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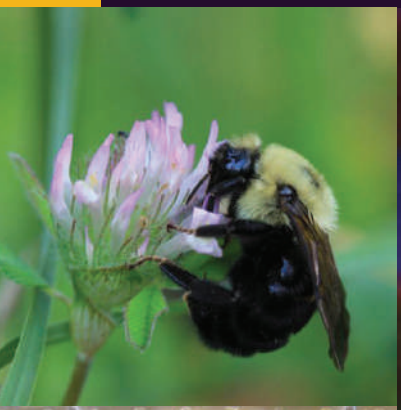

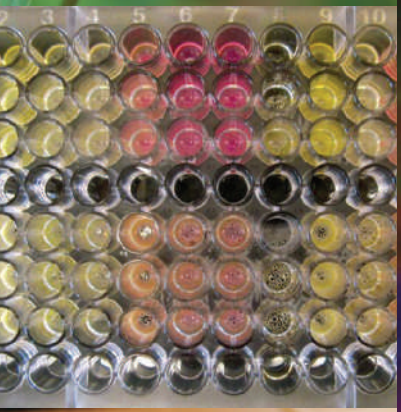

Plant Physiology

A. Malcolm Campbell
Christopher J. Paradise

MOMENTUM PRESS
APPLIED SCIENCES

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First published in 2016 by
Momentum Press®, LLC
222 East 46th Street, New York, NY 10017
www.momentumpress.net

ISBN-13: 978-1-94474-911-8 (print)
ISBN-13: 978-1-94474-912-5 (e-book)

Momentum Press Biology Collection

Cover and interior design by S4Carlisle Publishing Services Private Ltd., Chennai, India

10 9 8 7 6 5 4 3 2 1

Printed in the United States of America.

Abstract

This book examines three ways plants respond to their changing environment. The first example can be found in all plants. Despite the extreme changes in weather, plants have to stay where they are and respond to whatever nature produces. Plants have the capacity to respond quickly and yet they can evolve in a single generation. The second example addresses how an individual leaf has to respond rapidly and repeatedly to maintain the proper balance of carbon dioxide (CO_2) and water so that it can photosynthesize but not dry out. This delicate balance is governed by a pair of cells that regulate the size of openings on leaves. The final chapter examines a unique example of a leaf that can move fast enough to trap insects and digest them. This book presents data that led to our understanding of how plants function on different time scales.

Keywords

abscisic acid, paralogs, synteny, whole genome duplication, tetraploid, orthologs, deletions, inversions, insertions, ligules, epigenetic, complementary DNA, PCR, restriction enzyme, guard cells, stoma, voltage-gated K+ channels, osmotic pressure, cellular automaton, emergent properties, aquaporins, convergent evolution

Contents

Preface

This book about plant physiology is part of a thirty book series that collectively surveys all of the major themes in biology. Rather than just present information as a collection of facts, the reader is treated more like a scientist, which means the data behind the major themes are presented. Reading any of the thirty books by Campbell and Paradise provides readers with biological context and comprehensive perspective so that readers can learn important information from a single book with the potential to see how the major themes span all size scales: molecular, cellular, organismal, population and ecologic systems. The major themes of biology encapsulate the entire discipline: information, evolution, cells, homeostasis and emergent properties.

In the twentieth century, biology was taught with a heavy emphasis on long lists of terms and many specific details. All of these details were presented in a way that obscured a more comprehensive understanding. In this book, readers will learn how plants adjust to the challenges of their environments when they cannot move away to a better place, and some of the supporting evidence behind our understanding. The historic and more recent experiments and data will be explored. Instead of believing or simply accepting information, readers of this book will learn about the science behind plants responding to their environmental stresses the way professional scientists do—with experimentation and data analysis. In short, data are put back into the teaching of biological sciences.

Readers of this book who wish to see the textbook version of this content can go to www.bio.davidson.edu/icb where they will find pedagogically-designed and interactive *Integrating Concepts in Biology* for introductory biology college courses or a high school AP Biology course.

Acknowledgments

Publishing this book would not have been possible without the generous gift of Dr. David Botstein who shared some of his Breakthrough Prize with AMC. David's gift allowed us to hire talented artists (Tom Webster and his staff at Lineworks, Inc.) and copyeditor Laura Loveall. Thanks go to Kristen Mandava for project management and guidance on the publishing process. In particular, we are indebted to Katie Noble and Melissa Hayban for their many hours of help and attention to detail.

Kristen Eshleman, Paul Brantley, Bill Hatfield and Olivia Booker helped us with technology at Davidson College. We are grateful to administrators Tom Ross, Clark Ross, Carol Quillen, Wendy Raymond, Verna Case, and Barbara Lom who had confidence in us and encouraged us to persist despite setbacks along the way.

These books were the product of the shared labor of my two visionary coauthors Laurie Heyer and Chris Paradise. We shared the dream and the hardships and developed this book from scratch. My family has been very supportive and I thank Susan, Celeste and Paulina for their support and patience. I also want to thank Jan Serie, my pedagogical mentor, who taught me so much about the art and science of helping students learn. I benefited from the support of the Howard Hughes Medical Institute grant 52006292, the James G. Martin Genomics Program, and Davidson College. This book would not have survived its first draft without my students who endured the typos and the early versions of this book. These undergraduates participated in a bold experiment to see if beginners could construct their own knowledge, retain what they learned, and transform the way they see themselves and the discipline of biology. While many people said that beginning students were not up to the task, my students proved them wrong.

Introduction

This book presents three case studies about plant physiology. All three cases focus on homeostasis at the organismal level. The first case addresses how plants have evolved multiple mechanisms that allow them to adjust to their changing environments on multiple time scales. Genomic data will be examined that show why plants are the ultimate models of homeostasis in part because they are immobile. The second case study examines how plant leaves use paired cells to produce small openings that regulate water and CO_2 concentrations used by the entire plant. These **guard cells** change their shape rapidly in response to dynamic conditions such as temperature, sunlight and humidity. The final example confronts a common misconception that plants are not capable of moving on their own. The Venus flytrap not only moves, but moves quickly enough to trap insects between two lobes of modified leaves. This case study presents experimental evidence about how the plants know when to close and how they move quickly enough to trap their prey. All three of these examples illustrate how plants have evolved multiple dynamic mechanisms to maintain their physiology within a narrow range of acceptable limits.

CHAPTER 1

Plants Respond to Changes on Many Different Time Scales

Homeostasis By Gene Regulation

For most plants, locomotion is not an option, and therefore they must cope with their local conditions. Plants persist year after year despite drought, heat, high salt, flooding, and freezing. A plant's environment is always changing, and it must have the capacity to respond to whatever nature brings. What kind of adaptations have plants evolved to survive highly variable weather?

Homeostasis is an ongoing process to maintain a biochemical parameters within acceptable limits. When people get hot, they can sweat or move to a cooler place, but plants cannot use either of these mechanisms to maintain their temperatures. A group of German biologists wanted to determine how a model plant, *Arabidopsis*, responded to five different physiological stresses: high salt, osmotic shock, high doses of the stress hormone **abscisic acid** (ABA), cold, and heat. Through previous research, plant physiologists had documented several genes that altered their level of transcription when a plant was stressed. They chose four genes as representatives of the overall transcription stress response to five environmental stimuli. The four genes did not respond in unison with each stress. For example, genes 1 and 3 had similar transcriptional responses to cold and salt stress but very different from genes 2 and 4. The y-axis for their graphs used units of $\log_2$, which is an easy way to measure doublings of transcription. A $\log_2$ value of 1 indicated that the gene was induced twofold, whereas a value of -1 indicated twofold repression. Genes that did

not alter their transcription had a $\log_2$ value of zero. The investigators stressed genetically equivalent plants for either 1 hour or for 12 hours and compared the gene activity of all four genes.

The four stress-response genes did not respond consistently over time, nor did they respond uniformly to the five different stresses. For example, gene 1 increased its transcription between 1 and 12 hours for all five treatments. By contrast, gene 4 shows repression for the first hour of heat, whereas salt induced gene 4 after 1 hour and 12 hours of the same stress. From this small sample size, it is possible to determine that plant genes that have an immediate and transient response. A fast and transient response to environmental changes should be familiar. When a person walks into a room with a very different temperature, that person will immediately notice the stark temperature contrast, but after several hours, their body has acclimated to the new temperature. Other *Arabidopsis* genes altered their transcription for at least 12 hours, indicating that the encoded protein is probably needed to sustain a homeotic response to the new condition.

