy and trace photon fate until it is absorbed by a plant organ or the soil surface, or until it is reflected to the sky. Incident photon properties are sampled in distributions by using pseudo-random number generators: the spatial coordinates of the photon origin in the horizontal plane above the canopy is sampled in a uniform distribution, while the photon direction could be sampled from the sky radiance distribution. Ray–polygon intersection algorithms (Glassner, 1989) are used to determine collisions between photons and plant organs. After colliding with a polygon, photon scattering is decided according to leaf optical properties – both hemispherical and directional. As it is based on a stochastic process, the ray-tracing method needs millions of photons and allows computing uncertainty about flux estimations. This is regarded as the standard reference technique as it does not use any assumptions, except for uncertainties about input parameters and vegetation representation.

Third, radiosity methods allow taking into account radiation exchanges between vegetation elements, namely scattering. Exchange coefficients F_{ij} between scatterers i and j are computed using the solid angle made by the radiation receiver j viewed from the radiation source i, and directional optical properties of radiation source i. Total radiation flux I_j intercepted by radiation receiver j is thus

$$I_j = I_j^0 + \sum_i F_{ij} I_i \tag{3}$$

where I_j^0 is irradiance of receiver j resulting from interception of direct and diffuse incident radiation. I_j^0 is usually computed from a projection method. Equation 3 – written for each receiver j – provides a system of linear equations where the unknowns are irradiances of the vegetation elements. Given the potential high number of vegetation exchangers, efficient matrix computation algorithms and/or element clustering are used (e.g. Sillion, 1995; Chelle and Andrieu, 1998; Soler *et al*., 2003).

9.4.1.2 Rainfall interception

Rainfall interception by plant canopies involves processes similar to those involved in radiation interception. A fraction of incident rainfall directly reaches the soil surface through the gaps between the plant components. Intercepted rainfall may evaporate, or be redistributed by splashing, dripping and stemflow. Splashing and dripping may be regarded as 'rain scattering' processes because they alter the direction and size of droplets. Studies on rainfall interception have mostly been motivated by environmental purposes: water loss due to interception, erosion due to stemflow and dripping, disease survival due to wetness duration and disease dispersal due to splashing. As to plant architecture, direct throughfall and stemflow induce spatial variability of rainfall water at the ground surface (e.g. Ford and Deans, 1978) which has been correlated to the distribution of surface zone fine roots and soil water uptake (Bouten *et al*., 1992). Rainfall interception may, therefore, be regarded as the first step of water resource partitioning between plants, that is, due to their individual funnelling ability.

Theoretical treatment of rainfall interception has received much less effort than other microclimatic variables. Almost all models are based on that of Rutter *et al*. (1971), that is an equation for the balance of rainwater storage at canopy scale. However, some rainfall interception models using virtual plants have been proposed. The model DROP (Bussière *et al*., 2002) based on a projection method computes throughfall, stemflow and dripping, while Saint-Jean *et al*.'s model (Saint-Jean *et al*., 2004) deals with splashing from a Monte Carlo approach. For the two models, simulated spatial patterns of rainwater on the ground were in good agreement with measurements.

9.4.1.3 Momentum transfer

Like radiation, momentum is absorbed by plant components which act as passive momentum sinks due to the drag force. However, unlike radiation, local absorption of momentum has consequences for wind characteristics at larger distances, due to momentum transport by turbulent structures. Turbulence within canopies is mainly dominated by coherent structures with a spatial scale of several times the height of the canopy (Collineau and Brunet, 1993). Wind characteristics are also affected by the vegetation density, especially tree spacing. Heterogeneous or discontinuous canopies induce spatial variation of mean wind speed in the horizontal plane, as reported by Green *et al*. (1995) in the case of a forest stand and Daudet *et al*. (1999) within an isolated tree crown.

With regard to momentum absorption, all simulation models are based on the equation of momentum balance which is applied to horizontal layers or 3-D cells describing both the canopy space and the space above the canopy (see e.g. Wilson, 1989). Equation derivation shows that momentum balance is mainly driven by wind fluctuations.

Computations of wind distribution from virtual plants have never been proposed. Indeed, the gain resulting from a fine description of tree architecture would be very low, since turbulence occurs at scales larger than that of plant organs, and because

the assumptions used in the models are weak in comparison to those associated with canopy structure (Brunet, 1997).

Due to the complexity of momentum transfer, simpler empirical approaches have been proposed. In particular, Daudet *et al*. (1999) related horizontal wind attenuation within the crown of an isolated tree to the cumulated leaf area computed from crown edge along the wind path.

9.4.1.4 Scalar transfer

The heat and gas content in air are influenced by both plant structure and plant function. Plant structure passively affects the turbulent transfer of scalars via its action on wind characteristics, while plant function provides scalar sources or sinks, for example, of heat due to the energy balance of the tree components, water vapour due to transpiration, CO_2 in relation to photosynthesis and respiration and trace gases emitted or absorbed by the tree foliage (e.g. isoprene, NO–NO_2–O_3 triad). Both transport and production processes result in spatial variation of these scalars within tree canopies, especially along vertical transects in dense forest stands.

The starting point for modelling scalar transfer is the conservation law for the mass of the scalar entity (Raupach, 1989). Two approaches have been proposed. In the Eulerian approach, the conservation law is applied to a small volume fixed in space. Like momentum transfer, the resulting equation contains unknown terms of fluctuation correlation and then needs additional hypotheses for equation closure; but unlike momentum transfer, the equation includes a term of molecular diffusion occurring at solid surfaces, that is, due to the presence and functioning of tree components (e.g. transpiration rate for air moisture, net assimilation for CO_2).

An alternative to the Eulerian approach is the Lagrangian one, where the conservation equation is applied to a fluid particle, that is, an infinitesimal control volume moving with the fluid. This involves a transition probability function, that is, the conditional probability that a fluid particle lying at position x at time t was at position x_0 at time t_0. An analytical Lagrangian model was proposed by Raupach (1987) in the case of steady, homogeneous turbulence. In the case of a pine forest treated as a multilayer canopy, Ogée (1996) derived a system of linear equations relating the vertical profiles of scalar concentration and source to the vertical profiles of mean wind speed and turbulence. From our knowledge, no scalar transfer model has been proposed in the case of complex 3-D canopies. Authors however agree that Lagrangian theory only needs a rather crude model for the wind field (e.g. Wilson, 1989). Further details on both the Eulerian and the Lagrangian approach for scalar transfer within plant canopies can be found in the excellent reviews by Raupach (1989), Denmead and Bradley (1987) and Wilson (1989).

9.4.1.5 Accounting for gravity

In the case of gravity, the plant–environment interaction is obviously restricted to the plant perception and reaction without any feedback from the plant on this environmental constraint. While the perception mechanisms are still under investigation

and discussion (Haswell, 2003), models have been proposed to account for plant reaction to gravity. Under the influence of gravity, the form of a woody stem, which is initiated during its elongation by the direction of apical growth (Fisher and Stevenson, 1981), is modified by the combined effects of three phenomena: bending resulting from the additional self weight of stems and axillary loads, stem rigidification resulting from radial growth and secondary reorientation associated with wood maturation (Archer, 1986).

The deformation of a slender structure such as a stem can be simulated, applying the beam bending theory, under two main assumptions: (i) a perfect embedment at one end, and (ii) the stem remains in a plane (Timoshenko, 1953). In addition, torsions are usually neglected. In what follows, the notations are those proposed by Alméras (2001).

Let us consider a stem divided into n beam elements, the geometry of each element i in the initial state being described by its diameter D_i, its length L_i, and its curvature C_i^{ini}. All elements are made of the same homogeneous elastic material, characterized by its modulus of elasticity, E. The angle between the horizontal direction and the tangent at the embedment is given as Φ_0.

Assuming the validity of the 'small displacements' hypothesis during loading of each element and, therefore, that its length is unchanged, the calculation of new curvature of all elements allows one to rebuild the whole form of the stem. At the stem scale, the variation in total curvature $\Delta\gamma$ of the stem can be expressed as a function of the length L of the stem, its mean diameter D, its initial leaning ϕ, the mass of loads M, the relative mean position of loads along the stem p (in [0,1]) and the modulus of elasticity of the material E:

$$\Delta\gamma = \frac{-32g\,ML^2\,p^2\cos\phi}{\pi ED^4}$$

This formula shows that the variation in total curvature is sensitive to three main quantities, namely the diameter D, the position of loads on the stem and its slenderness L/D. In the case of large displacements, since the bending moments vary strongly during deformation, they must be recalculated in the deformed configuration, and a new stem form derived again from the deformations associated with the new moments. Different solutions have been proposed for this computation method. Alméras (2004) proposed an iterative resolution of the problem which can be subject to problems of convergence, but can be solved by the introduction of a damping factor (Craig, 1989). The method of Morgan and Cannell (Morgan and Cannell, 1987), based on the use of a transport matrix, differs by the ordering of operations, as moments are recomputed together with the shape reconstruction. As soon as a stable form has been reached, both methods should lead to the same solution. West *et al*. (1989) have proposed an alternative approach with an integro-differential equation technique based on the same physical principle, in the context of small displacements.

9.4.2 Transport models

Plant architecture, through the geometry of organs, is a major determinant of plant–environment interaction. On the other hand, plant architecture topology plays a crucial role in problems related to the transport of substances through the plant body. Mechanistic models of transport have been developed to account for various types of fluxes (principally water and carbon) that propagate in the plant structure. Here, we briefly mention how modellers account for these fluxes and give references to corresponding literature.

Water transport within plants has been studied empirically since the pioneering work of Zimmermann (1978). Models integrating the architecture as a hydraulic network have subsequently been developed to study the properties of such networks and how they allow fluxes to propagate within plants (Tyree, 1988; Jones, 1992; de Reffye *et al*., 1997; Früh and Kurth, 1999, Dauzat *et al*., 2001). All the approaches rely on the same general principle based on an analogy between hydraulic and electric fluxes. Leaf transpiration creates a difference of water pressure between the leaves and the soil. This gradient generates a flow of water that propagates bottom–up in the xylem tissues through the plant body. In a steady-state regime, each plant segment is a porous medium traversed by a water flow that is related to the water potential at its ends by the Darcy equation:

$$\Delta\Psi = \frac{1}{C} \cdot F$$

where $\Delta\psi$ is the water potential gradient, C is the hydraulic conductance of the segment and F is the water flux through the element. This equation, equivalent to Ohm's law for electric circuits, may be modified to integrate other properties of plant segments (e.g. by adding a capacitance to model the storage of water). Two types of methods have been used to solve this equation (in either integrated or differential form) on a tree structure, namely a finite element method (Fourcaud, 1995) and a finite difference method (Früh and Kurth, 1999).

Mechanistic models of carbon fluxes have also been developed to account for assimilate allocation inside the plant. A similar electric analogy is used in mechanistic models of the transport of sugars in the phloem. This approach corresponds to a mechanistic attempt to model interaction among plant organs considered as sinks for allocation of assimilates (Le Roux *et al*., 2001). Recently, approaches based on a similar principle were used to model the sieve tube circulation in roots (Bidel *et al*., 2000) and to study the coupling of water (in the xylem) and sugar (in the phloem) transport (Daudet *et al*., 2002).

9.5 Models of plant development

9.5.1 Dynamic systems with dynamic structure

Modelling the change in plant architecture during growth can be abstracted as modelling the change of a system throughout time. Traditionally, this falls into the theoretical

framework of dynamic systems. In this framework, systems are represented by a state whose variation in time draws a trajectory. The theory of dynamic systems concerns the rules that govern state changes (e.g. differential or partial differential equations linking the system variables with time) and the behaviour of the corresponding trajectories (periodic, asymptotic, chaotic behaviour, etc.). In the general framework of (discrete space) dynamic system theory, system states are represented by points in R^n, n referring to a fixed number of coordinates representing the system state variables.

Modelling the growth of a plant (or other biological structures) can be abstracted as a problem of modelling a dynamic structure whose state *and* structure changes over time. At each date, the resulting branching structure is the instantaneous support of the flow processes. In turn, the result of these processes drives the creation of new structure elements, and so on. The complexity of plant growth modelling (and more generally of biological structures) is in particular due to this feedback process between structure and function, for which there does not yet exist well-established (or understood) theories. This problem has been identified by Giavitto and Michel (2001) as being an instance of a new class of models, namely the class of dynamic systems with dynamic structure $(DS)^2$. There are basically two contrasted approaches to tackle the complex problem of modelling $(DS)^2$.

