FUNCTIONAL BIOLOGY OF PLANTS

Martin J. Hodson
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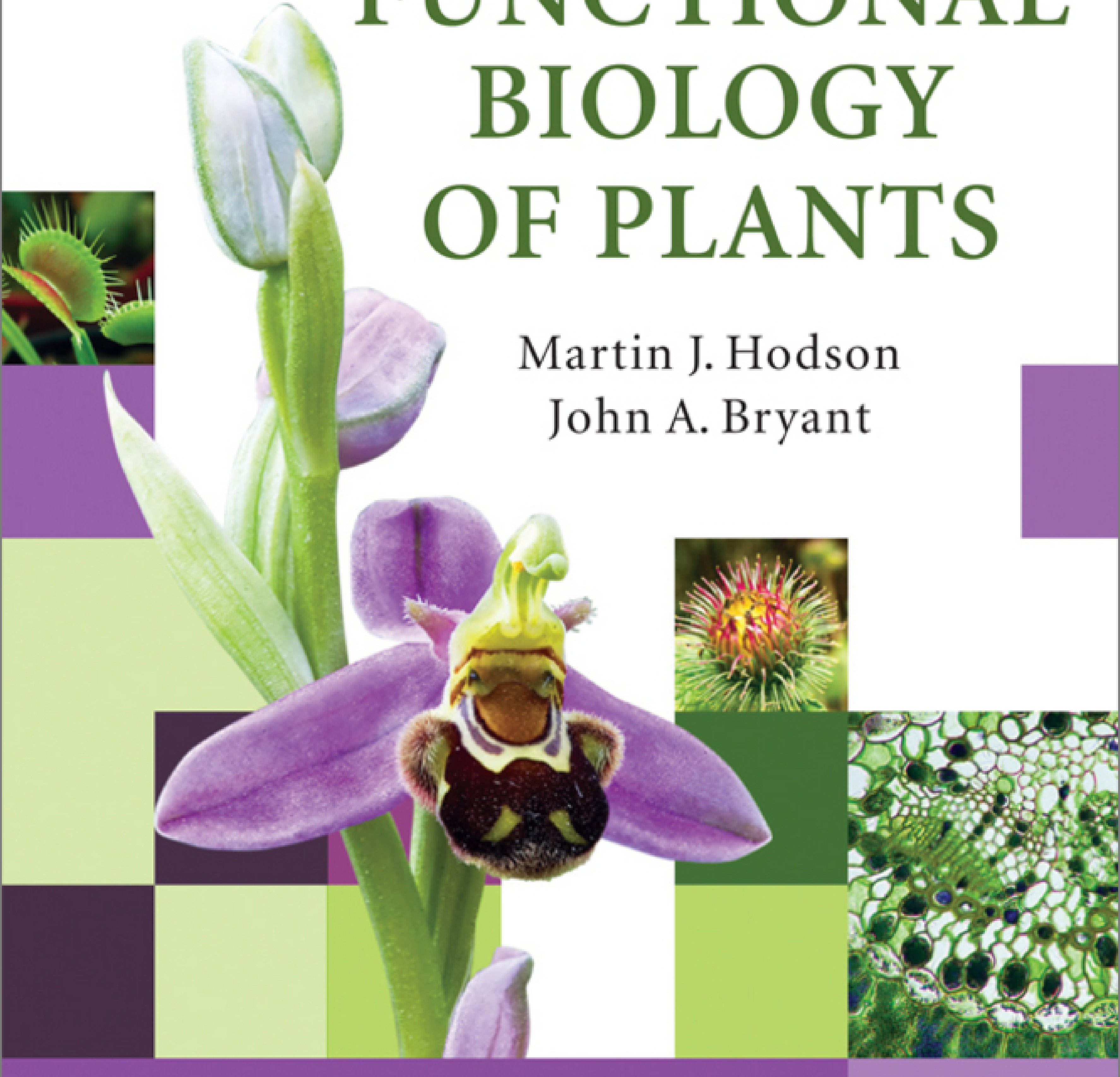

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Functional Biology of Plants

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MJH would like to dedicate this book to the three plant biologists who have been most influential in his career:

Dr. Helgi Öpik (Swansea University, Wales), who both taught me as an undergraduate and supervised my doctoral studies.