Once the investigators knew that their method worked as expected, they analyzed the gene activity of all 27,000 *Arabidopsis* genes. They were curious how many genes were induced or repressed for all five of their environmental stress conditions. They found that more genes were induced (35 genes) or repressed (66 genes) after 12 hours of stress, than were induced (7 genes) or repressed (15 genes) after 1 hour of all five treatments. These two sets of 101 and 22 stress-response genes might be core stress-response genes that are generic to all stress conditions and the genomic equivalent to first responders. After 12 hours of exposure, the 101 genes were differentially regulated, perhaps as a consequence of the 22 first responder genes at 1 hour. More genes were repressed by stress than were induced, which might indicate that physiological processes are temporarily slowed down during the stressful times.

A group of California botanists also stressed *Arabidopsis*, but these investigators compared the transcriptional response of leaves to roots. The investigators exposed the entire plant to three distinct conditions (cold, salt, and osmotic stress), but rather than grinding up the whole plant as the German team had done, the Californian team separated the leaves and roots before extracting and quantifying the messenger RNA (mRNA)

in each tissue. As the German team had done, the Californian botanists also compared two time points (3 hours and 27 hours) of continuous stress. They used Venn diagrams to show the overlap between the three treatments for both exposure times, as well as identifying the number of overlap genes for a given treatment at the two different times. It was not possible to determine from the Venn diagrams which genes were induced or repressed, but it was possible to compare numbers within a single Venn diagram and across different Venn diagrams.

It is not uncommon for biology students to think of plants as a homogeneous collection of cells. Upon reflection, however, it is clear that leaf cells are distinct from root cells, which means the two tissue types must differentially regulate their genes to make tissue-specific proteins. It is not necessary to know which genes were induced or repressed to reach some conclusions from the data. Comparing the two tissues for either 3 or 27 hours of stress, they discovered that leaves regulate their genes differently than roots do when exposed to each of the three stresses. Interestingly, the two tissues share the same number of genes (65) that were altered by all three stress conditions after 3 hours. It is not clear if the 65 genes were the same set of genes, but this is how research leads to new questions and more experiments to determine how many of the 65 genes were identical in the two tissue types.

The number of genes that only responded to 27 hours of cold in roots (1,111 genes) and leaves (1,094) was more than four times larger than any other single treatment. From the bottom row of Venn diagrams, they found that only about 15% (173 ÷ 1,111) of the cold-response genes from 27 hours were also induced or repressed after only 3 hours of exposure. The root-leaf comparison for cold, salt, and osmotic stress had very few genes in common for the two time points. Roots had only two stress-response genes, and leaves had only eight genes that changed transcription for short versus long exposure times of all three environmental stresses. The small number of genes indicates that most stress response genes are specific to the type of stress and that each stress requires a unique combination of genes for *Arabidopsis* to maintain homeostasis over its physiological responses. Producing a stress-specific response puts a large demand on the number of genes that plants must carry in their genomes. Most plants cannot move, and therefore their

Table 1 Number of genes that responded to only one environmental stress.

condition	number of genes
salt 1 hour	3
salt 12 hours	16
osmotic 1 hour	1
osmotic 12 hours	5
ABA 1 hour	1
ABA 12 hours	6
cold 1 hour	62
cold 12 hour2	13
heat 1 hour	290
heat 12 hours	59

Source: Based on Zeller *et al.*, their Table 2. 2009.

response to environmental stress must be based on genomic rather than behavior actions. The German investigators quantified the number of genes that were restricted to only one treatment condition out of the five that they tested (Table 1). The numbers in Table 1 for cold, salt, and osmotic stress do not match the equivalent list of unique genes the Americans presented earlier in this chapter in part because the German team examined whole plants, whereas the Californian team considered leaves and roots separately. In addition, the duration of stress differed between the two experiments. One striking aspect of Table 1 is that heat produced the largest set of treatment-specific gene responses but unfortunately, the California team only tested cold stress. It would be interesting to see if roots and leaves respond the same way to heat stress given that leaves are above ground but roots are below.

Homeostasis By Genome Duplication

One of the big lessons about plant stress responses and evolution is that plants deal with each type and duration of stress with different combinations of gene induction and repression. The implication of this discovery is that plants require many genes to maintain homeostasis (Table 2). Compare the number of genes in these selected plants and animals, and notice that genetically, animals are about half the size of plants. Homeostasis

Table 2 Current estimated number of genes for selected plants and animals.

plant species	# of genes	# of genes	animal species
golden delicious apple	57,386	27,918	puffer fish (*T. nigroviridis*)
soybean	46,654	27,200	frog (*X. tropicalis*)
poplar tree	45,654	22,808	human (*H. sapiens*)
rice	40,577	22,011	mouse (*M. musculus*)
grape	33,514	20,285	cat (*F. catus*)
corn/maize	32,540	19,375	worm (*C. elegans*)
Arabidopsis	27,228	16,822	lizard *(A. carolinensis)*
cucumber	26,682	13,601	fly (*D. melanogaster*)

Source: From public domain data.

is typically thought of as acting on a short time scale, but evolution of homeostasis is ongoing and involves changes in DNA information in populations over very long periods of time. Plants have evolved with bigger genomes than animals, because it is adaptive to have more genetic flexibility to respond to environmental changes (such as, stress) that they cannot walk away from. To help clarify what may have led to a doubling in gene number, consider the current record holder for the most genes—the diploid golden delicious apple with over 57,000 genes. A large, multinational consortium sequenced the golden delicious apple (*Malus domestica*) genome and annotated as many genes as they could based on DNA sequence comparison with previously characterized genes. In their analysis, the investigators noticed that many genes appeared to have **paralogs** within the genome. The recurrence of paralogs was widespread, so the bioinformaticists performed pairwise **dot plots** to align each apple chromosomes with the other 16 chromosomes to reveal the location of the paralogs. Many of the chromosomes exhibited **synteny**, which means two chromosomes have their paralogs in the same order and orientation. For example, chromosomes 3 and 11 had similar gene order on both ends of the two chromosomes, as shown by two diagonal lines separated by a gap between them. For a negative control, the investigators displayed a dot plot for two non-syntenic chromosomes, 7 and 13 and there was no apparent line in the graph.

By analyzing the genome of golden delicious apple, investigators began to understand at least one mechanism by which plants could have twice

as many genes as animals. A very large proportion of the apple genes are paralogs of other each other, which could be explained if an ancestral plant experienced **whole genome duplication**. For example, if a cell went through replication (S phase) but did not go through mitosis, then the **tetraploid** cell would contain paralogs for every gene. Based on the number of nucleotide changes between paralogous genes, the apple investigators estimated that the ancestral genome duplicated about 50 million years ago, which is about the same time that porpoises evolved from large land mammals. This seems like a long time ago, but 50 million years is a relatively recent given that the first land plants evolved about 700 million years ago. The investigators deduced a possible scenario that the apple's ancestor had nine pairs of chromosomes before whole genome duplication. For a period of time, these plants had 18 pairs of chromosomes, but over the past 50 million years, the chromosomes have merged, split, and mutated their DNA sequences. A portion of the ancient chromosome 18 merged into the middle of what used to be a duplicate copy of chromosome 8. The supporting evidence for this interpretation is that genes on chromosome 8 having paralogs on the two ends of 15, but the middle of chromosome 15 has paralogous on chromosome 2. Part of chromosome 18 did not get incorporated into the new chromosome 15, so some duplicate genes were deleted. Paralogs of the deleted genes from the ancient chromosome 18 would appear as having no paralogs in the modern genome, which was displayed as a gap in the chromosome drawings and the absence of dots in the middle of the graph.

Orthologs are two genes with very similar sequences found in two different genomes, and paralogs are similar genes found within one genome. By definition, whole genome duplication would produce a complete set of paralogs in the newly doubled genome. If a dot plot were produced from the newly duplicated genome, each plot would have been a perfect diagonal. However, over the last 50 million years, the apple genome has evolved through **insertions**, **deletions**, **inversions**, and other mutations. As chromosomes merged and split, they distributed their paralogs on more than one chromosome, as seen in golden delicious chromosomes 2, 7, 12, and 14. Once the plant had four alleles for every gene, then additional mutations allowed the plants to "experiment" by producing new alleles and even new genes with new functions. From these random mutations,

the apple has regions of its chromosomes that barely resemble their shared origins, as seen on the ends of chromosomes 5 and 10. Other duplicated genes were redundant and cost the organisms more energy to replicate than they provided functional benefits, so natural selection resulted in some lost paralogs. The golden delicious apple is an example of what happens long after genome duplication, but are there any modern examples of how genomes could double in a single generation? The next case study evaluates the best documented example of plant genome duplication starting with data from 1949.