9.5.1.1 Specific approaches

At one end, specific approaches tackle this modelling problem by designing a solution adapted to one or several particular hypotheses about plant growth. The system can integrate, for instance, a choice of space and time scales at which the model operates. The nature of its components is fixed: compartments, individual organs, voxels with different leaf densities, etc. The nature of the studied interaction between components and the growth rules are also usually predetermined. Stress is put, for instance, on a particular way of allocating carbon in the plant network (Pertunen *et al*., 2001), on a particular strategy to model light interception (Sinoquet *et al*., 2001) or on how the structure development is controlled (Barczi *et al*., 1997). In general, the system is designed to optimize the implementation of an idea or a concept.

There are a lot of advantages of developing such specific approaches, which explain their appeal and their wide use in plant architecture modelling. In particular, the system complexity can be optimized since only the necessary traits of the system have to be implemented. As a consequence, the system efficiency is often optimized. However, these approaches suffer also from a number of drawbacks. In general, the main data-structures representing the plant and the environment are specialized for the problem at hand and special tools have to be developed to manage, analyse or display them in 3-D. Each modelling group is then lead to develop its own tools, with particular emphasis on specific aspects of the problem. This takes a major effort in the overall model design. Although conceptually possible, this approach does not favour, in practice, the emergence of common

data-structures, tools, concepts and algorithms that could be developed, shared and reused by the modelling community. This is what generic approaches tentatively address.

9.5.1.2 Generic approaches: towards the definition of languages for morphogenesis

Alternatively, the problem of modelling plant growth can be embedded into the more general framework of modelling $(DS)^2$. In this case, the emphasis is put on the choice of a suitable paradigm to deal with questions raised by morphogenesis: how should $(DS)^2$ generic tools for 3-D representations, analysis and simulation be designed? What are the generic primitives that must be defined in order to enable modellers to describe growth of biological objects from a general perspective? In the context of plant growth modelling, this generic problem has lead researchers to design a language dedicated to the morphogenesis of tree-like structures.

The L-system approach (Lindenmayer, 1968; Prusinkiewicz and Lindenmayer, 1990) was designed to formalize the development of $(DS)^2$ string-like or tree-like structures. This generic approach lies in the class of 'rewrite systems' (Dershowitz and Jouannaud, 1990). A string rewrite system is a set of grammar rules that defines how a piece of a string can be replaced locally by a new string leading to a new overall string. In plant modelling, each letter of a string represents, for example, a bit of an axis (e.g. internodes, GUs, etc.), and changes in the axis structure due to a change in either time or in user viewpoint are described by rewrite rules. A developmental system is thus modelled by an initial system state, called the axiom, and a finite set of rewrite rules describing all the possible system state changes.

Grammar rules are usually defined for rewriting strings (like Chomsky grammars for modelling natural language). The possibility of applying rewrite rules to tree-like structures in a straightforward way relies on the possibility of encoding tree-like structures as strings, using brackets. Using the coding strategy described in Section 9.3, the growth of a plant can be described as the change of a bracketed string throughout time.

In the recent developments of L-systems (Prusinkiewicz *et al*., 1997, 2001; Karwowski, 2002), three kinds of rules are used. A first type of rule is used to transform string characters (representing plant components) over time. This process is called developmental derivation. A second type of rule is used to express the decomposition of components into subcomponents (decomposition rules). A third type allows the designer to define an end-user interpretation of the string obtained at each step, and also, for instance, a geometric representation of the computed string (homomorphism rules). These different types of rules are applied recursively to an initial string, the axiom, which defines the initial state of the growing object.

The following example illustrates the L-system approach and generates the growth of a simple branching system (Figure 9.12) that essentially consists of two rules of the first kind and a few rules that define geometry (homomorphism part; see

L-system 1). The first two rules express the growth of an apex over time (parameter t) which regularly produces an apex A($t+1$) on the top of a new internode I(t) and a new lateral apex A($t + 2$) with an alternate phyllotaxy p(t). Apices A transform into sexual organs S after a fixed number of differentiation stages. Axillary apices are generated at a particular time $t + 2$ greater than that of the principal apex, $t + 1$. Thus, this leads the apices from the bottom branches to enter the flowering state more rapidly. The developmental process starts from the axiom and applies the developmental rules to this initial string, thus obtaining, by 'derivation', a first string S_1 representing the plant development at time $t = 1$. A graphical interpretation of this string is then computed by applying the homomorphism rules to S_1 [L-system conventions used for graphical interpretation of strings are described in Prusinkiewicz and Lindenmayer (1990) and Mech (1997)]. Then, a second developmental derivation step is applied on S_1 and produces a new string S_2 representing the plant development at time $t = 2$. Again, a graphical representation of S_2 is obtained by applying the homomorphism rules to S_2. Figure 9.12 shows the graphical interpretations of strings S_0 to S_7, which represent the first steps of the plant development.

L-system 1

```
#define LIFETIME 10
#define GREEN 1
#define RED 2

Axiom: A(0)  /* initial plant state at time 0 */

A(t) :t< LIFETIME --> I(t) [p(t)A(t+2)]A(t+1)
A(t) :t>= LIFETIME --> S  /* Sexual organ */

homomorphism
I(t) --> F(LIFETIME-t)     /* I(t) is a segment (F) whose
                           length decreases with time */
p(t) :t%2 == 0 --> +       /* phyllotaxy to the left (+) for
                           even nodes */
p(t) :t%2 == 1 --> -       /* phyllotaxy to the right (-) for
                           odd nodes */
A(t) --> ;(GREEN)  @c(1)   /* A(t) is a green disk ( @c(1) ) */
S --> F(1);(RED)  @c(1.5) /* S is a short segment F(1) fol-
                           lowed by a slightly larger red disk
                           ( @c(1.5) ) */
```

This example shows that the development of rather complex tree-like structures can be modelled using very simple rules (here, two developmental rules only) which can be written or read by either computer scientists or biologists. For this reason, L-systems have been much developed and used in the context of plant growth modelling at the level of plant organs, where plants are $(DS)^2$ and naturally represented as tree-like structures, [see Prusinkiewicz and Lindenmayer (1990), Kurth (1994) and Prusinkiewicz (1998) for a review].

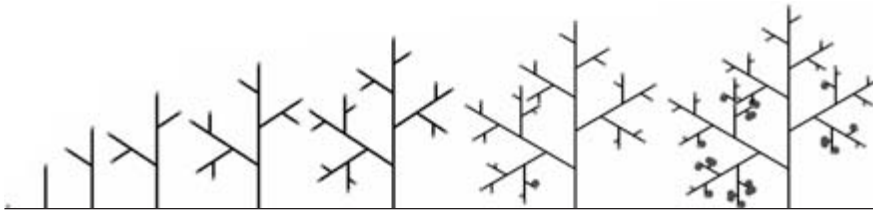

Figure 9.12 Developmental sequence of a simple tree. Meristems are represented by small disks and flowers by larger disks.

9.5.2 Descriptive models

Either specific or generic approaches have been applied to model the growth of plant structures from a purely descriptive point of view.

9.5.2.1 Bottom–up geometric approaches

Modelling stages of development in meristem activity. As described in Section 9.1, plants are made up of many similar organs at different scales: leaves, shoots, branches, reiterated complexes. From a dynamic perspective, this form of symmetry can be simply explained by the hypothesis that identical structures are produced at different locations within the plant structure and/or at some stages of development, by meristems in identical 'physiological states' or 'differentiation stages'.

To account for this hypothesis, the simplest model consists in considering that all meristems of a plant undergo the same series of differentiation stages. This series directly derives from the expression of the plant genotype in a given environment. In any given differentiation stage, meristems can possibly jump into a limited number of other differentiation stages, in which their physiological characters and growth potential will be different from that of the previous stage. The main modelling approaches of plant development rely on this hypothesis.

In the AMAPsim approach (Barczi *et al*., 1997), the authors use the notion of 'physiological age' of a meristem as a paradigm for modelling plant development. The nature of the variables that should define 'physiological states' of meristems has been particularly studied. The AMAPsim system explicitly relies on a description of the different stages of a plant meristem, and a fine control of the corresponding biological and morphological variables. In this approach, the series of differentiation stages carried out by each plant meristem is a sub-sequence of the complete sequence undergone by the main stem meristem, called the 'reference axis' (de Reffye *et al*., 1991).

In L-systems, the notion of meristem differentiation corresponds to the very idea of modelling a complex branching system using a reduced set of rules describing meristem behaviour at different ages. The transition between differentiation states is naturally captured by the notion of rewrite rule. If a meristem A is at date t in

'differentiation state' s, the meristem can jump at date $t + 1$ to the next 'differentiation state', say $s + 1$, or stay in the current state s depending on transition conditions, trans(s_1,s_2), for jumping from a state s_1 to a state s_2, that can be either internal to the plant or environmental. This framework can be sketched with the following L-system rules:

```
A(t,s) : trans(s,s+1)  --> ... A(t+1,s+1)
A(t,s) : trans(s,s)    --> ... A(t+1,s)
```

where the dots correspond to the meristem production when jumping from one state to the other. In the simple L-system example described above, we can see that a complex branching system can be generated with only two rules, which can be interpreted as two different differentiation states of the plant meristems. More complex examples are presented hereafter.

The meristem differentiation principle can be extended to tackle the development of more complex plant architectures in a descriptive way. For instance, to model the repetition of patterns in plant architecture (see Section 9.1) in a descriptive way, it is possible to design developmental rules at a macroscopic level and to generate the microstructure of patterns in a hierarchical manner. Prusinkiewicz *et al*. (2001) introduced the notion of decomposition in L-systems for this purpose. The following example illustrates this situation.

L-system 2

```
#define N1 3 /* Number of cycles for the first type of growth
             unit (GU) */
#define N2 3 /* Number of cycles for the second type of GU */
#define N3 2 /* Number of cycles for the third type of GU */
#define len(x) 1/(0.7*x^0.9) /* decreasing func of x for
                              x > 1 */

derivation length: 7

Axiom: [f(120)][+(90)f(60)][-(90)f(60)]A(1)

/* A plant is a series of N1 GUs of type U */
/* followed by a series of N2 GUs of type V */
/* followed by a series of N3 GUs of type W */
/* ended by a sexual organ S */

A(t) :t<=N1 --> U(t)A(t+1)
A(t) :t>N1 && t<=N1+N2 –> V(t)A(t+1)
A(t) :t>N1+N2 && t<=N1+N2+N3 –> W(t)A(t+1)
A(t) :t>N1+N2+N3 --> S

decomposition
maximum depth:10

/* Composition of the first type of growth units U */
U(t) --> M(t,1)          /* initializes a counter n for Us
                         internal modules */
```

```
M(t,n) :n< 2          --> I(t/4,1)[p(t)R][p(t+1)R]M(t,n+1) /*
                          two lateral long twigs */
M(t,n) :n>=2 && n<4 -->  I(t/4,1)[p(t)r][p(t+1)r]M(t,n+1) /*
                          two lateral short twigs */
M(t,n) :n==4          --> I(t/4,1)[p(t)A(t+2)][p(t+1)A(t+2)]
                            /* two lateral apices A older than
                                  the apex on the main stem */

/* Composition of the second type of growth units V */
V(t) --> I(t/4,2)[p(t)S][p(t+1)S]I(t/4,2) /* bears sexual
         organs S */
        I(t/4,2)[p(t)I(t/4,2)][p(t+1)I(t/4,2)]
        [p(t)A(t+2)][p(t+1)A(t+2)] /* bears lateral apices A */

/* Composition of the third type of growth units W */
W(t) --> I(t/4,3)I(t/4,3)[p(t)I(t/4,2)][p(t+1)I(t/4,2)]
         I(t/4,3)[p(t)I(t/4,2)][p(t+1)I(t/4,2)]

homomorphism /* defines the geometry of organs */
I(t,c) --> ;(c)F(6*len(t))   /* internode have decreasing
                              length as time proceeds */
p(t) :t%2 == 0 --> +       /* Phyllotaxy to the left for even
                          node index */
p(t) :t%2 == 1 --> -       /* Phyllotaxy to the right for odd
                          node index */
A(t) --> F(4);(15)@c(3)    /* an apex is represented as a
                          segment and a small disk */
S --> F(3);(4)@c(6)        /* sexual organs represented as
                          large disks */
r --> F(8)                 /* short twigs */
R --> F(15)                /* long twigs */
```

At the macroscopic level, an apex A produces a series of modules (e.g GU) of type U during N1 cycles, followed by a series of modules of type V during N2 cycles, and W during N3 cycles. The apex then differentiates into a sexual organ S. At each step, each macroscopic module appearing in the developmental string is decomposed into subcomponents according to the decomposition rules. Decomposition rules are applied after each developmental step, recursively, up to a specified maximum depth (here 10). Modules are decomposed into particular series of metamers. Modules of type U, for example, are made up of a first metamer bearing long twigs (R), followed by two metamers bearing short twigs, further followed by one metamer bearing two lateral apices A(t+2) older than that of the main stem apex A(t + 1) that can further develop. The decomposition of modules U, V and W is illustrated in Figure 9.13. The developmental rules thus express the series of differentiation stages (here U, V and W) of plant apices. The decomposition rules express at a finer level, the more precise structure of the portion of stem produced at these physiological states.