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Without their guidance and friendship, I would never have got as far as writing this book.

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Dr Cecil Prime (1909–1979) at Whitgift School, Croydon: a firm but caring school teacher whose love and knowledge of plants was infectious. This led me to study plants at university and I was grateful, as a 'first-generation' university student, for his continued interest and support during my undergraduate years.

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Preface

As we complete the manuscript of *Functional Biology of Plants*, many thousands of refugees, driven by drought and famine from the Horn of Africa, have found their way to camps in Kenya. Nowhere is it more obvious that people need feeding, yet it is also true to say that, with appropriate land use, the continent of Africa could become self-sufficient in food production.

This is not the place to discuss the political and economic challenges that will need to be faced; rather, we state that plant growth has never been so important. It may be true in some developed countries that students seem relatively uninterested in botany or plant biology, but it is equally true that we need to know more about plants and how they work, at least partly in order to harness and, indeed, to increase their potential in human nutrition. Thus we hope that this book will engender interest in the functioning plant.

We have not set out here to write a book about plant biochemistry or cell biology or molecular biology or genetics. Instead, after an introduction to plant function at those levels, we have attempted to show how activities at molecular and cellular levels are integrated and coordinated in the functioning of whole organs and of whole organisms – the plants themselves. In the later parts of the book, we place plants into their natural environments as they deal with abiotic and biotic stresses before considering, in the final chapter, the importance of plants in relation to some of the pressing problems facing humankind in the 21st century.

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1

CHAPTER 1

Origins

1.1 Plants – what are they?

We might simply define plants as photosynthetic eukaryotes – a description that would certainly include all the types of organisms that find their way into courses in botany or plant biology. However, as will become clear later in this chapter, such a definition brings together some very diverse groups whose common ancestor existed possibly as long ago as 1.6 billion years before the present time. These include glaucophytes (very simple unicellular aquatic organisms), all the different groups loosely known as algae and also the land plants, including the most advanced of these, the angiosperms (flowering plants), on which this book is mainly focused.

Charles Darwin, in a letter to Joseph Hooker, the Director of the Royal Botanic Gardens at Kew, described the origin of flowering plants as an 'abominable mystery'. They seemed at that time to appear in the fossil record without any obvious immediate precursors. Our understanding today, although somewhat more extensive than it was in Darwin's time, is still far from complete; the mystery is not yet completely solved. To appreciate this, it is necessary to go right back to the origin of cellular life and then of eukaryotes. It is a fascinating story.

1.2 Back to the beginning

For much of the 20th century, our knowledge of the history of life on Earth went no further back than the dawn of the Cambrian period – 'only' 550 million years ago. Fossils of quite sophisticated marine eukaryotes have been dated to that time and, during the Cambrian period itself, a very wide range of new lifeforms appeared. This flourishing of diversity in this period is known as the *Cambrian explosion*. However fascinating this is, it does not actually tell us of the earliest lifeforms.

Intense searches in pre-Cambrian rocks were conducted from the mid-1960s onward, but for many years failed to yield any fossils. However, one of those pivotal moments in science came when the American paleobiologist William Schopf identified fossil microorganisms dating back 3.5 billion (i.e. 3.5×10^9) years. Whether or not these represent the oldest living things on Earth is still not clear. Some paleogeochemists have suggested that there is chemical evidence of life processes in rocks dating back 3.8 billion years, while others are of the opinion that the chemicals that supposedly indicate some form of metabolism at that time could equally have arisen by non-biogenic processes. Nevertheless, Schopf's discovery unlocked the 'log-jam' and, since then, many more fossils have been found in pre-Cambrian rocks. Furthermore, paleogeochemical analyses have given us a good idea of what conditions on Earth were like during this period. To this we can add detailed knowledge of the molecular biology and genetics of organisms living today. All this has enabled scientists to build up a picture of the main features of the evolution of living organisms during the pre-Cambrian.