Watching Genome Duplication In Real Time

In the spring of 1949, botanist Marion Ownbey made a discovery that continues to shape our understanding of genome evolution and the definition of a species. While walking along the Palouse River near his workplace at Washington State University, Ownbey found a group of wild flowering weeds of the genus *Tragopogon* that had been brought to America from Europe. As a botanist, Ownbey was familiar with *Tragopogon*, but on this day, he noticed some new species growing among the more familiar species of *T. dubius* and *T. pratensis* (Figure 1). After further investigation, Ownbey realized that the species he had discovered, *T. miscellus*, was tetraploid and caused by the union of two diploid genomes from the familiar species of *T. dubius* and *T. pratensis*. Ownbey collected seeds and grew the progeny, which meant the tetraploid *T. miscellus* was fertile. In a single generation, a new species was formed as the result of genome duplication.

The first implication of Ownbey's discovery was a direct challenge to the common definition of a species. Are *T. dubius* and *T. pratensis* (each with 12 chromosomes) two distinct species, or are they a single species because they could mate and produce fertile tetraploid offspring? Both plants could mate with themselves and produce fertile diploid offspring with 12 chromosomes, but they could also mate with each other and produce fertile offspring with 24 chromosomes. Ownbey also determined that *T. miscellus*, with 24 chromosomes, could come in two different phenotypes with either long or short **ligules** (colored petal-like extensions of the flower). Furthermore, Ownbey was certain that the tetraploid species

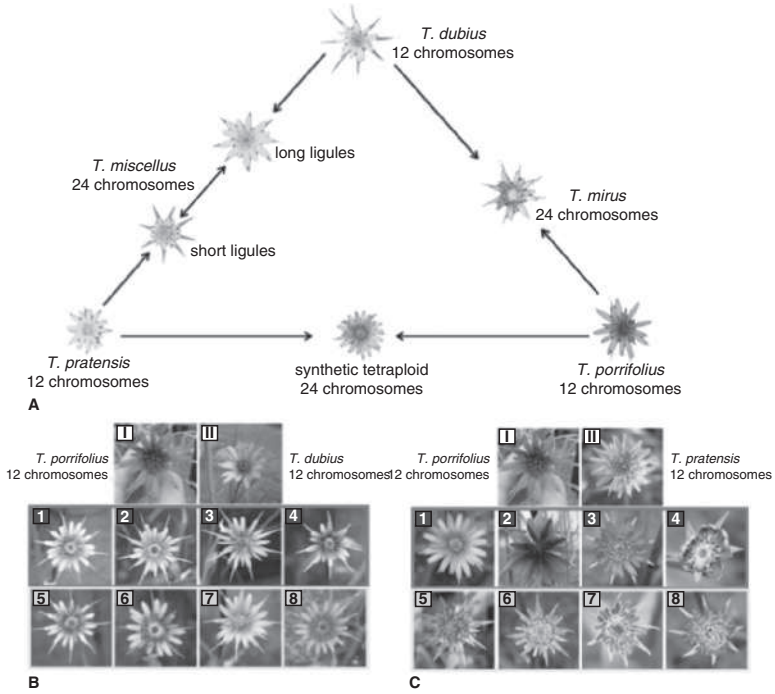

Figure 1 Modern tetraploids and their progeny. A, All of the plants are naturally occurring species except for the one synthetic tetraploid produced in the lab. B, Parental species are above and eight tetraploid progeny are below. The top rows are plants derived from* T. porrifolius *ovules and* T. dubius *pollen; bottom rows are products of the reciprocal cross. C, Parental species are above, and eight synthetic tetraploid progeny are below. As in B, top and bottom rows distinguish the color of the flower used for the ovules.

Source: Modified from Tate *et al.*, 2009b. American Journal of Botany 96(5): 979–988.

was produced spontaneously and independently many times in isolated meadows where the two diploid flowers grew in close proximity. The North American origin of the tetraploid species *T. miscellus* had to be very recent, which presented a unique opportunity to understand plant evolution and speciation. This once in a lifetime research opportunity caught the attention of a husband and wife team of botanists working at Washington State University 30 years after Ownbey published his first paper, and several years after Ownbey's death. Pam and Doug Soltis revolutionized the field of systematic botany, because they applied DNA technology to understand and classify plant evolutionary history.

The couple reread Ownbey's field notes and returned to the exact same sites and found that in some locations, the tetraploid *T. miscellus* had taken over the field; it was difficult to find specimens of either diploid parental flower. In other locations, the tetraploid had not survived, and only the diploid parents were present. When they searched a wider area, the pair of botanists also discovered another tetraploid species, *T. mirus*, formed by the union of *T. dubius* with a third diploid species called *T. porrifolius* (Figure 1A). As part of their innovative research methodology, the Soltises brought the plants into the lab and mated diploids *T. pratensis* with *T. porrifolius* to produce a "synthetic" tetraploid species not found in nature. The new synthetic tetraploid species was fertile but was not given a Latin name because it existed only in their lab. The Soltises published many papers to further our understanding of how plants maintain homeostasis at the genome level.

Through careful breeding experiments in the lab, the joint Soltis lab produced a range of phenotypes that varied, depending on which species donated the pollen and which species donated the ovule. When *T. dubius* was the maternal plant, the offspring have longer ligules (plants 5 to 8) than when *T. porrifolius* was the maternal plant (plants 1 to 4; Figure 1B). Similarly, progeny in Figure 1C had longer ligules if their mother was *T. pratensis* (plants 5 to 8) than if their mother was *T. porrifolius* (plants 1 to 4). The Soltis lab members realized that although many of the flower traits of the synthetic tetraploids varied from one individual to the next, the length of the ligules seemed to follow a more regular pattern. It appeared that the ligule length of *T. porrifolius* followed an **epigenetic** rule of inheritance because the parental source of the allele affected ligule length. Because of the results from their synthetic tetraploids, other investigators realized the botany community had a unique opportunity to understand evolution of a new species in real time. Watching evolution of a new species as it happened would shed new light how plant species respond to environmental changes. If a new tetraploid species were produced independently many times, would the genome evolve the same way repeatedly, or would each individual tetraploid species produce a unique genome combination in an effort to maintain homeostasis?

The investigators returned to natural populations of the flowers and collected many tetraploid individuals as well as individuals from the

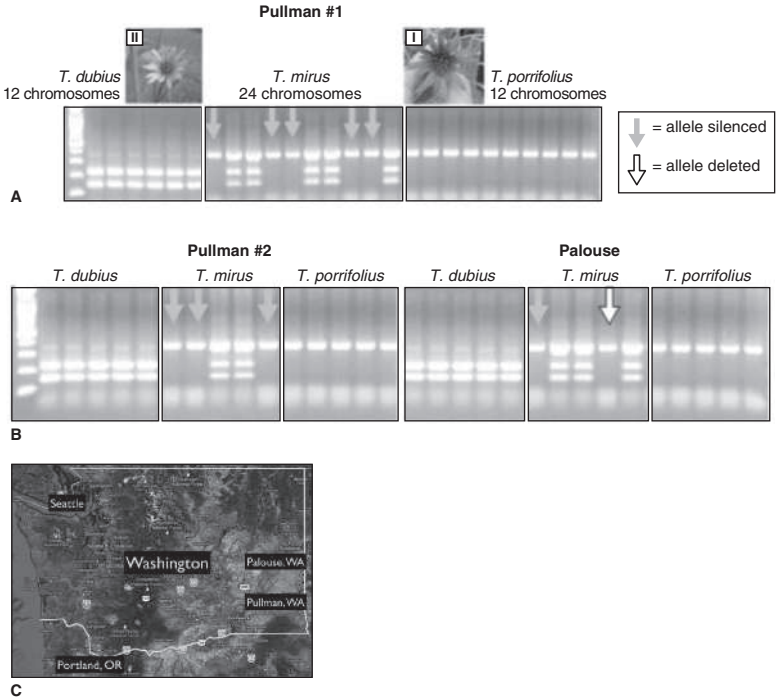

Figure 2 Detection of allele preferences. A, cDNA analysis from naturally produced tetraploid* T. mirus *and nearby parental stocks. Gray arrows indicate alleles still present in the genome but not transcribed; white arrow denotes paralogs deleted from tetraploid genome. B, Similar analysis for independent* T. mirus *and parental stocks from two different sites in the adjacent western Washington towns of Pullman and Palouse (C).