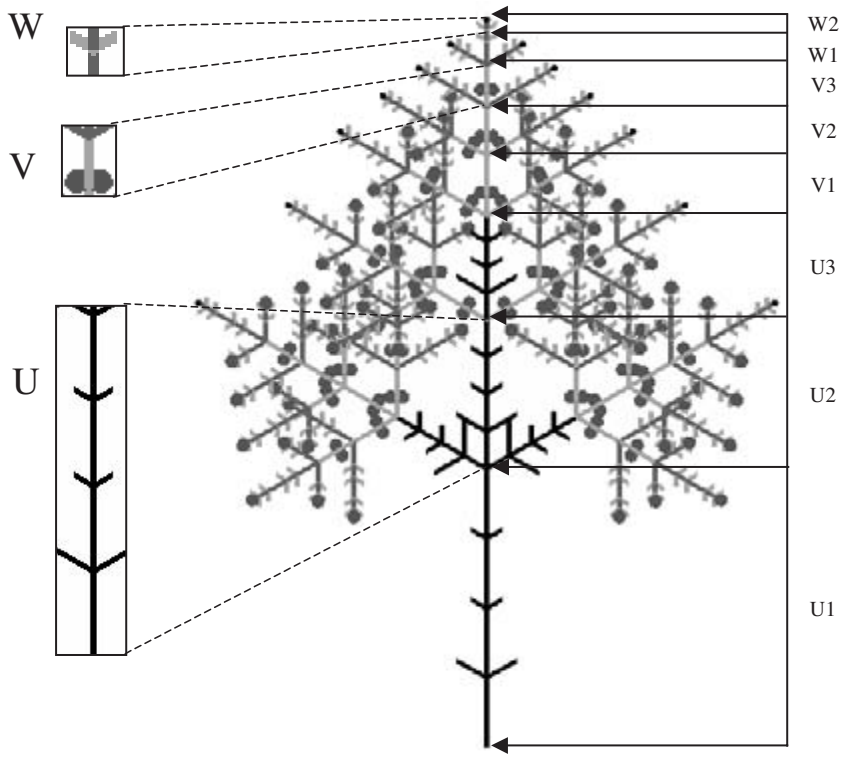

Figure 9.13 Generation of a plant architecture with a hierarchical organization using decomposition rules in L-systems. Meristems are represented by small disks and floral structures by larger disks.

Modelling branching habits. In Section 9.3.4.2, we considered the descriptive modelling of branching structures and we showed that a model with memory (like a Markov model) can be used to simulate branch distribution along an axis. Such a branching model can be simulated using a grammar formalism, basically, by attaching probabilities to the different rules, which thus models the process state change throughout time (Prusinkiewicz, 1998). The following L-system generates an ever growing stem, whose branching pattern is defined by a two-state Markov model with parameter $(p_1, p_2) = (0.85, 0.95)$ Figure 9.6d.

L-system 3

```
#define GREEN 1
#define RED 2

Axiom: I(0)

S(x) --> A(x) : 0.5  /* initial probabilities for the seed S
                        to enter */
```

```
S(x) --> B(x) : 0.5  /* either state A or B */

A(x) --> I[M(x)]A(1-x) : 0.85  /* Apex stays in state A with
                                  prob P1 */
A(x) --> IB(1-x)       : 0.15  /* Apex moves to state B with
                                  prob 1-P1 */
B(x) --> IB(1-x)       : 0.95  /* Apex stays in state B with
                                  prob P2 */
B(x) --> I[M(x)]A(1-x) : 0.05  /* Apex returns into state A
                                  with prob 1-P2 */

homomorphism

M(x) : x==0 --> ;(RED)+F(20)  /* if x==0 phyllotaxy is to the
                                 right */
M(x) : x==1 --> ;(RED)-F(20)  /* if x==1 phyllotaxy is to the
                                 left */
I --> ;(GREEN)F(2)           /* I is a green segment of
                                constant length 2 */
```

(Note that figures in this chapter are depicted in monochrome but software allows colour coding of segments)

The modelling of branch distribution along an axis can be combined with the modelling of meristem state activity as described in the previous section, by adding a parameter to the states describing the 'physiological state' to apices in states A and B.

9.5.2.2 Top–down geometric approaches

To build a complex form, a first possibility consists of mimicking the development of the form by simulating its growth in a realistic way (the activity of meristems for plants). In this case, the form emerges from the action of growth rules that locally specify the activity of each meristem in the plant. Controlling the overall shape of the resulting plant by such a 'bottom–up' specification of its growth process is particularly difficult and requires a lot of expertise from the modeller.

Where flexible control of plant shape is an issue, a different solution can be considered to design plant architecture. This solution involves to defining plant shape at a macroscopic level, and then using this shape as a coarse constraint to refine the component shapes at a more detailed scale, and so on. This is a 'top-down' approach since it proceeds from the design of the top-level shape of the plant and refines it progressively by modifying the geometric shape of its components until sufficient accuracy is obtained.

Using a top–down approach, the specification of how to modify the shape of different components in a complex form is a tricky issue. Prusinkiewicz *et al.* (2001) introduced the notion of positional information in this goal. Positional information defines component geometrical or biological information as a function of the location of this component within the entire structure. For plants represented as branching systems, the location of a component is naturally defined by the curvilinear abscissa of the component along its bearing stem. The curvilinear abscissa may be expressed using different units (centimetres, rank, etc.), Figure 9.14.

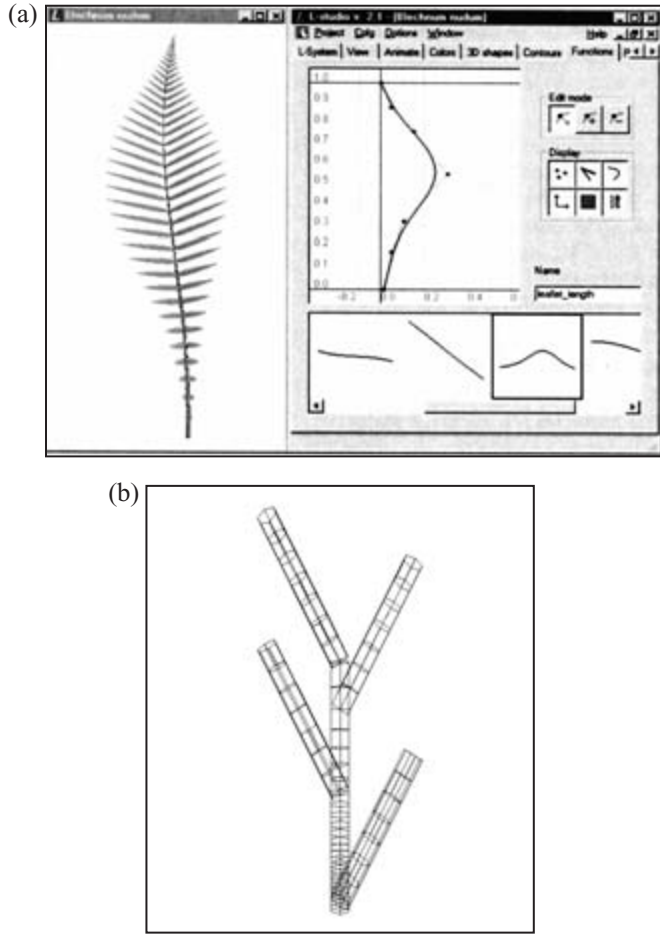

Figure 9.14 Top–down control of the shape of a branching system: the use of positional information. (a) In L-Studio (Prusinkiewicz *et al*. 2001), the designer uses 1-D functions to specify different shape variables as a function of the curvilinear abscissa along the main stem (here the length of the leaflets along the rachis). (b) A similar approach is used in AMAPmod to compute the geometric interpretation of a multi-scale tree graph using between-scale constraints, that is, equations linking component geometry to the geometry of their sub-components (Godin *et al*., 1999). Here, on the main stem, the length of internodes is defined as an increasing function of their rank along their axis.

The procedural specification of positional information is not always possible. Boudon *et al*. (2003) proposed a solution based on the construction of an MTG using an L-system that first defines the envelope of the more macroscopic components. Then, the algorithm recursively computes the envelope geometry of components from positional information (position along the main stem) and the geometry

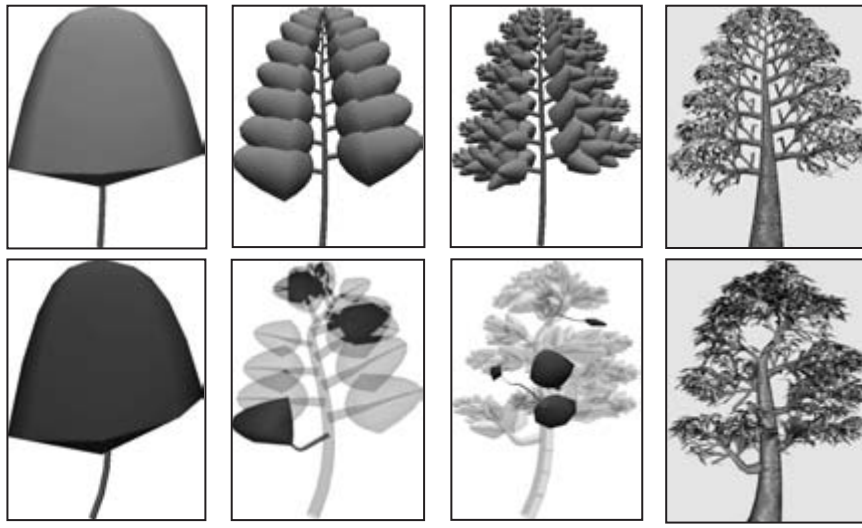

Figure 9.15. Top–down design of a complex form (a Bonsai). First, a general shape is defined for the tree envelope (top-left). This shape is used to generate a self-similar branching structure (top-right). Then, the designer modifies incrementally the initial self-similar structure at different levels of details (bottom, first three pictures) to obtain the final form with the desired accuracy (Boudon *et al*., 2003).

of their macroscopic constituents. The L-system algorithm then allows growth of the plant inside this empty shell of multi-scale envelopes. In the initial pass, the result of such a construct is a highly symmetric, fractal-like object (Figure 9.15, first line). To break this symmetry, the approach makes it possible to interactively modify, at each scale, the shape, size, orientation and other parameters of each branch envelope. Then the plant is recomputed using the L-system rules with the new envelopes. By progressively changing locally the plant geometry at increasing levels of details, the user can reproduce quite accurately complex vegetal forms, such that of bonsais (Figure 9.15, second line).

9.5.3 Reactive models

The study of reactive models was initiated by the pioneering work of Honda *et al*. (1981) and Borchert and Honda (1985). The authors analysed two theoretical hypotheses on the development of branching systems. First, exogenous (environmental) control was studied, where branch growth is affected by light availability (through self-shading for instance). Second, endogenous control was postulated, in which the different growth potentials at each point in the branching structure result from the physical competition between meristems for resources.

As opposed to descriptive models which are intended to capture the main structural traits of plant architecture and/or growth, reactive models are intended to capture

the plasticity of plant architecture due to either environmental changes or internal processes. They address the general question: how is plant form affected by internal or external events? In a review paper about plant architecture modelling, Prusinkiewicz (1998) called these models 'causal models'. In this section, we briefly recall the main approaches to reactive models [see Prusinkiewicz (1998) for a more exhaustive presentation], and sketch new models that have been recently developed.

9.5.3.1 Management of fluxes

Due to their role in water and nutrient supply to different part of plants, fluxes are generally considered as key parameters in the control branching system development. Based on observations by Zimmermann (1978) in the study of the hydraulic architecture of trees, Borchert and Honda (1985) made this assumption explicit by assuming that the development of a branching system is controlled by flux distributions within branching systems. At each bifurcation point i in the tree structure, the flux F_i in the mother branch is subdivided between daughter branches (indexes $2i$ and $2i + 1$) according to recurrence equations:

$$F_{2i+1} = F_i \cdot (1 - R) \cdot S_{2i+1} / S_{2i}$$

$$F_{2i} = F_{2i} - F_i$$

where R controls the ratio of flux in daughter branches due to their relative conductance ability, and the term S_{2i+1}/S_{2i} expresses the dependence of the flux distribution upon the relative leaf area supported by each daughter branching system. New branches are created for fluxes above a given threshold $F_{\min}$. Using these simple assumptions, Borchert and Honda showed that phenomena such as *apical dominance* can possibly emerge from competition between meristems by always supplying a better flux to the leader, which in turn determines a greater vigour.