So, life originated around 3.5 billion years ago (and possibly slightly earlier). The predominant, indeed probably the only, organisms then were similar to modern prokaryotes. Earth's atmosphere contained no free oxygen at that time, so these early bacteria were inevitably all anaerobic. Indeed, study of the properties of amino acids in modern anaerobic and aerobic organisms indicates strongly that the genetic code evolved under anaerobic conditions.

A good case has been made that the earliest cells were similar to today's Gram-positive bacteria and gave rise to two further lineages – the Gram-negative bacteria and the Archaea (or archaebacteria). The origin of the Archaea has thus been dated as occurring very early in the history of

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life. Fossil evidence indicates that photosynthetic bacteria (like modern cyanobacteria) first appeared about 2.8 billion years ago. The presence of photosynthetic organisms led to the '**great oxidation event**' (between 2.2 and 2.45 billion years ago), which was bad news for anaerobic organisms because it generated free oxygen, which was (and still is to an extent) toxic to them. This selective pressure led to the evolution of aerobic organisms, capable of using oxygen in energy generation, probably at least two billion years ago.

1.3 Eukaryotes emerge

The idea that chloroplasts and mitochondria may have been derived from bacteria was first mooted in the 19th century, but it was not until the 1960s that the idea received wider attention. Based on her studies in cell biology, Lynn Margulis proposed specifically that mitochondria were derived in evolution from aerobic bacteria that had been engulfed by anaerobic bacteria, establishing the lineage that led to modern eukaryotes. According to this view, the inner membrane of the mitochondrion represents the original plasma membrane of the engulfed bacterium and the outer mitochondrial membrane represents the plasma membrane of the original host cell (see Figure 1.1). A second engulfment, this time of a photosynthetic (cyano)bacterium, led to the lineage(s) of photosynthetic eukaryotes and eventually to plants.

It is fair to say that, although some scientists embraced it enthusiastically, the **endosymbiotic theory** was not widely accepted when Margulis originally proposed it. Nevertheless, there was interest in what was called the 'autonomy' of chloroplasts and mitochondria. DNA from these organelles was unequivocally identified, as was the whole range of protein synthesis 'machinery'. To all intents and purposes, these organelles appeared to be organisms within organisms – except that they had only a fraction of the number of genes needed to support independent life. If the endosymbiont hypothesis was correct, then transfer of genes from the endosymbiont to the host genome must have occurred during subsequent evolution.

Further analysis showed that a wide range of molecular biological features – including gene promoters, ribosome structure, sizes of particular types of RNA

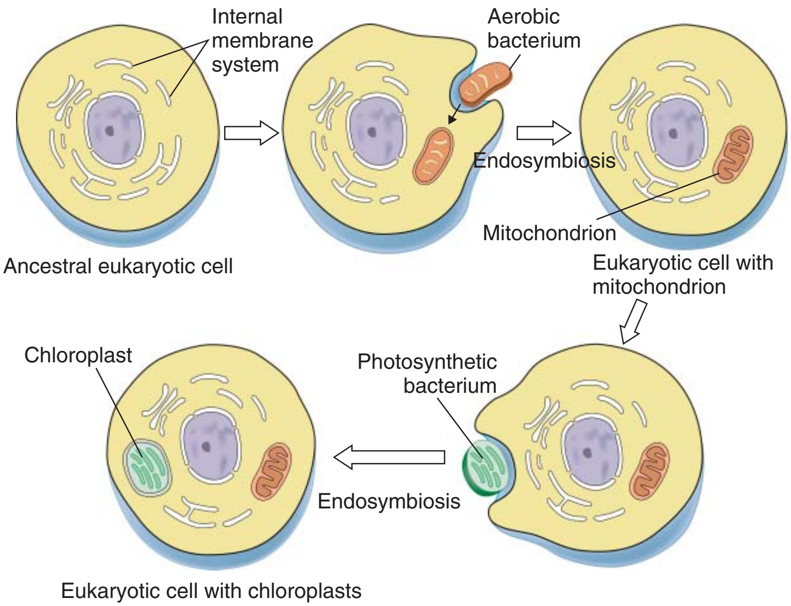

Figure 1.1 Diagram of 'engulfment' events leading to the formation of eukaryotic cells and then of photosynthetic eukaryotic cells. The original engulfing cell ('ancestral eukaryote') was almost certainly descended from an archaebacterium. It must have already possessed some features of eukaryotic cells, including a membrane system and possibly a nucleus (see text). Reproduced, with permission, from http://scienceisntfiction.blogspot.com/2011/04/endosymbiotic-origins.html