Source: Panels A–B from Koh *et al.*, 2010, their figure 4. Koh, Jin, Pamela S. Soltis and Douglas E. Soltis. 2010. Homeolog loss and expression changes in natural populations of the recently and repeatedly formed allotetraploid Tragopogon mirus (Asteraceae). BMC Genomics 2010, 11:97. Open access. Licensed under Creative Commons Attribution License 4.0 agreement. C. Image from Google Maps.

appropriate diploid parental species. All of the plants came from the nearby towns of Pullman and Palouse, WA, so every individual would have experienced similar environmental stresses (Figure 2). Once in the lab, they isolated mRNA from every specimen. The investigators converted the mRNA to **complementary DNA** (**cDNA**) and then used **PCR** to amplify one gene involved in carbon fixation. They digested the amplified cDNA with a **restriction enzyme** that cuts the allele from one species into two

fragments but does not cut the allele from the other species (Figure 2A). For example, from site #1 in the town of Pullman, the investigators analyzed the cDNA from six *T. dubius* diploids, ten *T. porrifolius* diploids, and ten *T. mirus* tetraploids. In the gel electrophoresis results, it can be seen that the parental *T. dubius* alleles appear as two bands, whereas the *T. porrifolius* parental species produced a single band for the same gene. The different banding patterns reveal differences in cDNA sequence, which indicate the parental alleles that were transcribed at the time of mRNA isolation. The cDNA banding is uniform within all individuals of the same species. When the investigators analyzed the results for the same gene in the tetraploid, they found that half of the individuals did not transcribe their *T. dubius* alleles, even though the alleles were still present in the genome (gray arrows in Figure 2). The biologists repeated their cDNA analysis with two more independent populations, Pullman site #2 and Palouse (Figures 2B and 2C).

Palouse and Pullman are only 15 miles apart (Figure 2C), so the tetraploids from all three populations experienced very similar environments. These plants are a natural experimental test of the question, "Can random events that lead to new species repeat themselves?" Based on the data in Figure 2, it can be seen that ten *T. mirus* plants silenced the *T. dubius* alleles but not the *T. porrifolius* alleles. What was the probability that half of the *T. mirus* plants would inactivate only their *T. dubius* alleles? Calculating the probability is similar to calculating the outcomes from the matings. To make the calculations easier, make a couple of simplifying assumptions. First, assume that the investigators chose plants randomly, and second assume that either both alleles or zero alleles will be silenced from a given parent. With these assumptions, the probability is 0.00017. In other words, it is highly unlikely that chance alone could explain how plants managed to produce the data in Figures 2. In one isolate from Palouse (white arrow), the tetraploid deleted the *T. dubius* paralogs from the tetraploid *T. mirus* genome. The botanists tested three other genes (not shown here); and every time, the gene from one parent was favored over the other. The mechanism of selectively silencing only one parent's alleles appears to be nonrandom.

From the data, it is not clear how the tetraploid was able to consistently silence *T. dubius* alleles but not *T. porrifolius* alleles. It could be that

each new tetraploid randomly silenced any two alleles, but the only plants to survive were those with both *T. porrifolius* alleles still working. Or it could be that one-sided allele transcription indicates epigenetic factors at work, although this seems less likely given that half of the plants did not silence their *T. dubius* alleles and they survived too. As often happens in science, research typically leads to more questions than answers. The botanists will continue their research, and maybe the reader will decide to pursue similar research to better understand evolution and natural selection.

It is difficult to explain why *T. miscellus* plants have either short or long ligules. However, the data indicate that ligule length is determined by which diploid species contributed the ovule and which diploid species contributed the pollen. Unlike gene inactivation in Figure 2, ligule length appears to be the consequence of epigenetic regulation and not a random genomic event, such as deletion or mutation. Despite years of looking, the Soltis lab has never found natural populations of tetraploid plants produced by diploids *T. porrifolius* and *T. pratensis*. The lab-generated, synthetic tetraploids can survive and reproduce based on breeding experiments in the lab, but so far nature has not produced equivalent tetraploids. Perhaps the two diploid parental species do not live in close proximity to each other, their progeny cannot survive outside of the lab, or these tetraploid plants do exist in nature but have not been discovered yet. Many aspects of biology are presently unknown but often become apparent with more research.

From the mating of two diploids, the tetraploid progeny show an impressive amount of variation in their flower color and morphology. Given that each tetraploid individual is a new species and that natural variation exists within the populations of both diploid parents, perhaps it should be expected wide variation in new tetraploids. Each tetraploid is a new experiment in evolution. Through natural selection, it would be predicted that some individuals will be more fit than others. Variation in the population and a changing habitat could lead to the extinction of some tetraploid individuals, whereas others will thrive and even outcompete their diploid parents. It would be possible to perform self-crosses of the tetraploids to determine if ligule length and other traits are inherited by Mendelian or epigenetic mechanisms. No matter which form of

inheritance was functioning biologists would discover new aspects of speciation and evolution, which would be publishable results.

It is common for people who argue against evolution to repeat a misconception that evolution is not a part of science because experiments in evolution are impossible to perform. This claim is false because botanists have been conducting research in *Tragopogon* evolution for over 60 years. To understand why tetraploids might be more fit than diploids, this chapter presented how investigators measured plant homeostasis in response to stress, which required many stress-specific genes altering their transcription within hours. The golden delicious genome data presented homeostasis on a time scale of millions of years after whole genome duplication of an ancestral species. The mechanisms of homeostasis are time-dependent and range from hours to years to millennia. The environment influences how species address the physical and chemical challenges caused by the inability of plants to move. Genomes can duplicate within a single generation, and genomes continue to evolve as natural selection rewards some genotypes with reproduction but others fail. If one wanted to design and perform experiments in evolution, it would be possible to expand upon the original research of Doug and Pam Soltis, or perhaps the reader will discover a unique system and become a pioneer in a new field.

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CHAPTER 2

Changes in Two Leaf Cells Affect an Entire Plant

Everyone has seen a wilted plant in need of water. Plants depend on sufficient water in their cells to prevent wilting. Wilted leaves are wrinkled and shriveled because their cells have lost too much water. Loss of water from plants is regulated in part by small structures on the underside of most leaves. Underneath most leaves is a pair of cells appropriately called **guard cells**, because each pair of cells guards an opening called a **stoma** (**stomata** is the plural noun). Plants face a difficult dilemma—they require CO_2 from the air, and they need to retain water (H_2O) in their cells. To complicate plant homeostasis, plants also need to allow some water to evaporate from their leaves in order to draw in more water through their roots and bring in soil minerals. To obtain CO_2, the leaves need to open their stomata to allow the gas to diffuse from outside the plant where CO_2 has a higher concentration, to inside the leaves where CO_2 is less concentrated. However, when a leaf opens its stomata, H_2O can diffuse from inside the leaf to the surrounding air if the humidity is low outside the leaf. Leaf cells need both CO_2 and H_2O to survive and leaves must be able to regulate the best times to open or close their stomata. The regulation of stomata opening is a tightly regulated homeostatic mechanism that this chapter will address by examining guard cells and their ability to change shapes.

Plants use sunlight and CO_2 to produce sugars and other molecules. Plants also need water for these vital biochemical functions and to maintain their overall posture and leaf structure. Therefore, it can be predicted that stomata are open more often on sunny days and when the humidity is high but closed more frequently during droughts (Table 3). How do guard cells "know" when to open and close? For drought-stricken plants,

Table 3 Environmental conditions and their effects on stomata.

environmental conditions	resulting stomata shape
bright light	opened
high humidity	opened
drought conditions	closed

roots send a chemical signal to the leaves triggering the guard cells to stay closed more often. How can paired guard cells be responsible for the hydration status of entire plants and respond to changing environmental conditions? This chapter's consideration of plant homeostasis of gas exchange regulated by cell shape begins with the chemical signals to close stomata. Later, the chapter will present how paired guard cells generate the physical force to open their stoma.

Plant physiologists had previously determined that root cells secrete abscisic acid (ABA) when they are stressed during times of drought. To determine whether ABA was the direct signal for guard cells to close their stoma, plant physiologists measured the diameter of stomata after various experimental treatments (Figure 3). Although these data show that the stomata were never fully closed, failure to fully close was an artifact of the experimental conditions; stomata can close completely when the leaves are undisturbed by scientists. In addition to treating the cells with ABA,

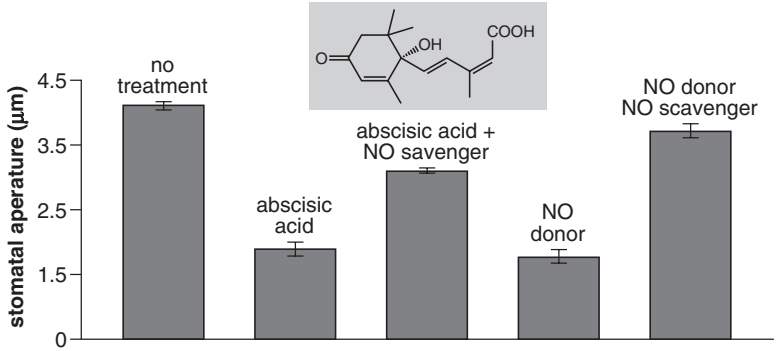

Figure 3 Chemical regulation of stomata closing. ABA and NO affect stomata by altering guard cell shape (error bars +/– SEM). Inset: ABA structure.