Subsequently, Prusinkiewicz *et al*. (1997) used Borchert and Honda's model to simulate the reaction of a plant whose aerial part is pruned out (or damaged) and that rapidly replaces the removed part thanks to the existence of an already well developed root part (Figure 9.16).

Recently, similar approaches attempting to model the flux distribution using an electric analogy have been developed. In the GreenLab approach, at each growth cycle, the plant is considered as a network of resistances that provides meristems with necessary substances to grow (Hu *et al*., 2003). The amount of fresh matter elaborated by the plant is directly linked to the plant structure by the equation:

$$Q(n) = E(n)/R(n)$$

where $Q(n)$ is the fresh matter produced at cycle n, $E(n)$ is the average biomass production potential (which depends on environmental factors) and $R(n)$ is the hydraulic resistance of the dynamic plant network (mainly due to leaves) which thus represents the effect of plant structure. Other models of plant growth use a similar electric analogy for modelling the competition process between sinks for

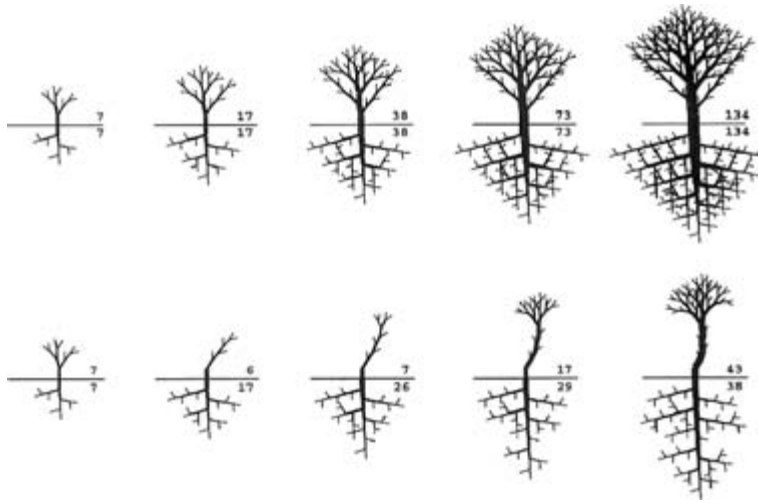

Figure 9.16 Illustration of a reactive system developed on the basis of Borchert–Honda's model. Top:development of a non-damaged plant (root and shoot systems have balanced size and development). Bottom: simulation of the reaction of the same plant to an accident on the aerial part [from Prusinkiewicz *et al.* (1997)],

supplies produced by sources through either xylem of phloem transport, (e.g. Bidel *et al.*, 2000).

9.5.3.2 Reaction to the environment

Reactive models also attempt to model the plasticity of plant architecture as a function of external factors such as light, water, wind, human/animal/vegetal interactions. Light has certainly been the most studied environmental parameter. Interaction between light and plant growth is usually estimated by locally computing the light received by a leaf or a group of leaves (see Section 9.4.1.1) in a given region of space and which was then transformed into photosynthetic production and later into new organs using various types of tentative rules (Greene, 1989; Blaise, 1991; Takenaka, 1994; Mech and Prusinkiewicz, 1996).

More recently, the dynamic interaction between plants and gravity has been investigated. Models have been introduced to take into account diameter growth of stems and wood maturation in the prediction of stem shape variations under gravity. Fournier and collaborators (Fournier *et al.*, 1991a, b) clarified the application of mechanical principles to the calculation of the deformation of a growing stem, providing two formulations which differ by whether the effect of maturation wood is taken into account or not.

Two simplified formulations have been proposed to calculate the variation in curvature during diameter growth, including either the load variation or the effect of wood maturation (Alméras *et al.*, 2004). The first model can account for the

interaction between the variation in diameter and the loading or unloading (e.g. at harvest) of the stem. Let us consider a stem loaded with a mass M and whose curvature variation $\Delta\gamma$ is estimated as previously defined in Section 9.4.1.5. Assuming that material properties in the new wood layers are the same as in the old wood layer, the change in total curvature $\Delta\gamma'$ after a subsequent change of both load ΔM and diameter D, can be simply expressed as a function of previous bending $\Delta\gamma$:

$$\Delta\gamma' = -\Delta\gamma \frac{\Delta \mathrm{M D}^4}{\mathrm{M}\,(\mathrm{D} + \Delta \mathrm{D})^4}$$

The second model accounts for the production of reaction wood in the stem during diameter growth, represented as an asymmetric field of maturation stresses. Let us assume that (i) reaction wood is located in a radial band of angular extension β, (ii) the modulus of elasticity E is uniform over the section, (iii) the intensity of maturation stress depends only on the nature of the wood and (iv) small variations of diameter ($\Delta D \ll D$). Under these conditions, the total curvature variation of the stem is given by:

$$\Delta\gamma'' = \frac{16\,\varepsilon\, L\, sin(\beta/2)\,\Delta D}{\pi\,(D + \Delta D)^2}$$

where $\varepsilon = \varepsilon_{\mathrm{TW}} - \varepsilon_{\mathrm{NW}}$ denotes the difference in maturation strains between normal and tension wood. This analysis emphasizes the strong dependence of the change in total curvature on a change in diameter in both models. This suggests that the timing of diameter increment should be carefully studied during the growing period to predict the final shape of stems in plants (Alméras *et al*., 2004).

At plant level, particular computation strategies have been developed to compute recursive changes in axes geometry induced by gravity on a structure that changes throughout time (DS^2); two basic solutions have been studied. Fourcaud and Lac, (2003) separated two aspects of the problem. A first module carries out the growth computation while an external module uses a finite element method to compute the mechanical constraints on the structure provided at each time step by the first module. In turn, the external module provides the growth module with an updated geometry. Based on similar mechanical equations, a different approach was proposed by Jirasek *et al*. (2000) who used L-systems as an integrating framework to merge the resolution of mechanical equations and the computation of plant development (in particular, the discretization of the plant into components is used for both growth and mechanical computations). At each time step, the computation of mechanical constraints is treated as a series of outward (geometry update pass, from plant base) and inward (load update pass, from plant periphery) data fluxes, until convergence of constraints and geometry.

Most of the work on biomechanical modelling of the form of woody stems has been conducted on forest species, and in the context of ecology (Castera and Morlier, 1991; Fournier *et al*., 1991a, b, 1994; Niklas, 1994), with a particular focus on the change in form and stress distribution of trunks of adult trees. The time unit

is usually the year (Fournier *et al*., 1991a; Fourcaud and Lac, 1996) and the effect of intra-year dynamics of loading and rigidification has been disregarded. Qualitative validations of the concepts used in the above models have been proposed (Castera and Morlier, 1991). A confrontation between the mechanical model and experimental data has been achieved in the context of biomechanics of regulation (Fournier *et al*., 1994). A specific modelling approach has been carried out on fruit trees by comparing contrasted varieties of apricot tree by taking into account both large displacements and tension wood effect (Alméras, 2001; Alméras *et al*., 2004). These studies showed that the main factors involved in the final shoot form were, first, its initial geometry (in particular, its slenderness and inclination) and, second, the distribution of loads along the shoot. This suggested that the variables related to shoot morphology are the first targets to evaluate the propensity of shoot bending across a wide range of genotypes.

Prusinkiewicz (1998) discusses a number of other forms of interaction with the environment, where plant growth is affected either by global properties (such as day length, daily temperatures) or by local properties (micro-environment, light, presence of obstacles, other plants, varying soil resistance or pruning), with possible loops in the information flow between plant and environment.

9.5.3.3 Integrated reactive models

In the last decade, a number '*functional–structural models*' [term coined by Sievänen *et al*. (1997)] have been designed to integrate various knowledge sources into reactive systems. Depending on the focus of the approach, the model may be made up of different types of sub-models. Biophysical phenomena (such as light interception) are usually well-understood and can be integrated in the overall model without the help of adjustment parameters. For internal processes such as transport, allocation, photosynthesis, etc., the situation is more complex since we do not precisely understand their exact physical and chemical nature. For these processes, additional adjustment parameters are usually required. This can be done to determine the resistance of a branch segment, to set up a conversion ratio between light and photosynthesis, to define an allocation strategy of assimilates to different organs, to fill a reserve compartment, to define a leaf growth rate as a function of temperature, etc. These parameters are used to adjust the model to observed data. They can express, for example, different types of empirical correlations between variables of interest. In some extreme cases, there may only be a qualitative knowledge about a particular phenomenon or even no knowledge at all.

For instance, designs with a qualitative basis have been conducted using different levels of combinations of mechanistic and empirical approaches on maize, (Fournier and Andrieu, 1998) sugar maple (Pertunen *et al*., 2001) cotton (Hanan and Hearn, 2002) root systems (Drouet and Pagès, 2003) sunflower (Rey, 2003) various types of plants (Hu *et al*., 2003), and on peach tree (Allen *et al*., 2004). The aim of these models is to use plant architecture as a support to design and test the spatial integration of different processes: light capture, photosynthesis, respiration,

flux circulation, allocation of assimilates, organogenesis (location and nature of new organs), organ development, etc. Due to the difficulty in determining parameters and their overall complexity, these models are not intended to have great predictive power, at least in the first generation. However, the building of such complex models is absolutely essential to improve understanding of the interactions between the different processes, as well as to enable identification of knowledge gaps and to explore the hierarchy of parameters through sensitivity analysis.

9.6 Conclusion and perspectives

In this chapter, various aspects of the modelling of plant architecture have been reviewed: observation, representation, analysis, statistical/process-based models, descriptive/reactive models. All these approaches have in common the fact that they use a spatial 3-D representation of the plant. The main current effort is in the integration of these different pieces of knowledge into consistent modelling frameworks. These functional–structural approaches face several aspects of the system complexity: (i) *complexity of the biological system*, in particular due to the high variability and plasticity of plant growth, and to the multitude of interweaved scales at which physical, ecophysiological and morphogenetic phenomena occur, (ii) *complexity of integrating various sources of knowledge*, possibly at different time scales, into one consistent modelling framework; (iii) *computer simulation complexity* which necessitates management of numerous dynamically changing and interacting parts.

These questions are currently being discussed within the functional–structural plant model research community (Godin *et al*., 2004a) where new approaches emerge:

- To tackle the lack of a modelling framework for developing integration of structure and function, new modelling paradigms are being developed and tested. This is illustrated, for instance, by the intermediate-level approach (not completely mechanistic or descriptive) based on a systematic flux-based representation of the various phenomena at different scales [Renton *et al*., (2004) and detailed in Renton (2004)], and in the adaptation of theory of variable aggregation to support scaling in plant processes (Franc, 2002; Mäkelä, 2003).
- Understanding the effect of genes in the development of plant form. The large number of recent results obtained in both molecular and cellular biology enables us to consider a new approach of developmental biology based on modelling at a cellular scale. As Sussex and Kerk put it: *Much architectural diversity results from the varied growth patterns of apical meristems. Current research is showing that meristem growth patterns are regulated genetically and hormonally, and the genes that control these processes are being identified and characterized* (Sussex and Kerk, 2001).

Today, several teams are building models of meristem development, organ growth and hormone signals, to grasp the role of different parameters in the control of phenomena like phyllotaxy, meristem maintenance and response to environment [see Prusinkiewicz (2004) for a review].

- Design of new languages is also emerging for modelling the morphogenesis of structures computationally more complex than tree graphs. Not all structures can simply be encoded into strings. In particular, discrete structures with so-called cycles (structures for which there exists pairs of components linked by more than one pathway) cannot be simply represented by strings. At the scale of tissues, for instance, structures correspond to complex 2- or 3-D objects. In an attempt to generalize L-system to model the development of discretized surfaces, Prusinkiewicz and colleagues introduced a language, called VV, for rewriting meshes of triangles (representing tissues). They applied this generic system to the problem of modelling the growth of apical meristem and the emergence of phyllotactic patterns (Smith and Prusinkiewicz, 2004). Another attempt of such a generalization lead Giavitto and Michel to design a generic language, called MGS, as an experimental generic approach to $(DS)^2$ rewriting (Giavitto and Michel, 2001). Like L-systems, MGS enables the description of string, multiset and tree-like structure rewriting, but it also allows manipulation of new $(DS)^2$ complex structures such as regular arrays, grids, *n*-dimensional Voronoi complexes, simplicial complexes and membranes (Giavitto and Michel, 2002). The possibility of using such languages in the context of plant architecture modelling is currently being tested to model the growth of meristems (Barbier de Reuille *et al*., 2004).