and the initiation of protein synthesis in plastids and mitochondria – resembled much more the equivalent features in bacteria than those of the major genetic system in the eukaryotic cells that contain the organelles. Further, the plastids of glaucophytes have a peptidoglycan wall, similar to the cell walls of cyanobacteria. All this is, of course, consistent with the endosymbiotic hypothesis and, by the time Margulis published her book *Symbiosis in Cell Evolution* in 1981, the hypothesis was accepted by the majority of biologists.

Further research during the past three decades has further confirmed the validity of the hypothesis, and it is now firmly stated that eukaryotes arose by the engulfment of an aerobic α-proteobacterium. Whether the 'host' cell was an archaean or a eubacterium is a matter for discussion. However, comparisons of biochemical mechanisms involved in DNA, RNA and protein synthesis, and of the sequences of genes and proteins, suggest a close relationship between the eukaryotic and archaebacterial clades. The authors of this book thus favour an archaebacterial origin for the eukaryotes, as shown in Figure 1.1, but there are some who believe that eukaryotes and archaebacteria are sister clades, having diverged from a common ancestor. Whichever of these two views one holds, there are still further problems to consider, of which we highlight three:

- First, there are some 60 clear differences between the organization, activity and structure of eukaryotic and prokaryotic cells. One of these differences is that prokaryotes are incapable of phagocytosis. However, the engulfment of a proteobacterial cell by an archaebacterial cell, a key part of the endosymbiont theory, would have been achieved by phagocytosis. So, either we envisage that a sub-group of ancient archaebacteria had already acquired some eukaryote-like features, such as phagocytosis, or that merger of two cells occurred by an unknown process.
- The second problem concerns another of these major differences, namely the sequestration of the main genome inside a complex organelle – the nucleus. With this came specific mechanisms for the division and segregation of the genome in the processes of mitosis and meiosis (the latter arising as part of the evolution of sexual reproduction). There has been much speculation on the evolution of the nucleus, but to date no really convincing hypothesis has emerged. The origin of this major feature of all eukaryotic cells remains totally mysterious.
- The third problem is that of the age of the eukaryotic lineage. The 'molecular clock' approach uses comparisons of sequences of genes and proteins in diverging lineages. Assumptions about rates of mutation, based on rates in living organisms, give an estimate of when lineages diverged from each other. This method places the origin of the eukaryotes at between 1.9 and 2.0 billion years ago, and there is some support for this dating from the fossil record. Most paleobiologists accept this dating, but there is a small group who contest it vigorously, suggesting that the eukaryotic lineage is much younger, dating back 'only' 800–900 million years. The authors of this book accept the majority view.

1.4 Photosynthetic eukaryotes – the first 'plants'

The emergence of photosynthetic organisms and the resulting 'great oxidation event' provided the selective pressure for the emergence of aerobic organisms and the establishment of the eukaryotic lineage. However, we can say with some justification that the arrival of photosynthetic eukaryotes was even more significant. This large and now diverse array of autotrophic organisms, ranging from simple single-celled organisms to huge forest trees, has had a greater effect on the world's ecosystems than any other, and thus the engulfment of a photosynthetic cyanobacterium by an early aerobic eukaryote was a key step in the development of life on Earth.