Source: Modified from Neill *et al.*, their figure 2.

they also applied a compound that produced nitric oxide (NO) molecules in plants, or a scavenger chemical that removed NO molecules from leaf tissue. In a second experiment, the same investigators microinjected a fluorescent dye into guard cells that glows red in the absence of NO and glows green in the presence of NO. The negative control in this experiment was the injection of a plain green fluorescent dye in the absence of any additional chemical treatment. The purpose of this negative control was to demonstrate that the dye by itself did not cause the guard cells to produce a more open stoma.

Both ABA and NO lead to a closing of stomata (Figure 3). However, notice that when ABA was used in conjunction with a NO scavenger, the stomata did not close as much. If ABA was the only chemical signal, then the NO scavenger would have no effect. Similarly, when NO was provided by the investigators in conjunction with a NO scavenger, the stomata failed to close. Therefore, it appears that ABA stimulates the production of NO, which causes the stomata to close. This conclusion is supported by the data from their second experiment where ABA led to an increase in NO inside guard cells (increased green fluorescence). But perhaps ABA contributed to closing stomata in the absence of NO because the stomata closed more with ABA plus NO scavenger than with NO plus scavenger. Alternatively, the NO scavenger might not have worked with 100% proficiency. When interpreting experimental results, it is important not to over interpret the results or reach a firm conclusion from a single experiment. Guard cells may rely on two different chemical signals (ABA and NO) to know when to close their stoma. ABA could act directly on the guard cells and/or stimulate the production of a second chemical other than NO that causes guard cells to reduce the size of the stomata. The data are insufficient to support the conclusion of only one chemical causing the stomata to close.

In contrast to drought-induced ABA production, bright light can stimulate the opening of stomata (Table 3). Investigators wanted to determine what caused the guard cells to open their stoma when exposed to light. Among the many measurements they made, one was a measure of membrane potential the same way action potentials are measured in neurons. First, investigators impaled individual guard cells with tiny electrodes to measure the membrane potential in guard cells exposed to dim light.

After 2 minutes, the investigators turned on a bright white light and continued to measure the membrane potential of the same guard cell. After 15 minutes of bright white light exposure, the lights were dimmed again, and the guard cell was measured for another 10 minutes. Photographs of the guard cell being measured were taken while the bright light was on. Light travels very quickly, but its effect on guard cells takes about two minutes before it is possible to detect any change in cytoplasmic charge. While the white light was shining on the guard cells, the cytoplasm became more negatively charged. The increase negative charge in the cytoplasm could have been caused by the loss of positive ions or the accumulation of negative ions; these data are inconclusive. Once the light was turned off, it took at least 10 minutes for the cytoplasm to return to its original charge.

In a second experiment, the investigators used different colors of light to see if guard cells preferentially responded to one color more than others. They tried red and then green with no response, and they finally tried blue light, which produced a robust response and greatly increased the ion conductance rate across the guard cell's plasma membrane. The data did not identify which ions are moving, just that ions were moving. In a second experiment, they turned on the blue light for 30 seconds, waited 15 minutes, and then turned on the blue light a second time. To better visualize the conductance of the guard cells in response to the second exposure of blue light, they used software to mathematically subtract the response to a single dose of blue light from the double light exposure. Guard cells responded vigorously to blue light but not red or green. When stimulated with blue light, protein channels in the plasma membrane of guard cells increased their permeability to ions. When guard cells were stimulated a second time, they increased their ion conductance by about 2/3 of the initial response with ions moving through membrane protein channels. Guard cells were unable to double their conductance rate of ions with the second light exposure. Unlike a neuron's action potential, which is an all-or-none response, guard cells produced a stepwise response up to a limit with more ions moving into the cytoplasm after a second light stimulation.

Figure 4 showed that ABA causes guard cells to close their stoma. Plant physiologists suspected that potassium ions (K^+) played a key role in guard

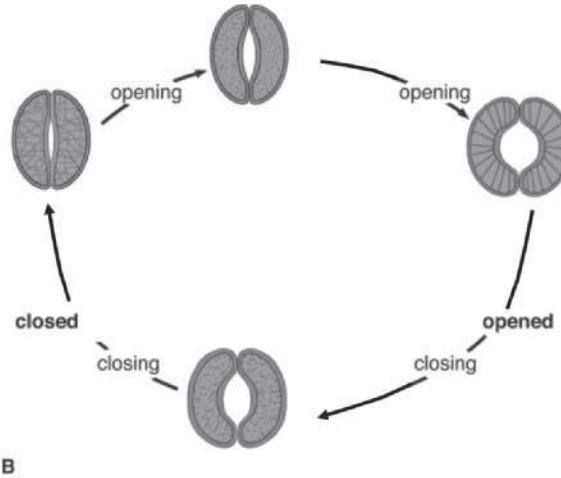

Figure 4 Actin cytoskeleton rearranges inside guard cells. Drawings of four different pairs of guard cells in different phases of closing or opening their stomata. Actin appears as lines inside the cells.

Source: Modified from Gao *et al.*, 2009, their figure 4. Gao, Xin-Qi, Xiu-Ling Wang, *et al.* 2009. Dynamics of vacuoles and actin filaments in guard cells and their roles in stomatal movement. Plant, Cell and Environment. Vol. 32: 1108–1116. © 2009 Blackwell Publishing Ltd. Modified with permission from John Wiley and sons.

cell shape. They quantified the amount of K^+ inside guard cells after being exposed to blue light and later when the cells were exposed to ABA. When exposed to light, the K^+ concentration was high, but it drifted lower over the first 30 minutes after the light was turned off because the cells had been removed experimentally from their leaves. When the guard cells were subsequently exposed to ABA, K^+ left the guard cells at an accelerated rate shortly after a high dose of ABA treatment and more slowly after a low dose.

In addition to ABA, very high leaf levels of CO_2 also trigger the closing of stomata. Consistent with their experimental data, the concentration of K^+ also went down with each exposure to experimentally manipulated CO_2 levels. However, after the second exposure of CO_2, the stomata opened again but the K^+ concentration continued to go down, whereas the sucrose concentration increased at the same time the stomata opened. All of these data initially might have seemed confusing to the plant physiologists.

When light strikes the guard cells, their cytoplasm gets more negative and the stomata open. When ABA within guard cells rises and the stomata close, the cytoplasm of guard cells gets more negative because K^+ ions leave the cytoplasm. How can opening (light) and closing (ABA) of the stomata both lead to increased negativity of guard cell cytoplasm? What investigators eventually learned was that light causes guard cells to pump H^+ out of their cytoplasm, which increases the negative charge of the cytoplasm. The rise in negative charge **allosterically** modulates **voltage-gated K^+ channels** that allow K^+ to enter guard cells. Guard cells swell due to the increased **osmotic pressure** created by the rise in cytoplasmic K^+. When stimulated to close by ABA and NO, K^+ flows out of the guard cells through a different set of ion channels, which reduces the osmotic pressure and allows the guard cells to relax and thus close their stoma.

When leaves were exposed to very high levels of CO_2, the leaf tissue accumulated the CO_2 and later the guard cells closed their stomata once the leaves had accumulated enough CO_2. When the CO_2 was reduced to low levels, the guard cells opened their stomata again after the concentration of CO_2 inside the leaf was reduced. Both doses of increased CO_2 cause the guard cells to close in synchrony with the drop in K^+ concentration in guard cells. However, after the second high dose of CO_2, the guard cells opened their stomata but K^+ did not rise. For this second round of stoma opening, the sucrose concentration rose instead of K^+. Increases in cytoplasmic sucrose also increased the osmotic pressure, which caused the guard cells to swell and open the stomata. In short, osmotic pressure regulates guard cell size which in turn regulates stomata diameter and leaf hydration status.

Once again, the data might appear counterintuitive. It seems logical to predict that swollen guard cells would cause their stoma to close, but the opposite happens. Swollen guard cells lead to the opening of stomata, not closing (Figure 4). In the color micrographs, green molecules were actin proteins that comprised part of the cytoskeleton. When thinking of skeletons, it is common to imagine a static structure, such as the human skeleton or the exoskeleton of insects and crabs. The microscope used to capture the images was capable of focusing on only a thin slice of the whole cell and causing the other portions of the cell to be hidden from view. It was possible to see small circular chloroplasts inside the cytoplasm in the

photographs. The green actin cytoskeleton in the middle of two guard cells was visible at different stages of opening or closing their stoma as redrawn in Figure 4. In another set of images, entire cells were imaged so that all of the actin in the cytoplasm appeared white and the rest of the cell was black. The investigators studying the cytoskeleton of guard cells provided line drawings in Figure 4 to help interpret the photomicrographs.