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10 Applications of plant architecture
Haute cuisine for plant developmental biologists

Nick Battey

Hors-d'oeuvre: tender asparagus in melted lemon and Parmesan butter

I hope with this menu to titillate your interest in the applications of plant architecture, and to spice things up with some thoughts on uses of developmental knowledge in a practical context. A keen awareness of how different kinds of plants work underpins many centuries-old horticultural and agricultural techniques for controlling plant architecture. This knowledge is needed for the exploitation of the particular part of the plant of commercial value, whether it be root, shoot, flower or fruit, or even overall aesthetic form. Indeed, exploitation is as diverse as the variety of plant architectures. The rose can ramble impossibly and still be venerated for it (Figure 10.1). The yam buries its treasure in the ground (Figure 10.2), while the hop towers 5 m high and requires elaborate wirework to accommodate its effervescent twinings (Figure 10.3). The grapevine gnarls its fists at decades of pruned confinement; yet, Joseph Paxton built a glasshouse in the image of the Amazonian water lily, in deference to its extravagant growth (Figure 10.4).

What is a developmental biologist to make of all this? How does it help to know about *SHOOTMERISTEMLESS, REVOLUTA, AERIAL ROSETTE 1, SCARFACE* and the hundreds of other genes that generate form in *Arabidopsis*? If we know, for example, that *LATERAL SUPPRESSOR* from tomato and *Arabidopsis* have analagous functions that are crucial for axillary meristem establishment (Greb *et al*., 2003), how can this help to improve practice in commercial crop production? Is it enough to tell the asparagus grower, whose livelihood depends on successful shoot production, that similar genes are likely to be important? No. We need to see the underlying principles, and, based on these, devise strategies for useful application of our knowledge.

In agriculture, major issues have been yield and crop quality, and we shall see that significant contributions have already been made through manipulation of plant architecture. In horticulture, where perennial crops are prominent, most of the existing systems for modification of plant architecture are concerned with the control of plant size and the accommodation of timing of meristem transitions to flowering, or into active growth. Labour cost, a prime problem for growers in the developed world, is closely connected to this need for regulated plant development; so,

Figure 10.1 *Rosa filipes* 'Kiftsgate' at Kiftsgate Court, Gloucestershire, UK, from which it takes its name.

Figure 10.2 *Dioscorea rotundata*: yam tubers at harvest. Photograph courtesy of Peter Craufurd (The University of Reading).

Figure 10.3 Hop training system. In the United Kingdom, the wirework support for the coming season's hop growth was traditionally erected and maintained by stilt men. Photograph courtesy of the National Hop Association of England.

I explore here the likely future economic benefits of molecular understanding in this area. Overall, the intention of this brief gustatory tour through the architecture of the plant world is to indicate the potential for applications of plant developmental biology in matters of practical significance.

The wine list

The grapevine illustrates an important principle of manipulation of architecture in temperate perennial fruit crops: flower buds (and therefore fruit) are borne on last year's (or older) shoots. Although this is not invariably true (subtropical citrus and olive are notable exceptions) it is typical, and it occurs because axillary meristems of leaves from the current year's growth initiate inflorescences that will not emerge

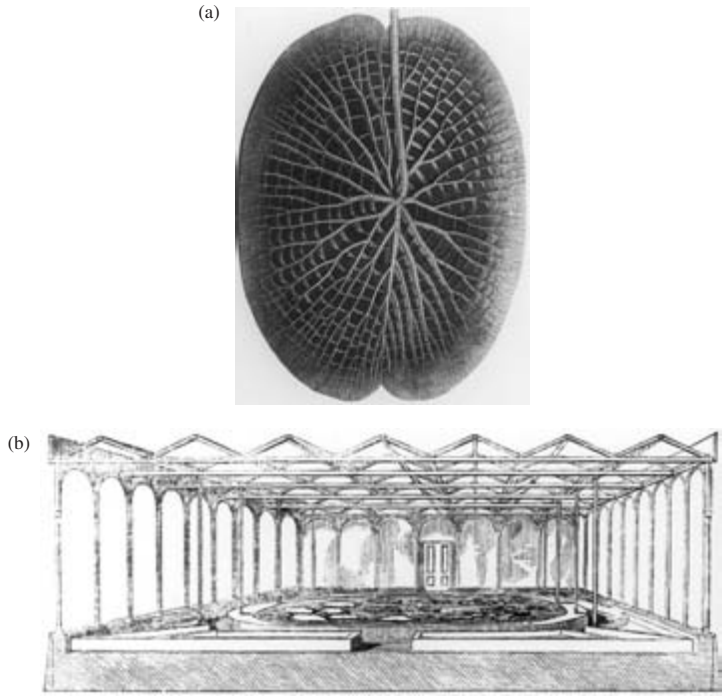

Figure 10.4 (a) Underside of the leaf of *Victoria amazonica* (illustration by William Sharp, in Allen, 1854). Reproduced with permission of Royal Botanic Gardens Kew Library. (b) Interior of the lily house built by Joseph Paxton to accommodate *V. amazonica*. The roof design was inspired by the cantilever principles of the leaf's architecture (see Chadwick, 1961; Colquhoun, 2003). Reproduced with permission of the Illustrated London News Group.

until the following year. Figure 10.5a shows how this works in grapevine [for further details see Pratt (1974) and Mullins *et al*. (1992)]. Shoots grow rapidly in the spring and the axillary meristem associated with each leaf forms the 'prompt bud'. This immediately grows out to form the summer lateral. The first leaf of this lateral shoot is a prophyll and its axillary meristem forms the 'latent bud'. This latent bud does not grow out, but initiates two to three prophylls, about ten leaf primordia and three inflorescences. In the axils of the prophylls, secondary and tertiary latent buds are formed. The whole complex of buds-within-a-bud is visible as a very swollen 'eye' by the autumn, when the summer lateral abscises (Figure 10.5b). In the spring, the primary latent bud grows out and the inflorescences emerge; secondary and tertiary buds, typically, play little further role. In the axils of the leaves of the shoot derived from the latent bud, the cycle of prompt and latent bud formation is repeated.

Why are the inflorescences initiated one year, yet grow out in the next? This is because, as in many temperate woody perennials, inflorescence initiation and development take a very long time – about 14 months (Lavee *et al*., 1967; Buttrose, 1974). This has been investigated at the molecular level in grapevine

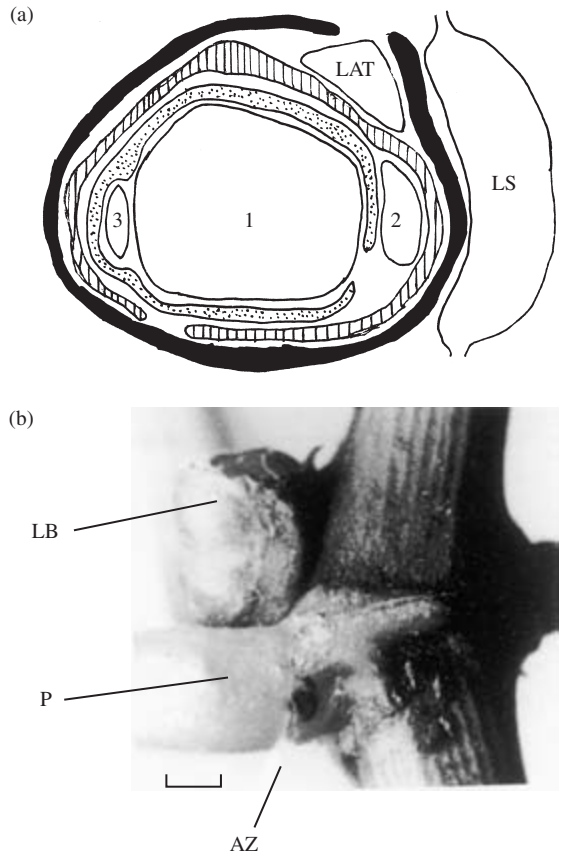

Figure 10.5 Axillary bud development in grapevine. (a) Transverse section through a compound bud ('eye') of Concord grape (*Vitis labruscana*) as it appears after abscission of the subtending leaf (leaf scar, LS) and summer lateral (LAT), in the autumn. The first leaf of the summer lateral is a prophyll (solid black area) and subtends the primary latent bud (1); the secondary and tertiary latent buds (2 and 3) are in the axils of the two basal prophylls (vertically hatched and stippled) of the primary bud. Redrawn from Pratt (1974) and Mullins *et al*. (1992). (b) Grapevine cane in autumn before abscission of the leaf (P, petiole; note abscission zone, AZ) subtending the summer lateral, which has abscised. LB is the 'eye', or complex of latent buds enclosed by the prophyll of the summer lateral, and shown in section in (a). Reproduced courtesy of Mullins *et al*. (1992) *Biology of the Grapevine*, with permission of Cambridge University Press.

(Carmona *et al*., 2002; Calonje *et al*., 2004), and in kiwifruit which, like the grapevine, flowers on 1-year-old shoots and requires an extensive support system. Putative kiwifruit orthologues of the floral meristem identity genes *LEAFY* and *APETALA1* show a bimodal pattern of expression, first during floral evocation in late spring of the first year, and again during floral differentiation about 10 months later (Walton *et al*., 2001).

The early evocation appears to be crucial in determining the overall architecture of the kiwifruit plant, because it prevents continued vegetative development in meristems in the middle nodes of the axillary bud (Figure 10.6). This means that the following year, when this bud grows out, flowers are located in the middle of the shoot. The most distal meristems of this shoot in turn differentiate into axillary buds containing flowers in their middle nodes, and in the natural habitat would repeat the cycle the next season. The consequence of this is the trailing, vine habit.

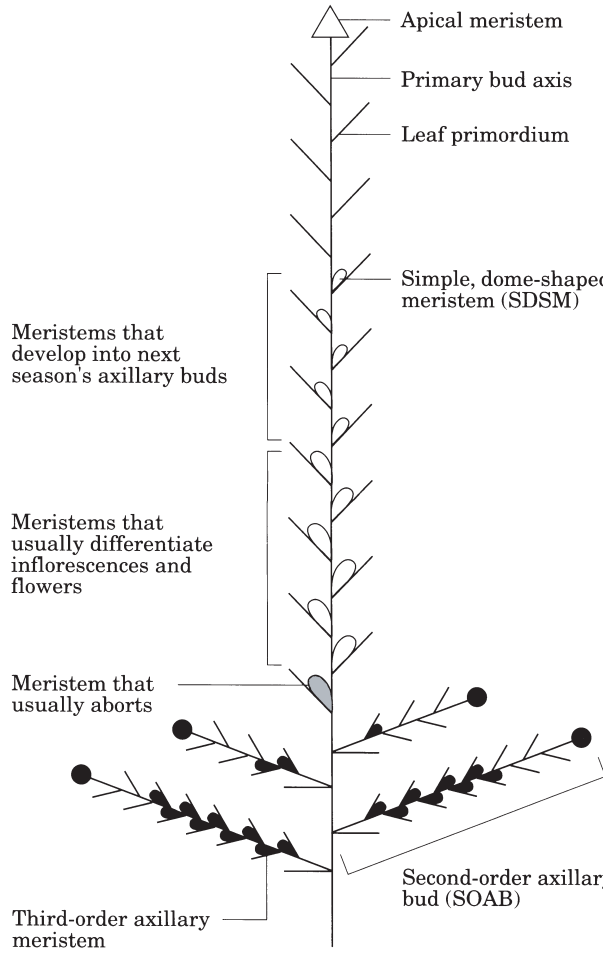

Figure 10.6 Axillary bud development in kiwifruit (*Actinidia deliciosa*). The diagram shows a mature first order axillary bud from node 15 of a kiwifruit vine. Note the varying developmental fates of the meristems subtended by the leaf primordia within the bud. Reproduced with permission from Walton *et al*. [Published in *Annals of Botany* **80** (1), 13–21 (1997)].

In commercial production, the second-order axillary buds at the base of the structure depicted in Figure 10.6 are key: they can be used as replacement shoots to carry the following year's crop. The factor that determines whether a meristem undergoes evocation or remains vegetative appears to be its size (Walton *et al*., 1997). Because this has such a strong influence on the architecture of the plant, it will be of great interest to discover how it connects to the decisive event of *LEAFY/APETALA1* expression, and floral evocation.

This kind of developmental pattern drives the training and pruning methods used to manage and optimize fruit production. There are many such methods, particularly for grapevine, with its long history of production (Mullins *et al*., 1992). The variations reflect not only tradition, but also different approaches to optimizing light interception and dealing with the growth habits and vigour of particular vine varieties/species (for further details see Jackson and Looney, 1999). I shall briefly describe the double guyot system – a traditional French system – to show the principle. The double guyot is well-suited to steep hillsides and intensive vineyard production, and involves cane pruning in which the canes that have fruited in the summer are cut out in the winter (Figure 10.7). New canes are tied in and these flower and fruit the next summer. It is, therefore, a replacement pruning system and has a similar developmental basis to that discussed for kiwifruit above.