Eukaryotes had split relatively rapidly into two groups: the unikonts (with one flagellum[i]), which gave rise to animals and fungi; and the bikonts (with two flagella). It was among the latter that photosynthetic ability was acquired, approximately 1.6 billion years ago. The Australian cell biologists Geoffrey McFadden and Giel van Dooren leave us in no doubt about the significance of this event:

> *'This fusion of two cell lineages . . . brought the power of autotrophy to eukaryotes and descendants of this partnership have populated the oceans with algae and the land with plants, providing the world with most of its biomass'.*

---

[i] The Greek word *kontos* actually means 'barge-pole' or 'punt-pole' and gave rise to the English word *quant.*

From this foundational step, there arose several of the groups that we included in our earlier loose definition of plants, including the green plants (see Box 1.1).

Box 1.1 Abundance of green plants

The role of plants in contributing to biomass is clearly seen by considering *cellulose* (Chapter 2, section 2.2.1). This polysaccharide component of the cell walls of nearly all photosynthetic eukaryotes is the most abundant organic compound on Earth.

Furthermore, the most abundant protein in the world and the most abundant naturally occurring polar lipid in the world are both associated with photosynthesis. The protein is the primary carboxylating enzyme, *ribulose bisphosphate carboxylase oxygenase* (also known as Rubisco; see Chapter 7, section 7.4.5), while the lipid, *monogalactosyl diglyceride* (MGDG), is an essential component of the chloroplast thylakoid membrane (see Chapter 2, section 2.5.2). It is ironic that many biologists are unfamiliar with these two important molecules.

However, the story does not end there. There are many photosynthetic eukaryotes, some of them loosely classified in the past as algae, in which the plastids do not have the 'classical' double membrane but instead have four (or in some groups, three) membranes round them. Where did these complex plastids come from? Detailed sequence analysis of their genes and the genes of 'conventional' plastids indicate strongly that *all* plastids arose from a single ancestral source – the originally engulfed cyanobacterial cell. Study of the extra membranes round these complex plastids shows that they originated when a non-photosynthetic eukaryote engulfed a photosynthetic eukaryote.

The extra membranes round these plastids thus represent the plasma membranes of the engulfed cell and of the host. The major event of this type was the engulfment of a red algal cell, which led to lineages that include cryptophytes (which still carry a relic of the nuclear genome of the engulfed cell, the nucleomorph, with approximately 500 genes in a much reduced genome), the dinoflagellates (which have lost the host-derived outer plastid membrane), the brown algae and the diatoms.

In some of the lineages arising from this secondary symbiosis, the plastid has been lost or is much reduced. The Apicomplexa, a phylum that includes the malaria parasites (*Plasmodium* species) provide examples of this. Until the evolutionary origin of this group was understood, the possession of plastids by these organisms seemed very bizarre. The organisms are, of course, non-photosynthetic; over the course of evolution, their plastids (known as apicoplasts) have lost all the components of the photosynthetic machinery. However, they still have an important role in fatty acid metabolism and are essential to the life of the organism.

Finally in this section, it is noted that there have certainly been more than one of these secondary symbioses. The current view is that three such events took place in total, the other two involving engulfment not of red but of green algal cells. One of these events gave rise to the euglenoids (e.g. *Euglena gracilis*), which, like the dinoflagellates, have lost the outermost of the four chloroplast membranes. The other event led to the emergence of the chlorarachniophytes, which, like the cryptophyte lineage arising from the 'main' secondary symbiosis, have retained the vestiges of the engulfed cell's genome in the form of a nucleomorph.

1.5 The greening of Earth – plants invade the land

The evolutionary 'journey' from the first living organisms to the emergence and initial diversification of photosynthetic eukaryotes, discussed here in the space of a few paragraphs, covered a period of well over two billion years (the secondary symbioses described above are dated by different authorities at some time between 1.2 and 0.55 billion years ago). All the events described took place in water and, even today, 40–70 per cent of the world's primary production (based on photosynthesis) occurs in marine environments (despite the fact that the total 'photosynthetic biomass' of marine photosynthetic organisms is only about 0.33 per cent of the total). Admittedly, photosynthetic prokaryotes – cyanobacteria – are responsible for a large proportion of the CO_2 of that fixed in marine environments, but marine algae of various lineages, and especially diatoms, are also very important.