Figure 4 allows the reader to generate a good mental image of two guard cells regulating the opening of a single stoma. A good analogy is to cup one's hands together with palms facing each other. Close the opening between the hands by pressing the palms together. To open the simulated stoma, curve one's hands outward so that the leaf can exchange gases with the environment. However, it may be less clear how two cells could regulate the hydration status of one whole leaf, not to mention an entire tree. In addition to guard cells changing their shape, paired guard cells communicate with other pairs in ways that no one fully understands yet. It may seem unusual that a fundamental area of biology is not completely understood by anyone, but that is the essence of science. All scientists learn what others have discovered and then move into the new areas so they can make discoveries too. By designing experiments and interpreting data, the reader can uncover new information that at some point could become part of a book.

To understand the physiological challenge guard cells face, imagine a large building with many windows. At each window stands a person who closes the window when someone enters the room and opens the window when no one is in the room. All of the windows are opening and closing with no coordination as people walk around the building. Meanwhile, the heating and air conditioning system of the building is trying to regulate the temperature one room at a time, which affects humidity of the entire building. A chaotic building with windows opening and closing periodically results in the heating and air conditioning system wasting a lot of energy fighting a losing battle of climate control. The chaotic building is analogous to a leaf or plant trying to regulate its H_2O and CO_2 status if the guard cells could not communicate with other paired guard cells. What if some parts of the leaf were in the shade but other parts were exposed to sunlight? What if one guard cell mistakenly swells in size and opens its half of the stoma but environmental conditions favor a closed stoma?

It would be beneficial to the plant if guard cells could sense the status of neighboring guard cells and make a collective "decision" of when to open and close stomata.

Biologist, Keith Mott, and physicist, David Peak, at Utah State University studied guard cells as part of a large network of many guard cells performing a coordinated task of regulating whole leaf physiology. At the beginning of their 5½ hour time-lapse movie showing a portion of a leaf, most of the stomata were opened as indicated by the dark gray coloration. Over the time course of their experiment, stomata gradually closed at the top of the area as indicated by the increasing light gray coloration. The open or closed stomata status of an area flickers between the two states but a general trend emerged that this portion of the leaf was changing from mostly open stomata (dark gray) to mostly closed stomata (light gray). Because it was hard to distinguish light and dark gray patches by eye, the two investigators used computer-generated colors to represent patches of cells that had changed from open to closed (yellow), closed to open (red) or no change (black). From the colorized data, they could see patches of stomata flicker back and forth between open and closed. By the end of the 5½ hours, a trend emerged showing closed stomata gradually sweeping from top to bottom in this patch of leaf cells. Plants lack neuronal control of their stomata, so it appears that guard cells may be using a type of chemical signaling to generate a local network logic. Logic networks can be modeled with as a **cellular automaton** (pronounced au-tám-a-ton). One example of a cellular automaton is a logic game invented by mathematician John Conway called the "game of life," which is easy to find on the Internet.

By studying open and closed stomata closely, biologist noticed that the guard cells were attached to each other at both ends. As the guard cells swelled, their ends could not move, so the extra volume caused the guard cells to bend outward. This is similar two placing palms together and then curving the palms to create an opening just as swelling guard cells opened to form a stoma. When examining the guard cell actin cytoskeleton in open and closed stomata (Figure 4), it was possible to see that the cytoskeleton was dynamic and rearranged to facilitate the outward bowing of the guard cells to open a stoma. Cellular swelling

and a restructuring of guard cell cytoskeleton allowed guard cells to regulate stomata diameter.

Because plants lack a central nervous system to coordinate cells over distances, plants require different types of communication and coordination systems to ensure that guard cells are all working together. If leaf tissue were low on CO_2 or the environment had high humidity, then it would benefit the leaf if all the stomata functioned as an interconnected network doing the same task at the same time. Leaves appear to use a type of cellular automaton because a patch of guard cells that changes from open to closed stomata can communicate this change to its neighbors. If a majority of the neighboring guard cells have also closed their stomata, then open stomata will be converted to closed stomata. Leaves exhibit a type of tissue coordination where a critical mass must be present to convert a patch of guard cells from one state to another. The use of cellular automaton to explain coordinated regulation of stomata should not be considered a 100% accurate model of all guard cell behavior. But a good model does not have to be correct to be useful. The model helped biologists understand the gradual transition from open to closed as indicated by the increasing number of yellow patches in the color coded time-lapse movie. Any red patches surrounded by yellow patches would convert to a closed yellow patch in the next time step. The cellular automaton of stomata behavior functions as a cellular fail-safe mechanism that prevents leaves from drying out simply because one pair of rogue guard cells mistakenly left their stoma open.

Guard cells exhibit two **emergent properties** at the cellular level to maintain homeostasis of the plant. First, these paired cells function as signal transducers converting information from their environment into cellular changes in volume and cytoskeleton structure. These cellular changes regulate the diameter of stomata, which in turn regulate the amount of CO_2 and H_2O in leaves and the entire plant. Second, guard cells appear to use a form of cellular automaton to provide a fail-safe mechanism so that an entire leaf is not vulnerable to wilting because of a few errant guard cells. Plants maintain CO_2 and H_2O homeostasis through complex cellular functions without any neurons. Guard cell behavior is surprisingly complex and plays a critical role in organismal homeostasis of plants.

Chapter 3 will examine a plant that can move fast enough to catch a fly. The physiology that allows plants to move quickly is contrary to how most people think of plants.

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CHAPTER 3

Venus Flytraps Move Quickly to Trap Prey

Watching a Venus flytrap (*Dionaea muscipula*) capture a fly in its trap is fascinating. It is even more fascinating once one understands the mechanism that allows the plant to be the predator and the animal to become its prey. The Venus flytrap lives in bogs in North and South Carolina and nowhere else in the world. The soils in which they live are poor in nitrogen, and being carnivorous is an adaptation for obtaining nitrogen for use in amino acids and nucleic acids. Trapped insects are digested by enzymes released by the plant, which then absorbs the released nutrients. This chapter investigates how a plant uses specialized cells to respond to external stimuli that cause the leaf to move fast enough to trap the fly. In order to understand the trap's function, this chapter will begin with the structure of the leaves.

Each Venus flytrap plant has multiple traps that are specialized leaves which can be seen by a quick search of the Internet. All of the green parts of the plant are photosynthetic, but photosynthesis does not supply nitrogen to the plant. The trap consists of two lobes hinged at a midrib, and the red coloration helps attract certain insects. Along the margins of the lobes are glands that secrete nectar, which also attracts insects. At the edges, long guard bristles can be seen that prevent insects from escaping once the trap is shut. The key to making the trap work are six to ten small trigger hairs. A trap snaps shut when an animal bends at least two trigger hairs in succession or deflects the same trigger hair twice within 20 seconds.

DiPalma and colleagues wanted to understand how the trigger hairs caused the trap to shut so quickly. Based on previous work by others, they focused their attention on the electrical properties of Venus flytraps. The investigators connected two electrodes to the outer surface of traps and

Table 4 Amplitude and duration of the first and second action potentials after stimulation of trigger hairs on Venus flytraps. Averages for 31 leaves with standard errors in parentheses. Amplitude is in millivolts and duration is in milliseconds.

first action potential				second action potential			
depolarization		post-depolarization		depolarization		post-depolarization	
amplitude	duration	amplitude	duration	amplitude	duration	amplitude	duration
11.2 (0.8)	0.24 (0.1)	10.4 (0.8)	0.76 (0.1)	14.6 (0.7)	0.13 (0.02)	8.4 (0.9)	0.65 (0.07)

Source: From DiPalma et al. 1961. DiPalma JR, Mohl R, Best W Jr. (1961). Action potential and contraction of *Dionaea muscipula* (Venus flytrap). Science 133(3456):878–879. Reprinted with permission from AAAS.

used an oscilloscope to monitor the changes in electrical potential. They also used a strain-gauge transducer to monitor the trap's closure. Then they brushed trigger hairs to cause the traps to close. After over 100 trials, the scientists found that the leaves rarely closed with one stimulus or three, and most took only two brushes of trigger hairs at 2 second intervals to close. Action potentials exhibited a distinct depolarization phase when the graph went up, followed by a post-depolarization phase where the electric potential moved below the original resting potential.