The key feature is that three canes in the centre are saved each year. After the fruited canes have been cut out, the best two of these three are bent through 90° to form the replacement 'arms' of the vine. In the subsequent year, the axillary shoots from the latent buds on these canes grow vertically and the inflorescences (and hence grape bunches) are presented at a convenient height for picking. The bunches are also, given judicious summer pruning of excess leaves, well-positioned for light interception to ripen the fruit. Crucially, the system accommodates the prolonged period of inflorescence development within the latent buds. This protracted development means that spring weather is critical for the following year's crop. For example, the Muscat grape fails to initiate flowers if spring temperatures are below 20°C (Buttrose, 1969).

This example of the double guyot system shows that understanding the control of developmental rate is very important for viticulture. If flowers grew out in the year they were formed, it might be possible to design a simplified (and less labour intensive) system. This change has been partially achieved in raspberry, because of the availability of naturally occurring autumn-fruiting cultivars (Carew *et al*., 2000). The traditional, biennial raspberry is similar to the grapevine and kiwifruit in that it initiates flowers one season and these grow out the next. Hence the need for elaborate and costly training systems to separate vegetative and fruiting canes. The introduction of autumn-fruiting cultivars, which flower and fruit all in one year, means that a simpler support system is required, and the canes can be cut down to the base at the end of each season – a much cheaper means of production. It may be that once the appropriate fruit quality characters have been achieved, these cultivars find an increased market. There is still, however, a need for a long growing season to accommodate the autumn-fruiting raspberry.

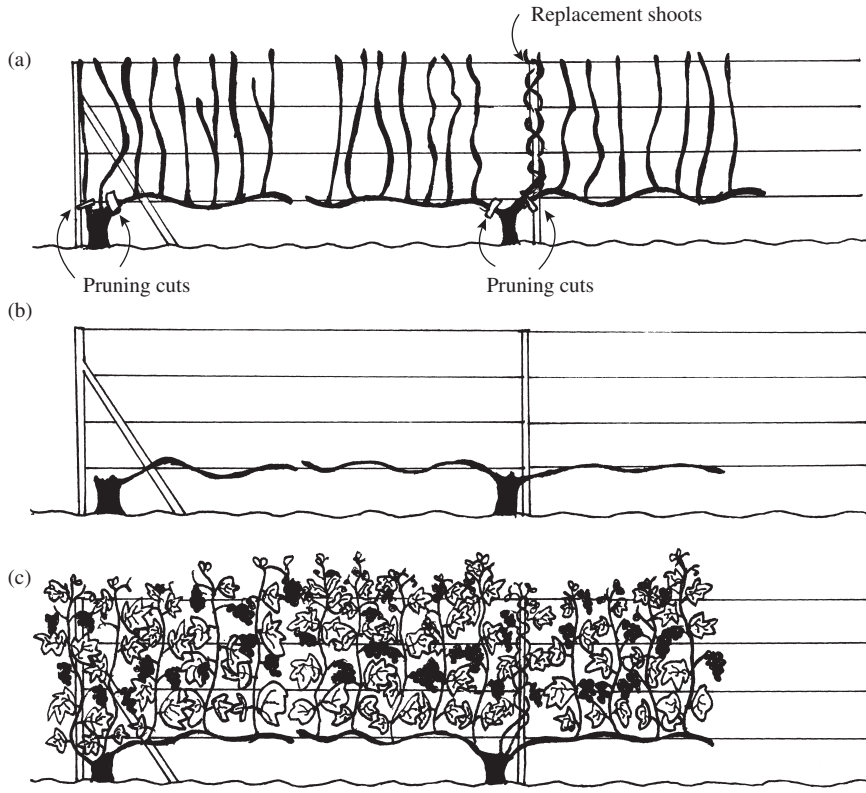

Figure 10.7 Double guyot training system for grapevine. (a) Winter. After leaf-fall, the canes that fruited the previous season are removed by pruning cuts. Three canes have been trained up the support post during the season ('replacement shoots') and are not removed. (b) The two strongest replacement shoots are bent down to the horizontal and tied to the lowest wire. The third replacement shoot is discarded. (c) The following summer. The lateral buds on the tied-down replacement shoots have broken and grown vertically to display inflorescences (and hence fruit bunches) at a convenient height for light interception and harvesting. Note the replacement shoots wound around the support post. The latent buds formed in the axils of the leaves on these shoots will provide inflorescences for the next season's crop. Diagrams adapted from Jackson (1986).

Interestingly, in tropical species such as lychee and mango, which have a number of growth (flush) cycles per year, flowers are initiated and emerge shortly after bud growth begins (Batten and McConchie, 1995). This rapid and relatively continuous process of flowering in association with shoot growth means that, in general, pruning and training in tropical subjects are primarily concerned with optimizing photosynthesis in relation to fruit load, and presentation of fruit for harvest, rather than ensuring a regular cycle of bud development across the seasons, as described above for temperate species.

Starter: rosemary and Taleggio stuffed tomatoes on a bed of herbs

The tomato plant shows very striking variation in habit. Although there are many subtle distinctions between cultivars, they can be divided into indeterminate and determinate types (Atherton and Harris, 1986). The habit of the weedy progenitor of the cultivated tomato would have been indeterminate, and this character is still present in most of the varieties used in glasshouse production of tomatoes for the fresh market. These vines can grow to be 10–11 m tall, and elaborate training systems have been devised to accommodate them (Figure 10.8). Fruit production occurs in the terminal 3–4 m and the indeterminate habit leads to a prolonged cropping season. In contrast, the breeding of the determinate character into the tomato underpinned the development of the outdoor, mechanically harvested crop for processing. The bushy, squat habit of these tomatoes allows an over-row harvester to pass above the plants, undercutting at the top of the root system. The whole plant is then shaken so that the fruits fall on to a conveyor belt and are sorted before transport to the processing plant. Associated with the determinate habit is a concentrated flowering/fruiting phase that suits the economics of mechanical harvesting where a single cropping operation is often desirable.

The determinate habit arises as a result of a recessive allele at the *SELF-PRUNING (SP)* locus (Yeager, 1927; Stevens and Rick, 1986). Its effect is to terminate growth of the main axis and lateral axes that break subsequently, gradually reducing the number of leaves within successive sympodial units from three, to two, and then to none as two successive inflorescences are produced (Figures 10.9a,b). The *SP* gene

Figure 10.8 Tomato production system. Glasshouse tomatoes showing indeterminate habit and elaborate support system. Photograph courtesy of Martin Emmett (The University of Reading).

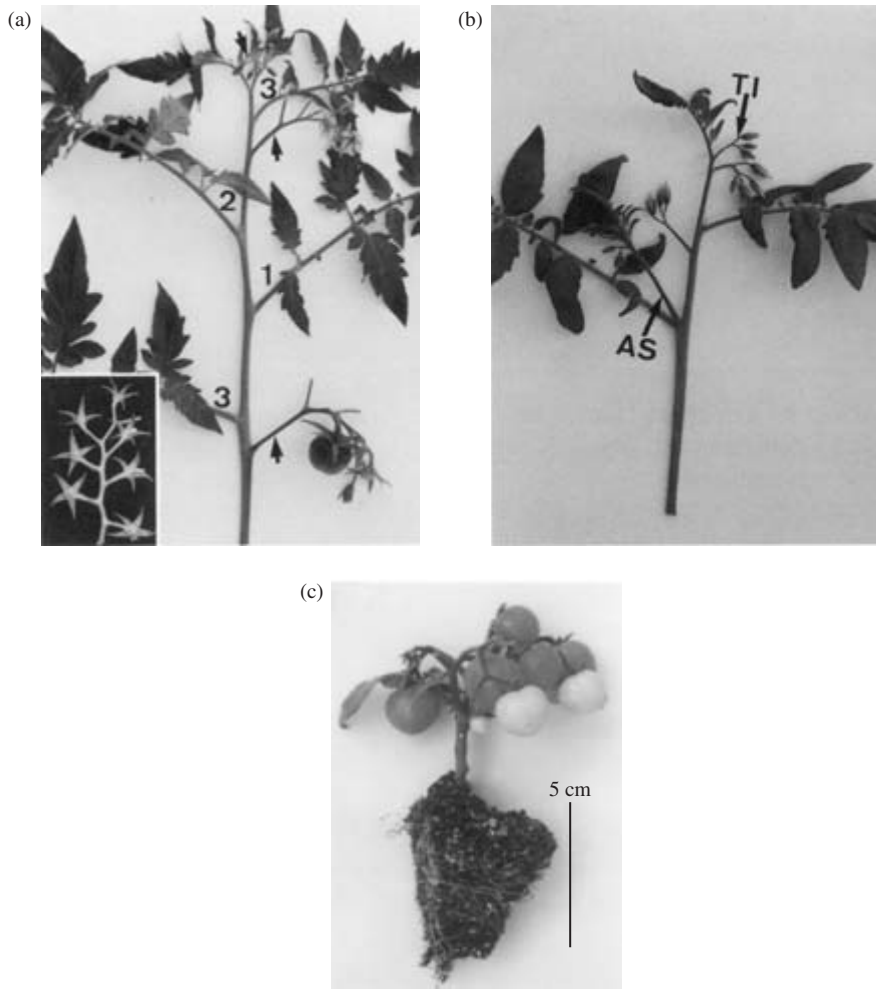

Figure 10.9 Indeterminate and determinate habits in tomato. (a) Shoot of an indeterminate type. A sympodial unit consists of three leaves (labelled 1, 2, 3) and an inflorescence; leaf 3 appears above the inflorescence of its sympodial unit because its petiole is united with the stem of the next unit. The arrows indicate the inflorescences of three consecutive units. (b) Shoot of a determinate (*self-pruning*) type. Only one leaf separates the two inflorescences, and the uppermost inflorescence (T1) terminates the shoot. Growth is continued by the axillary shoot (AS) below, leading to the bushy habit. Reproduced with permission of Pneuli *et al*. [Published in *Development* **125**, 1979–89 (1998)]. (c) Micro-Tom, a dwarf determinate cultivar bred for gardening but with architectural qualities suitable for a model fruit crop. Reproduced with permission of Meissner *et al*. [Published in *The Plant Journal* **12** (6), 1465–72 (1997)].

is described as the orthologue of *CENTRORADIALIS/TERMINAL FLOWER 1* (*CEN/TFL1*) from *Antirrhinum/Arabidopsis* because it has extensive sequence similarity and regulates shoot determinancy like these genes (Pneuli *et al*., 1998). It is clear, however, that *SP* acts on the underlying mechanism that leads to sympodial growth, and that it does not regulate the length of all phases, as has been postulated for *CEN* and *TFL1* (Ratcliffe *et al*., 1998, 1999). The juvenile phase is unaltered in the *sp* mutant, as is inflorescence structure (Pneuli *et al*., 1998). *SP*, therefore, ensures the cycling between vegetative and reproductive phases that is characteristic of indeterminate tomato. How this function relates to *CEN/TFL1* plants is still unclear, but a major role of *SP* is to prevent the allocation of some meristematic cells to inflorescence fate. In doing this, it allows indeterminate growth to persist.

The *self-pruning* mutation is one of two or three genes mutated in Micro-Tom (Figure 10.9c), a miniature-dwarf-determinate cultivar of *Lycopersicon esculentum* bred for gardening and now advocated as a model system for developmental analysis of fruit crops (Meissner *et al*., 1997, 2000; Emmanuel and Levy, 2002). Here, breeding for architecture to suit a commercial niche has contributed to fundamental analysis of plant development.

Main course: pea and Pecorino risotto with saffron

Just as the development of field-grown processing tomato was underpinned by the breeding of determinate varieties, so modified stature played a crucial role in the process of rice improvement. The Green Revolution of the 1960s and 1970s, in which yields of both wheat and rice showed spectacular increases, was underpinned by the introduction of semi-dwarfed cultivars. In these high-yielding types, the shorter stems were capable of supporting the increased grain load. In both crops, the semi-dwarf trait results from the presence of mutant alleles at particular loci (*REDUCED HEIGHT* in wheat; *SEMIDWARF1* in rice) and these interfere with gibberellin biosynthesis or signalling [for a review see Hedden (2003)]. In the Chinese cultivar Dee-geo-woo-gen, the source of the semi-dwarf trait used in breeding the commercial *indica* cultivars of the Green Revolution, the *sd1* allele contains a 383-bp deletion and therefore encodes an inactive GA20-oxidase (Ashikari *et al*., 2002; Sasaki *et al*., 2002). The wild-type allele is needed for maximum vegetative growth, but the GA20-oxidase is partially redundant with other enzymes regulating GA biosynthesis. This is why its loss leads only to semi-dwarfism, and it makes the trait of particular practical value, because it is not too extreme (Hedden, 2003).