As a habitat, water has one major disadvantage for photosynthetic organisms: the deeper the water, the less light there is. Light may be reflected off the water surface, it may be scattered by particles in the water and it is absorbed by the water. The speed at which the latter happens depends on the wavelength of the light; light at the red end of the spectrum is absorbed before light at the blue end of the spectrum. Thus, in clear water, red light penetrates only to about 15 metres, whereas blue light may reach 100 m. There is therefore a zone – the **euphotic zone** – in which light penetration is adequate to support photosynthesis. In general, shallow water occurs on the margins of land masses and, in this primal history of photosynthetic eukaryotes, the land represented a major niche (actually, of course, a wide array of niches), endowed with a much better light environment.

Although better access to light was an obvious advantage, there were also obvious disadvantages. The need for water in order to maintain life meant that the possibility of desiccation was a serious problem. Water is also the medium into which algae release their gametes. Sexual reproduction on land would be more difficult. Furthermore, immersion in water made for easy uptake of nutrients and also provided support for the larger organisms.

Successful conquest of the land needed solutions to these problems and, based on fossil evidence, this did not occur until between 450 and 490 million years ago. It was another defining event in the history of planet Earth, albeit an event that unfolded slowly. There are now at least 370,000 species of land plants. Their evolution and diversification led to dramatic changes in Earth's environment, including a reduction in the concentration of carbon dioxide in the atmosphere, which resulted in a lowering of the planet's surface temperature. Linda Graham refers to all this as a '*quiet but relentless transformation of terrestrial landscapes*' which initiated the development of new ecosystems and the provision of niches for the evolution of other organisms.

In the transition from water to land, we see a major change in the predominant lifestyle. The aquatic ancestors of the land plants, in common with the majority of modern aquatic photosynthetic eukaryotes, were protists. Most protists are single-celled; the relatively few multicellular forms have little in the way of cellular differentiation, even though some (such as kelps) are very large. Some more complex protists, including the kelps and other brown algae, possess a region of dividing cells, equivalent to the meristems of land plants. The organization of these protist meristem-like regions is simpler than it is in land plants, with fewer possible planes of division.

Simpler protists are capable of, and in many circumstances do undergo, asexual reproduction. In those forms that also reproduce sexually (i.e. by the fusion of gametes), a meiotic division is necessary somewhere in the life cycle. In the simplest examples, this occurs in the zygote, straight after fertilization, but in many protists there is an alternation of generations in which a lifeform that produces gametes alternates with a lifeform that produces spores.

In contrast to the protist life style, we see in land plants the **embryophyte** lifestyle. Embryophytes are multicellular, with clear cellular and tissue specialization. Dividing cells are organized in regions known as **meristems;** meristematic cells possess more than two cutting planes and can thus generate three-dimensional structures. All embryophytes exhibit alternation of generations and possess antheridia (male gametophyte organs) and archegonia (female gametophyte organs) or the equivalent of these structures. Above all, their embryos are **matrotrophic**, meaning that for all or part of their period of existence they are closely associated with maternal tissues, from which they draw nutrients and signalling molecules.

The simplest, and probably the most primitive, embryophytes, the mosses and liverworts (Bryophyta) are still extensively reliant on water. They have no obvious means of restricting water loss and there are no specialized water-conducting cells. The plants also require water to enable the male gametes to swim to the female gametes within the archegonia in order to bring about fertilization. Modern bryophytes are desiccation-tolerant (i.e. they can recover from severe dehydration) and it is likely that this was also true of the earliest members of this group.

So how and when did these early land plants arise? Study of the cell biology and ultrastructure of modern green algae and bryophytes shows that the bryophytes resemble more the charophyte algae than the chlorophyte algae. For example, in both charophytes and bryophytes (and indeed in all embryophytes), the mitotic spindle is persistent and mitosis is open. The cell wall between daughter cells is laid down via a structure called the **phragmoplast** (see Chapter 2, section 2.12.2), involving a cleavage furrow with a microtubule array oriented at 90° to the plane of cell division.