The plant physiologists recorded differences in the first and second action potentials, as described by two aspects of the graphed responses: the amplitude and duration of the curves (Table 4). Amplitude equaled the top of depolarization curve and the bottom of the post-depolarization curve. The average time delay between the second stimulus and the onset of contraction was 0.6 second (+ 0.05), and it took an additional 6 to 7 seconds to attain maximum tension in the trap.

Remarkably, the action potentials in this plant were very similar to action potentials in mammalian neurons. There was a depolarization phase followed by repolarization and hyperpolarization of the membrane potential. Stimulation of trigger hairs produced action potentials, two of which are necessary for trap closure. The investigators concluded that the second action potential that caused closure of the trap had a faster and stronger depolarization; the depolarization peak occurred much more quickly than in the first action potential. There were some differences between the two post-depolarization phases, but they were not statistically significant. DiPalma and colleagues concluded that the major difference was in the depolarization phase. The electrical signal activated a mechanism that consumed adenine triphosphate (ATP) and caused changes in the cells

in the midrib, leading to closure of the two halves of the leaf and trapping the unwitting insect.

A different group of plant physiologists wanted to determine whether it was the electrical signal or some other aspect of the trigger hairs that caused trap closure. Volkov and colleagues studied the localized electrical signal generated from the bending trigger hair. The investigators inserted very thin electrodes into leaves—the negative end was inserted into one of the two lobes and the positive into the midrib. Now they were ready to repeat DiPalma's experiments to induce and measure action potentials but with a new variation. This time, the physiologists varied the strength of the charge applied to leaves. When an electrical pulse was applied between the electrodes, the traps closed in about 0.3 seconds. The mean charge that could close the trap with one pulse was 13.63 μC + 1.51 (μC = microcoulombs). Alternatively, the investigators could administer a succession of smaller charges until they added up to the full charge. Small charges accumulated in the flytrap until a threshold was reached and the trap closed. The scientists measured the distance (d) between trap lobes relative to the maximum distance (d_{max}) when fully open in response application of small charges. When the ratio d/d_{max} equaled 1, the trap was fully open.

Volkov and colleagues suspected that trap closure was caused by rapid movement of ions into or out of particular cells in the midrib. For example, if positive ions flowed into a cell through voltage-gated ion channel, the increased ion concentration would draw water into the cell. The increased volume of the cell would cause a change in cell shape due to the higher water pressure on the cell walls. Volkov and his colleagues applied ion channel blockers to plants and found that they caused traps to close two to three times more slowly when stimulated either with electrical pulse or physical stimulation of trigger hairs. Regardless of the voltage applied, it was impossible to stimulate trap closure when electrodes were attached to both lobes, as opposed to a lobe and the midrib.

Volkov and colleagues showed that electrical stimulation causes trap closure, which further supported the importance of action potentials as the mechanism for signaling closure. And when the ion channel blockers were added, the traps closed more slowly. The cells along the central rib are essential to trap closure. Thus it is reasonable to hypothesize that the

change in shape in midrib cells is caused by ion flow as a result of the action potential generated by deflection of trigger hairs. It seems clear that the Venus flytrap uses voltage-gated ion channels to quickly alter the osmotic pressure in cells along the midrib. Although there are still some missing pieces to fully explain how the trap closes, this chapter has provided some evidence that ion channels, water, internal pressure, and changes in cell shape are involved.

Rapid trap closure requires membrane proteins that facilitate movement of water (such as, **aquaporins**), which are transmembrane channel proteins through which water can pass. If intracellular water pressure plays a role in trap closure, then aquaporins are likely to be an essential component. Other investigators have found that protons are pumped into the spaces between cells of the midrib when traps close. The extracellular space around midrib cells would become more acidic, and the acid can break down the cell walls. Weakened cell walls would make those cells less rigid and more flexible. Venus flytrap cells allow calcium ions (Ca^{2+}) to enter the cells when they are depolarized. Aquaporins would let water flow into the midrib cells with elevated Ca^{2+} concentration. With the weaker cell walls, the midrib cells swell rapidly, causing the hinge area to enlarge and push the two lobes together. Acidification of the hinge cells at the midrib probably requires proton pumps to move H^+ out of the cells. Therefore, it would be expected to be a requirement of ATP to power the proton pump for trap closure.

In the 1970s, Mitchell Jaffe at Ohio University examined the role of ATP in trap closure. Other plants with rapid movements consume ATP during movement, and they have an increased efflux of positive ions from cells immediately preceding movement. Jaffe tested the effects of 100% O_2, 100% CO_2, or laboratory air by placing flytraps in gas-tight chambers (Table 5). He also tested the effects of light or darkness on trap closure. In each treatment, Jaffe stimulated trap closure by touching trigger hairs with a small object. Jaffe measured the rate of trap closure and computed the angle between the two lobes of the trap, where 0° would be fully closed and a fully open trap would have an angle greater than 90°. Jaffe also measured ATP concentrations in the midribs of traps before and after making them close. He surgically removed the midrib tissue and rapidly extracted all of the ATP for quantification. Jaffe found 950 + 40 μM ATP

Table 5 Effect of various treatments on rate of trap closure in Venus flytraps after stimulation of trigger hairs. Rate of closure is in degrees changed per second. Numbers are averages for 20 traps +/– 1 standard deviation.

treatment	rate of closure
dark pre-treatment for 20 hours, then . . .	
darkness	39 ± 19
light	129 ± 37
stimulated after 30 min darkness, in atmosphere of:	
air (0.03% CO_2, 20.5% O_2)	20 ± 2
100% CO_2	2 ± 0
100% O_2	82 ± 30

Source: From Jaffe 1973 in text table. The role of ATP in mechanically stimulated rapid closure of the Venus's flytrap. Jaffe MJ.1973. Plant Physiology 51:17–18. Reprinted with permission from American Society of Plant Physiologists.

in midrib tissue before closure and 650 + 50 μM ATP in midrib tissue after closing.

Venus flytraps closed more rapidly in light than in dark, more rapidly in 100% O_2 than in laboratory air, and very slowly in 100% CO_2. Mitochondria require O_2 to produce ATP. Likewise, the light reaction of photosynthesis produces ATP that can be used in carbon fixation. The two treatments that promoted ATP production led to faster closure of traps. The drop in ATP concentration from before to after closure is consistent with the conclusion that ATP is required for trap closure. ATP is consumed when protons are pumped from the cytoplasm to the extracellular space, which causes the cell walls to soften.

Although the full mechanism of trap closure is not yet known, this chapter has presented several aspects of Venus flytraps that relate to homeostasis at the organismal level. The Venus flytrap is using ion and water balance to change its cell shape quickly so that it can trap animals. The animals are digested to provide nitrogen that the plant is unable to obtain from its mineral-poor soils. The plant uses feedback mechanisms to generate the time-dependent processes of rapid trap movement. The plants are small, and the region of lowered pH outside cells causes the entire trap to fold along the midline to compensate for its nutrient-poor habitat. Venus flytraps are not the only carnivorous plants along the sandy east coast of the Carolinas. Sundew and pitcher plants are carnivorous plants

that use very different mechanisms to capture their animal prey, which is an example of convergent evolution. **Convergent evolution** happens when multiple species evolve similar traits but through different means. In this case, three distantly related species evolved different mechanisms to acquire nitrogen by digesting animals. The sundew and flytrap both use movement to facilitate trapping their prey, but the pitcher plant merely drowns its victims in a pool of digestive enzymes.

Ethical, Legal, Social Implications: Correcting Misconceptions Is Difficult

Very few people have a deep understanding of the core concepts in biology. This book employs a constructivist approach, meaning that the reader uses data to build a personalized understanding of the concepts and connects new knowledge to prior experiences. Research has shown the constructivist approach is the most successful way to develop understanding rather than simple factual recall. Grant Wiggins and Jay McTighe characterized deep understanding by saying that students should be able to explain, interpret, and apply their new knowledge. Furthermore, understanding allows students to have a perspective on the information. These learners are aware of their own limits of their knowledge and can empathize with people who have different perspectives. By integrating the data with Ethical, Legal, and Social Implications readings, this book provides readers with a mechanism to reach full understanding.

One key barrier to meaningful understanding for many people is prior knowledge founded on a misconception as understood by experts in the field. In order to correct a misconception, it is important to confront a person's incorrect perception with conflicting evidence that leads to intellectual tension. Consider a very common misconception related to Venus flytraps: Plants do not move. Most people would say plants can be moved by wind, water, animals, and so on, but they are not capable of moving on their own power. Based on what was learned in Chapter 3, it is clear that plants can move fast enough to trap a fly. Can most people catch a fly with their hands?