MONOCULM1 is another rice gene with a negative effect on plant height, but its more significant, primary function is to regulate the normal formation of tillers (Li *et al*., 2003; see Chapter 4). It may be a master regulator of a network of genes that influences axillary bud formation and includes the rice orthologue of the maize gene, *TEOSINTE BRANCHED1*, and the homeobox gene, *OSH1*. Elucidation of the network may herald a new phase in the manipulation of architecture in this crop

because tiller production is an important determinant of grain yield (Yan *et al*., 1998). It is therefore of particular interest that *MONOCULM1* is a member of the GRAS family of VHIID proteins, and homologous to *LATERAL SUPPRESSOR* from tomato and *Arabidopsis* (Schumacher *et al*., 1999; Greb *et al*., 2003). This family of regulatory gene products includes *RHT, GAI* and *D8*, which now have well-characterized roles in mediating GA signalling. Although *MOC1*/*LS* are on a separate branch of the family, they may also act by controlling GA responsiveness in the leaf axil (Grbić, 2002), suggesting further commercial gains may arise from controlling the effects of this plant hormone.

Against this background, it is germane to consider less favourable rice-growing regions where Green Revolution cultivars have had less impact. Farmers in these regions (the uplands and rainfed lowlands, as opposed to irrigated areas, of south-east Asia) have continued to use traditional varieties, for example, where deep flooding is sufficiently long-term to make short stature inappropriate (Fujisaka, 1999). As Fujisaka observes, breeding for plant architectures relevant to very specific local conditions may be the next big challenge to be addressed.

Knowledge of the rice genome sequence and of the developmental subtleties of the rice plant should help to achieve this objective. Agronomic methods also complement advances in breeding because there are very pronounced effects of environment on the architecture of the rice plant; for instance, wider plant spacing encourages tillering. In addition, increased physiological understanding of the response to flooding will be valuable: flooding-induced stem elongation is mainly due to ethylene accumulation, which reduces ABA, which in turn increases tissue responsiveness to GA (see Chapter 3). There is also likely to be an iron toxicity dimension to the depressed yields associated with long-term flooding (Hengsdijk and Bindraban, 2004).

While the history of rice breeding illustrates the advantages of short stature, the pea plant shows, perhaps surprisingly, the disadvantages that leaves can have for overall crop performance. The pea leaf has a complex architecture, with basal stipules, leaflets and tendrils (Figure 10.10a). Wild progenitors of the pea plant gained their support from other plants, and so invested poorly in stem-support architecture. As a consequence, the unsupported pea crop has a tendency to fall over, making harvesting difficult. The potential of the semi-leafless trait was therefore explored. In semi-leafless types, tendrils replace leaflets as a result of the *afila* mutation (Figure 10.10b), and these extra tendrils wind around neighbouring plants, providing much improved mutual support (Snoad, 1974; Pyke and Hedley, 1985). A concern with these types might be reduced photosynthetic capacity due to reduced leaf area. However, the combined light interception of the remaining leaflets, along with the tendrils, stipules, stems and ultimately pods, is apparently sufficient for photosynthetic efficiency. Furthermore, pea plants normally make such extensive vegetative growth that seed development can be negatively affected. In one study, the semi-leafless character reduced leaf area by 40% and seed yield was increased by 10–20%; this benefit was considered to result from better light penetration into the canopy and prolonged stipule photosynthesis (Guillon *et al*., 1982; Cousin *et al*., 1985). A detailed analysis of a series of pea lines showed clearly that the

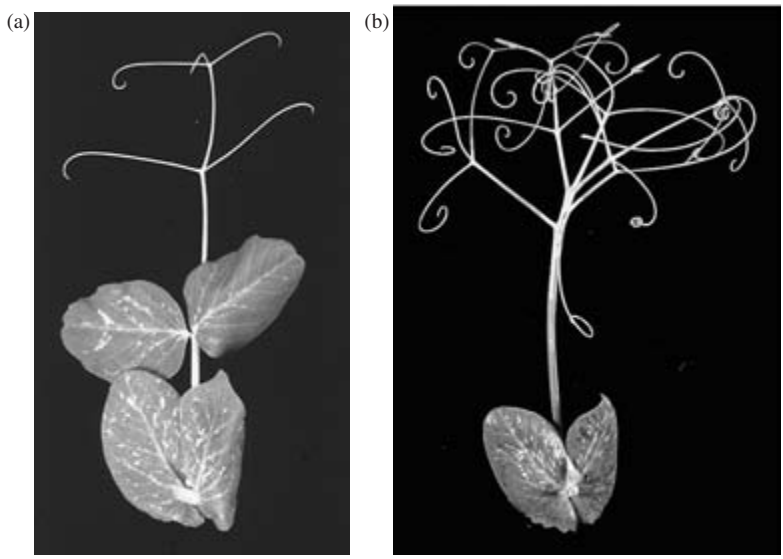

Figure 10.10 Architecture of pea leaves. (a) Wild-type pea leaf with a pair of basal stipules, petiole, and a blade made up of a pair of proximal leaflets, two distal pairs of tendrils and a terminal tendril. (b) *Afila* mutant leaf: the leaflets are converted to tendrils but the stipules remain. © American Society of Plant Biologists and reproduced with permission of Gourlay *et al*. [Published in *The Plant Cell* **12**, 1279–94 (2000)].

semi-leafless character was not detrimental to cropping and emphasized the improved standing ability of crops with this trait (Pyke and Hedley, 1982, 1983a,b). Many cultivars of commercial significance now include the *afila* semi-leafless trait (Heath and Hebblethwaite, 1985).

Dessert: individual apple tarts with strawberry coulis

Since the middle of the last century, apple growers have been focused on the orchard management goal of controlling vegetative growth. This encourages flowering early in the life of the tree and provides smaller trees that are easier to prune, spray and harvest (Figure 10.11). Sexual precocity has been an objective in its own right, because fruiting limits vegetative vigour. Although plenty of work has been done on the physiology of flowering in apple (Buban and Faust, 1982; Dennis, 2003), it is through the use of rootstocks that most progress has been made in limiting vegetative vigour and engendering this precocious flowering. The economic benefits – of rapid, consistent cropping and limited tree size – are very significant where labour and land costs are high (Webster and Wertheim, 2003).

Rootstocks are now used in most tree crops, including citrus, walnut and avocado, but it is in Rosaceous fruit trees (e.g. apple, pear, plum and cherry) that they have been

Figure 10.11 Intensive apple production. Reproduced courtesy of East Malling Research.

evaluated in most detail (Rom and Carlson, 1987). The rootstock provides a root system, while the scion (the desired cultivar, such as 'Cox' or 'Golden Delicious') is grafted on to it, typically at about 30 cm above soil level. The rootstock is an aid to propagation because it is usually easier to root than the scion cultivar; it can provide disease resistance or tolerance to unfavourable environmental conditions; and, most noticeably, it can profoundly influence growth rate and ultimate tree size (Figure 10.12). Thus, the extremely dwarfing apple rootstock M27 gives a tree not more than 1-m tall, compared with a size of around 7–10 m for a tree on its own roots; and the widely used stock M9 gives a tree only 25–35% of full size (Ferree and Carlson, 1987; Webster and Wertheim, 2003). These stocks also induce precocious flowering: a significant yield can be obtained from a 2–3-year-old dwarfed tree, whereas, on their own roots, apples will not normally flower until several years later.

The physiological causes of the dwarfing effects of rootstocks are poorly understood (Lockhard and Schneider, 1981; Webster and Wertheim, 2003). This dwarfing is not as simple as many of the single-gene gibberellin-related internode length traits discussed earlier and in Chapter 3. Trees on dwarfing stocks have fewer growing points that extend for a shorter period than those on a more vigorous stock

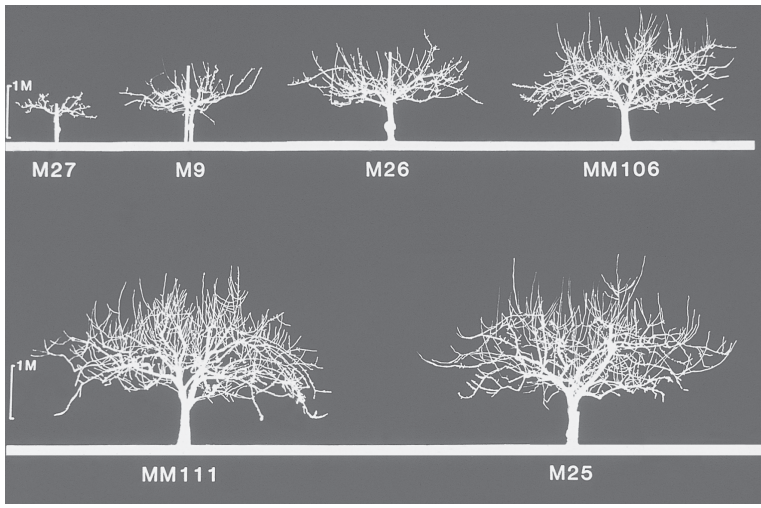

Figure 10.12 Rootstock effects on tree size in apple. The 'M' and 'MM' abbreviations refer to the Malling and Malling-Merton rootstock series. Reproduced courtesy of East Malling Research.

(Avery, 1969). The root system has an even slower growth rate than the scion, and this may limit overall tree growth. This is consistent with the observation that dwarfing stocks favour the diversion of photosynthates to the fruit (Avery, 1970). It has been difficult, however, to establish a clear chain of cause and effect for the underlying dwarfing process.

One concept that has received general support is that dwarfing stocks alter the translocation of water, nutrients and/or hormones (Jones 1971, 1986). This may be because rootstock anatomy and physiology are specialized for slow growth and development. Thus, Colby (1935) notes that M9 is naturally a low-branching shrub, adapted to semi-arid conditions, rather than a tree. M9 was originally known as the 'true Paradise' apple and has a long history of use for dwarfing (Hatton, 1920). The name 'Paradise' derives from Pairidaezai – a Persian park or garden, emphasizing this dwarf character. From a physiological point of view, it is of interest that the Paradise apple has been recommended since at least 1681 for vigour control *as a small stem piece* ('interstock') inserted between a non-dwarfing stock and scion (see Parry and Rogers, 1968). This ancient observation offers one clue to the rootstock mechanism.

Parry and Rogers (1968) showed the degree of growth control provided by a range of interstocks (Figure 10.13); in this study, M9 was equally dwarfing as an interstock or as a rootstock. In general, the longer the M9 interstock, the bigger the effect (Roberts and Blaney, 1967). A related observation is that a dwarfing effect can be produced by only a ring-graft of bark (Roberts, 1949). Lockard and Schneider (1981) studied the dwarfing effects of bark grafts of the semi-dwarfing stock M26 and proposed that basipetal auxin movement was the key process affected. Soumelidou *et al*.

(1994a) showed that the velocity of basipetal auxin transport was reduced in M9 compared to that in the vigorous rootstock MM111; it was suggested that this reflected a lower capacity for auxin efflux from transporting cells. Evidence consistent with poor auxin movement across the developing graft union was also found (Soumelidou *et al*., 1994b), suggesting a general importance of reduced auxin movement in the dwarfing effect. Kamboj *et al*. (1997) presented evidence consistent with these results for both M9 and M27. Given the great strides in understanding the mechanisms of auxin transport (Friml and Palme, 2002) and its function in relation to vascular patterning (Scarpella and Meijer, 2004) in *Arabidopsis*, it is time to revisit these long-standing issues about rootstock effects in tree crops. It is pertinent, however, that a dwarfed phenotype has now been described for poplar overexpressing the gibberellin catabolism gene GA2 oxidase (Busov *et al*., 2003). This suggests an alternative approach that could be used for dwarfing tree fruit crops; it will be of interest to

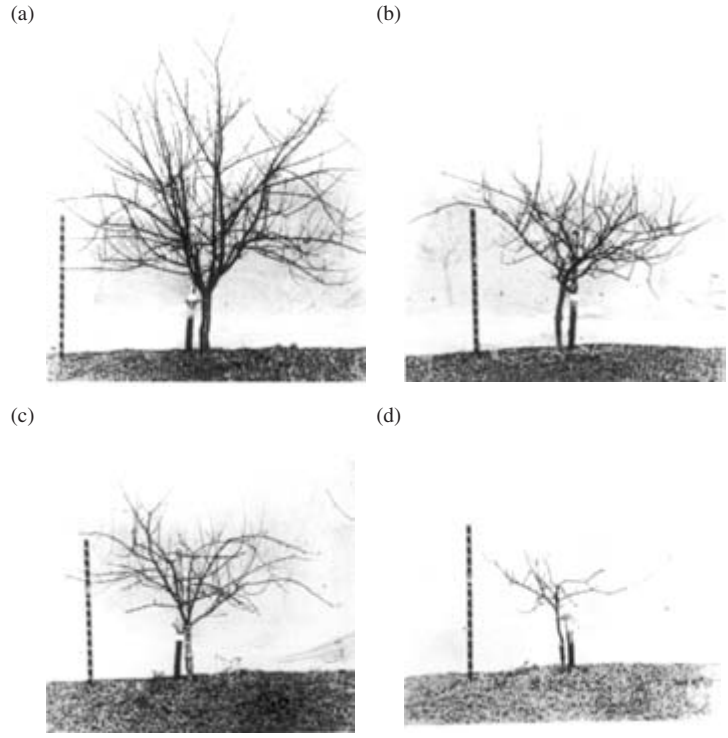

Figure 10.13 Control of vigour in apple trees by use of an interstock. All the trees are 6 years old and consist of a scion of Cox's Orange Pippin and a rootstock of MM104; the interstock is MM104 (a), M20 (b), M9 (c) and M27 (d). Reproduced from Parry and Rogers (1968) Dwarfing interstocks: their effect on the field performance and anchorage of apple trees. *J. Hort. Sci*. **43**, 133–46, with permission from the Editor and Trustees of *The Journal of Horticultural Science and Biotechnology*, who hold the copyright.

discover whether the required effect on precocity will also occur, particularly in view of the delayed flowering observed in GA2-oxidase overexpression mutants of rice and *Arabidopsis* (Sakamoto *et al*., 2001; Schomberg *et al*., 2002).