There are also clear biochemical similarities between charophytes and embryophytes, while molecular phylogenetic analysis, based on gene sequences in nuclear, plastid and mitochondrial genomes, places the charophytes as a sister group to all embryophytes. Furthermore, extant charophytes have rudiments of the matrotrophic embryo, in that there are cellular

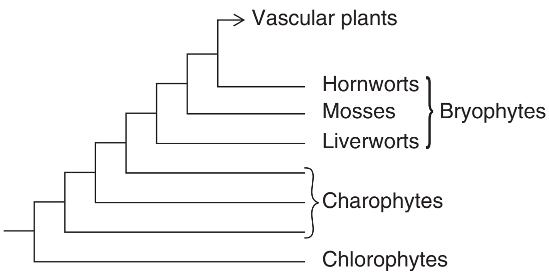

Figure 1.2 Diagram illustrating the positions of the chlorophytes and charophytes in the ancestry of embryophyte land plants.

interactions between haploid maternal cells and diploid zygotes that are thought to be involved in nutrient transfer. They also possess cell wall polymers that inhibit fungal degradation. In particular, a polymer laid down in charophyte zygotes resembles strongly the sporopollenin present in the cell walls of seed-plant pollen. All these data suggest that embryophytes and charophytes are descended from a common ancestor which itself had arisen by divergence from the chlorophytes (Figure 1.2).

Although the family tree for the earliest embryophytes appears clear enough from the data based on extant species, the fossil record is much less helpful. The main problem is that the earliest fossil evidence (consisting of tetrads of spores) for embryophyte land plants dates back about 450–490 million years, to the mid-Ordovician period (see Table 1.1) whereas the earliest known fossil charophytes occur in rocks from upper Silurian strata, dating back about 414 million years. Thus we have no clear picture of the immediate ancestor of the embryophytes. We do not know whether the embryophyte lifestyle evolved in an aquatic environment, or whether charophytes invaded the land before the origin of embryophytes. The existence today of many species of both chlorophyte and charophyte algae that live in terrestrial habitats (albeit still needing water for sexual reproduction) certainly shows that the latter was possible. Nevertheless, from our point of view as we follow the journey from the earliest living organisms to flowering plants, the main point is clear: the land was invaded.

There is still some discussion about which of the three bryophyte lineages – hornworts, mosses or liverworts – represent the earliest land plants. Although there is some support (mainly from comparative anatomy and morphology) for the view that hornworts were the earliest land plants, studies of genome structure, of gene sequences and of particular biochemical mechanisms in extant plants, point to the liverworts.

Table 1.1 The geological periods.

Period	Years before present
Quaternary	1.8 million to present day
Tertiary	66.4 million to 1.8 million
Cretaceous	144 million to 66.4 million
Jurassic	208 million to 144 million
Triassic	245 million to 208 million
Permian	286 million to 245 million
Carboniferous	360 million to 286 million
Devonian	408 million to 360 million
Silurian	438 million to 408 million
Ordovician	505 million to 438 million
Cambrian	570 million to 505 million
Pre-Cambrian	4.5 billion to 570 million

For example, in common with charophytes, the immediate progenitors of land plants, the mitochondrial DNA of liverworts lacks a particular type of intron[ii], the type II intron (see Chapter 3, section 3.2.1). All other bryophytes and all vascular plant groups possess three mitochondrial type II introns, although there have been subsequent losses in some lineages within these plant groups. Indeed, those who use molecular data in constructing phylogenies suggest that such data settle the question beyond doubt, so that is the position we take here: the earliest land plants were liverworts, from which mosses and hornworts diverged. The latter eventually gave rise to vascular plants (see next section).

1.6 Embracing the terrestrial lifestyle

While terrestrial habitats may indeed provide a good light environment, they also pose some strong challenges for living organisms. The lifestyle of modern bryophytes almost certainly typifies the way in which the earliest multicellular land plants dealt with those challenges. Such a lifestyle is successful in its own way, in its own ecological niches, but it can hardly be said to have conquered the land. Invasion is different from conquest.