James Wandersee and his colleagues published a list of assertions related to misconceptions and what it takes to overcome these

erroneous perceptions of the natural world. Some of Wandersee's assertions include:

- People approach learning new information with a diverse set of misconceptions.
- Misconceptions are tenacious and are difficult to remove from a person's understanding of the world.
- Misconceptions have many sources, including personal experiences and formal instruction.
- Teachers often share the same misconceptions with their students.
- Instruction that addresses a misconception indirectly can lead to unintended learning outcomes and new misunderstandings.
- Instructional methods that dislodge misconceptions can be very effective classroom experiences.

With an in-depth understanding of biology in general, and the fast movement of Venus flytraps in particular, it is possible to teach others in a meaningful way. The first step is to hear and identify the misconceptions. What does a friend think about the ability of plants to move? With an appreciation for a different perspective, it is possible to confront a target audience with data that do not align with their view of the world (for example, plants cannot move on their own). The objective is to provide sufficient evidence until they become uncomfortable holding on to their perception and grow dissatisfied with what they had held to be true. Using data, one can provide them with a context to connect the new information with an existing understanding that is true (e.g., some plants are carnivorous, capture animals, and digest them). People can be given the Venus flytrap data to solve a logical problem their misunderstanding produces. The plant must move quickly if it is to capture a fly. Once one example is provided, several more examples can be provided that generate new insights for them (e.g., non-carnivorous plants can move too). For example, there are video examples on the Internet that can be shared:

- Venus flytrap shutting
- Plants moving to a new location

- Sunflowers twirling
- Sensitive plant closing
- Sunflower leaning toward the light
- Morning glory twisting
- Unicellular algae swimming
- Volvox colonies swimming

This book was designed to maximize a deep understanding as defined by Wiggins and McTighe. By minimizing the amount of jargon and providing real data, the reader has been encouraged to construct a personal understanding in a way that goes beyond simple comprehension. In 1956, Benjamin Bloom enumerated six hierarchical levels of understanding, from easiest to most complex:

1. knowledge
2. comprehension
3. application
4. analysis
5. synthesis
6. evaluation

Evaluation is the highest level, because one must be able to synthesize new information and judge the validity and quality of new information to see if the new information is credible or not. It is unwise to believe everything one sees. With digital photography and software tools, it is easy to generate realistic looking images that are completely fictitious. As a student of biology with a deep level of understanding, the reader needs to judge information to prevent being fooled by science fiction.

There are many topics subject to misconceptions, such as prokaryotes do not have internal membranes and all prokaryotes are smaller than eukaryotes. Everyone is susceptible to misconceptions, but the key to being a scientist is to let the data do the convincing. Keep an open mind but retain healthy skepticism when hearing claims that are inconsistent with the data. Help the community by sharing a deeper understanding of biology. Use data, not doctrine or ideology, to shape an understanding of the natural world. Ask for evidence, and share the data so that everyone can address

misconceptions directly; otherwise the misconceptions will persist and people will work under a cloud of ignorance. Policies and personal decisions should be based on well-founded understanding of the world and how it functions.

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Conclusion

This book presented three different cases of plant homeostasis at the organismal level. Genomes process information and allow individuals to adapt to rapidly changing environments when they cannot move away and seek shelter. The physiological responses must be rapid, but genomes can evolve over longer times as successful individuals reproduce with their beneficial alleles. When an individual plant is stressed, it must induce some genes and repress others. Proteins affect most homeostatic processes, which means the genome must be able to sense and respond to environmental changes in a timely fashion. Some genes generically respond to all stresses, whereas others respond only to particular changes, such as heat, salt, or osmotic stress. Within a single generation, some plants can double their genomes with four alleles for every gene. With the new paralogs, plants have the "luxury" to let some alleles change their DNA sequence and produce proteins with new functions. Segments of chromosomes can be deleted, inverted, inserted into other chromosomes, or gradually mutated. Natural selection provides a feedback mechanism so that the better suited variants can reproduce and propagate their genome alterations.

Plants must balance their dependence on CO_2 and H_2O and only open their stomata at the right times. Any rogue guard cells could open at the wrong time and kill the leaf it's on. However, through a computer-like program using cellular automata logic, stomata open and close in a coordinated fashion in the absence of a nervous system. Plants, like the Venus flytrap, have evolved a neuron-like capacity to signal when prey has entered its carnivorous trap. The combination of H^+ ions, ATP, Ca^{2+}, and water respond to trigger hair movement fast enough to catch a fly. All of these physiological processes maintain optimal conditions using time-dependent processes. Individual plants contend with their size and the environment to solve the chemical and physical challenges they face. All of these processes require energy that plants make more efficiently than most other species.

Glossary

abscisic acid. a plant hormone used in many signaling pathways, including stress-induced ones.

allosterically. a protein's function is changed, allosterically modulated, when any molecule or element binds non-covalently to a protein and alters the protein's shape.

aquaporins. integral membrane proteins that form channels through which water molecules pass rapidly.

cDNA. complementary DNA is generated experimentally using reverse transcriptase to produce DNA from mRNA template.

cellular automaton. it employs a set of rules and logic to determine whether the central unit should maintain its current state or change to the alternative state.

complementary DNA. cDNA, DNA generated by reverse transcriptase that is complementary to mRNA.

convergent evolution. convergent evolution is a process in which organisms evolve similar structures or functions, even though the species are not closely related evolutionarily.

deletions. loss of a segment of DNA that could range from one nucleotide, to millions.

dot plots. graph that displays sequence similarity between two DNA or protein sequences.

emergent properties. characteristics that become apparent at a higher level of biological complexity due to interactions among lower level components.

epigenetic. epigenetic changes to DNA are chemical modification that do not change the sequence of DNA nucleotides.

fail-safe. it is a mechanisms designed to prevent catastrophic consequences when a simple part stops working properly.

guard cells. paired cells on leaves that regulate the opening and closing of stoma.

insertions. gain of a segment of DNA that could range from one nucleotide, to millions.

inversions. flipping a segment of DNA 180 degrees with no loss of nucleotides.

ligules. petal-like showy parts of flowers that varied in length in the genus Tragopogon.

orthologs. two genes with very similar sequences found in two different genomes.

osmotic pressure. osmotic pressure is the pressure exerted by water through a semi-permeable membrane separating two solutions with different solute concentrations.

paralogs. duplicate genes found within the genome of a single species.

PCR. polymerase chain reaction, enzymatic amplification of a segment of template DNA as defined by two primers to amplify both strands one billion times in thirty iterative temperature cycles.

restriction enzyme. protein enzyme that binds to a particular sequence of DNA and cuts, or restricts, both strands of the DNA at or near the recognized sequence.

stoma. a single opening between two guard cells that allows leaves to passively exchange gases by diffusion with the environment.

stomata. plural noun form of stoma.

synteny. it occurs when two or more chromosomes have the same genes in the same order, which is easy to detect in dot plots.

tetraploid. cells have four copies of each chromosome instead of two copies, as in diploids.

voltage-gated K+ channels. integral membrane proteins that are normally closed but can open when the membrane potential near them changes which causes the channel to open and allow K+ ions flow down their concentration gradient.

whole genome duplication. a dramatic and rapid change in DNA content of an organism where the entire genome is duplicated as in S phase, but the cell/nucleus does not divide resulting in twice the previous amount of genomic DNA.

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Plant Physiology

A. Malcolm Campbell • Christopher J. Paradise

This book examines three ways plants respond to their changing environment. The first example can be found in all plants. Despite the extreme changes in weather, plants have to stay where they are and respond to whatever nature produces. Plants have the capacity to respond quickly and yet they can evolve in a single generation. The second example addresses how an individual leaf has to respond rapidly and repeatedly to maintain the proper balance of carbon dioxide (CO_2) and water so that it can photosynthesize but not dry out. This delicate balance is governed by a pair of cells that regulate the size of openings on leaves. The final chapter examines a unique example of a leaf that can move fast enough to trap insects and digest them. This book presents data that led to our understanding of how plants function on different time scales.

A. Malcolm Campbell teaches biology at Davidson College, NC. He received national and international education awards: Genetics Society of America (2013); American Association for the Advancement of Science (2012); and American Society for Cell Biology (2006). He was the founding co-editor in chief of CBE Life Sciences Education; founding director of Genome Consortium for Active Teaching (GCAT); and member of the American Society for Cell Biology governing council (2012–2014).

Christopher J. Paradise is professor of biology and environmental studies at Davidson College. He teaches introductory biology, ecology, entomology, and topical seminars on ecotoxicology and renewable natural resources. He also occasionally leads a study abroad program in India. His research evaluates anthropogenic factors that influence insect biodiversity at a variety of scales. His current research interests include effects of land use patterns on pollinator communities in parks.

MOMENTUM PRESS
APPLIED SCIENCES

ISBN 978-1-94474-911-8
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