While vigour control has been a preoccupation of growers of apples and other tree fruits, the problem for strawberry growers has been the squat construction of their crop plant. A range of ingenious architectural solutions has been devised to overcome this natural dwarfness, in the interests of management efficiency and reduced harvesting costs (Figures 10.14a–c). But, all are expensive to set up and maintain; an alternative solution is suggested by a mutant, long-stemmed wild strawberry *Fragaria vesca arborea* from the island of Madeira (Staudt, 1959; Figure 10.14d). Genetic and physiological analysis suggested that this phenotype might result from a mutation in a repressor of gibberellin synthesis (Guttridge, 1973). Stem growth in the mutant was not sufficiently strong for the plant to be self-supporting, but this example suggests that a new strawberry architecture could be achieved with appropriately targeted breeding.

Coffee served with Deglet Noor

Over rich Arabian coffee served with dates – fruit of the desert and architectural inspiration to the ancient Egyptians (Figure 10.15a) – there is a little time to reflect on the wider applications of plant architecture. In gardens, we form and maintain plants in the shapes we consider characteristic: a top-worked willow that cascades downwards from a vertical stem is a botanical contradiction created for our diversion by the skill of the grafter (Figure 10.15b). The eccentricity of the corkscrew hazel (*Corylus avellana* 'Contorta') adds a bizarre twist – a talking-point – to a garden (Figure 10.15c). Topiary is perhaps the most obvious, and certainly one of the most extreme, examples of contrived plant architecture (Figure 10.16).

In the landscape, plant architectures are often deliberately created: the Lombardy poplar was probably a natural mutant of the black poplar (*Populus nigra*) selected by enterprising farmers in the Po Valley (Li, 1958). Traditional English woodlands are a consequence of active management of tree form, created and maintained for centuries by coppicing, suckering or pollarding (Rackham, 1995); by the time of the Domesday book (1086), there was probably no wildwood (i.e. unmanaged woodland) left in England. Even the towering Big Trees of Yosemite (*Sequoia gigantea*) were rapidly appropriated as symbols both of American national destiny and individual spiritual redemption (Figure 10.17; see Schama, 1996).

Take a step further, into the ornate magnificence of Gothic buildings and we stand among plants turned to stone, adapted and exaggerated into architecture that expresses a powerful idea about the relationship of man to nature. This idea was summarized by Friedrich von Schlegel in response to his visit in 1804 to Cologne cathedral (Figure 10.18): 'The essence of Gothic architecture consists in the power of creating, like nature herself, an infinite multiplicity of forms and flower-like

(a)

(b)

Figure 10.14 (a)–(c) Production systems designed to overcome the problems created by the small stature of the strawberry plant. Photographs courtesy of Alex Wagstaffe (The University of Reading). (d) *Fragaria vesca arborea* alongside *Fragaria vesca* of same age. Reproduced with permission of Staudt, G. 1959. Eine spontan aufgetretene Grossmutation bei *Fragaria vesca* L. *Naturwissenshaften* **46**, 23, Fig.1 © Springer.

(c)

(d)

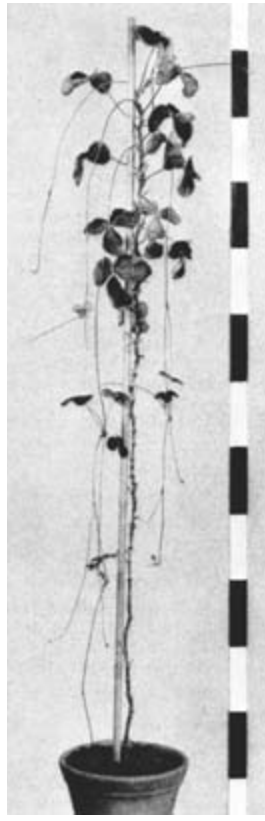

Figure 10.14 *continued*

Figure 10.15 (a) Egyptian column with a palm capital, from a temple built by Rameses II (fourteenth century BC). Reproduced with permission from Simon, H. (1978) *The Date Palm: Bread of the Desert*. Dodd, Mead & Co., New York. © Copyright The British Museum. (b) *Salix caprea* 'Kilmarnock'. This is naturally a shrub or ground cover plant, but commercially is usually grafted on a stock at about 2 m to give the weeping tree form. Photograph courtesy of Pat Breen, Oregon State University. (c) *Corylus avellana* 'Contorta' showing crooked stems; the leaves are also contorted. Photograph courtesy of Pat Breen, Oregon State University.

decorations. Hence the inexhaustible and countless repetitions of the same decorative details; hence the vegetable element'. This enthusiasm was connected to the view of a Gothic building as a work of nature with an underlying organic unity, first articulated by Goethe 30 years earlier, enraptured by Strasbourg cathedral with its facade of a 'thousand branches, million twigs and leaves like the sand on the shore' (Robson-Scott, 1965). Thus, Goethe considered that aesthetic beauty requires truth to nature; and that profusion should, therefore, reflect a deeper coherence.

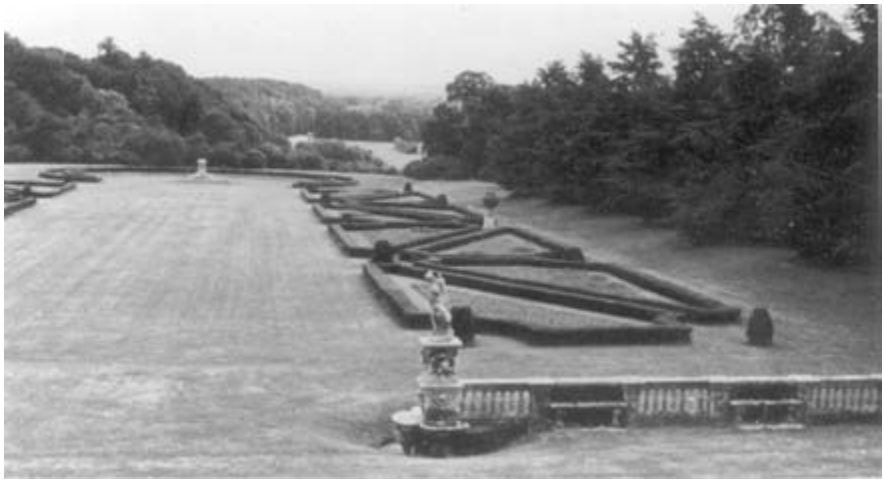

Figure 10.16 Topiary. The parterre at Cliveden House, Berkshire. Reproduced from Hadfield, M. (1971) *Topiary and Ornamental Hedges*. A. & C. Black, London.

Figure 10.17 The Great Trees, Mariposa Grove, Yosemite National Park, USA. Painted by Albert Bierstadt in 1876. The painting is in a private collection and is reproduced here from Schama, S. (1996) *Landscape and Memory*. Fontana Press, London.

Figure 10.18 Cologne cathedral, Germany. Photograph courtesy of the Cologne Picture Archive.

The palm features literally in the Regency design of John Nash at the Royal Pavilion in Brighton, and even more exotic is the Dunmore pineapple from a slightly earlier period (Figure 10.19). Plant architectures were also the basis for many of the designs of William Morris and the Arts and Crafts movement, and of Charles Rennie Mackintosh, although Mackintosh's use in particular was highly abstracted (Figure 10.20). It has even been suggested that while Mackintosh admired nature for the way in which beauty and utility are combined, in his own work, form outranked function (Robertson, 1995). In general, though, it has been the balance and harmony of form and function in plants that has influenced architects and designers. Here, to finish, is the famous assertion of Louis Sullivan (1896) concerning building design and construction, and the law that should bind nature and architecture; though it may seem extravagant and even fanciful now, it is a

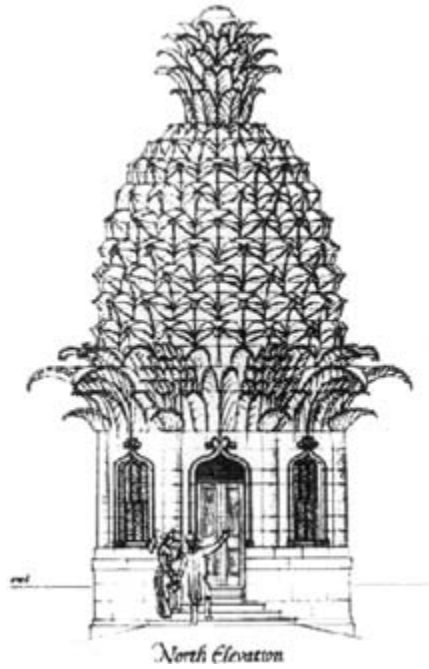

Figure 10.19 The Dunmore pineapple. The pineapple was a sign of hospitality in its native America when it was discovered by Christopher Columbus, and its rarity and expense led it to have the same symbolic role in polite society in Britain in the seventeenth and eighteenth centuries. This role as a status motif achieves perhaps its most fantastic expression in the stone pineapple built by the Earl of Dunmore as a garden folly in 1761 at Dunmore Park near Airth, Stirlingshire, Scotland. Reproduced with the permission of the Royal Commission on the Ancient and Historic Monuments of Scotland.

useful guide to thinking about how plants are designed, or could be redesigned for both aesthetic and practical purposes:

Whether it be the sweeping eagle in his flight, or the open apple-blossom, the toiling workhorse, the blithe swan, the branching oak, the winding stream at its base, the drifting clouds, over all the coursing sun, form ever follows function*, and this is the law. Where function does not change form does not change. The granite rocks, the everbrooding hills, remain for ages; the lightning lives, comes into shape, and dies in a twinkling.*

It is the pervading law of all things organic and inorganic, of all things physical and metaphysical, of all things human and superhuman, of all true manifestations of the head, of the heart, of the soul, that the life is recognizable in its expression, that form ever follows function. This is the law.

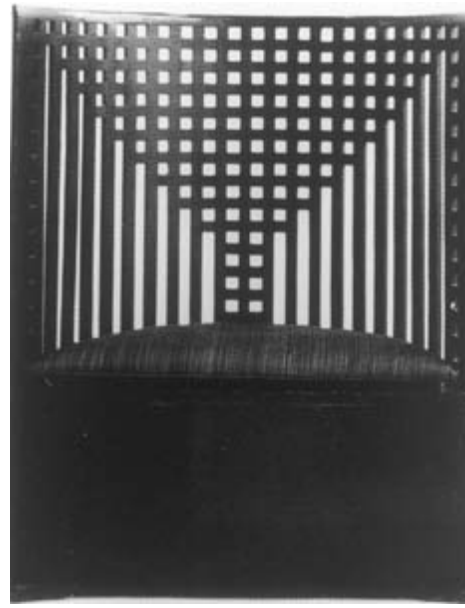

Figure 10.20 Chair designed by Charles Rennie Mackintosh for the Willow Tea Rooms, Glasgow, 1904. The tree form has been abstracted to an arrangement of squares and rectangles. Reproduced with permission from Robertson P. (1995) *Flowers: Charles Rennie Mackintosh*. HN Abrams, New York.

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