[ii] An intron is a sequence of DNA that interrupts the coding sequence of a gene (see Chapter 3, section 3.2.1).

Nevertheless, the popular view of early land plants is one of conquest. We are very accustomed to reconstructions and artistic presentations showing a rich flora of vascular plants. The dominant forms differ according to which geological period is being portrayed, but the common feature is that it is *vascular plants* which make up these fossil forests. Conquest, rather than just invasion of the land, required a number of adaptations, including mechanisms or structures for prevention of water loss and for movement of water within the plant. Furthermore, the selective pressure to seek the light also led to the need for support as many plants evolved an upright stance.

In modern floras, symbiosis between green plants and soil-dwelling fungi features very strongly, as seen in different types of **mycorrhizae** (see Chapter 5, section 5.8). It now seems likely that mycorrhizae, and possibly other forms of symbiosis, were important in helping green plants to invade the land. Mycorrhizae identical in form to modern vesicular-arbuscular mycorrhizae have been discovered in association with *Aglaophyton major*, a very early Devonian land plant, suggesting that nutrient transfer mutualism (symbiosis) may have been in existence when plants invaded the land. This would have aided green plants in exploiting nutrient-poor substrates.

Evidence for the early evolution of vascular plants comes from fossils, from new, less destructive techniques for investigating fossil structure, from comparative anatomy and physiology of extant plants and from molecular phylogenetic studies. These studies provide strong evidence that the hornworts were the immediate ancestors of vascular plants. It is interesting that hornworts can exert some degree of control over water loss and gas uptake because they possess stomata, an important adaptation to life on land and a feature found in all vascular plants (see Chapter 9, section 9.4).

The evidence for a single origin (‘monophyly’) of the vascular plants comes both from comparative morphology and from an increasing array of DNA sequence data. What is not so clear is the position in the evolutionary tree of some fossil plants found in a remarkable assemblage in the Rhynie chert in Scotland. These fossils, which include *Aglaophyton*, *Horneophyton* and *Rhynia*, possess some features of vascular plants but also retain several bryophyte-like characteristics.

The earliest true vascular plants were the lycopsids or lycophytes. These first appeared in the late Silurian period. Modern members of the group include quillworts (*Isoëtes*), *Selaginella* and club mosses (*Lycopodium*; see Figure 1.3). Today they are relatively scarce, but in the Carboniferous period they were a dominant group, with tree lycopods forming extensive forests. The ability to grow as trees reflects the dual function of vascular tissue, both as a means of conducting water and nutrients throughout the plant and as a means of support of large aerial structures (see Chapters 5 and 6). Tree lycopods eventually became extinct in the Permian period, but they left a legacy, providing the bulk of the material from which coal was formed.

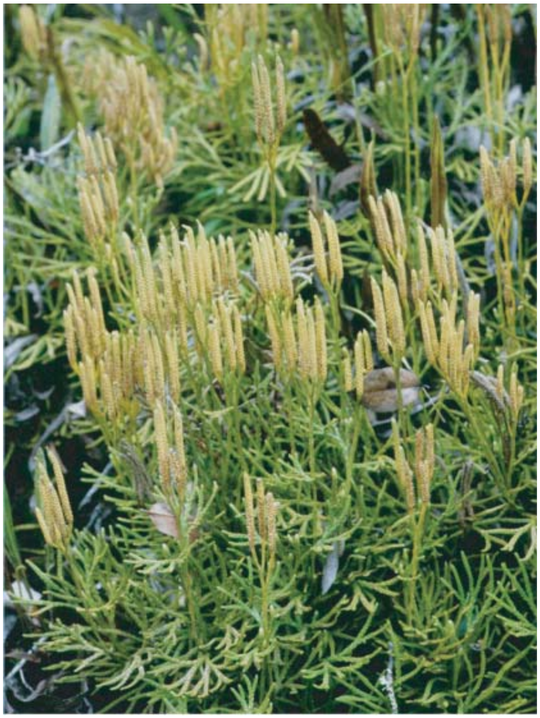

Figure 1.3 *Lycopodium thyoides*.
Photograph by Dr Gordon Beakes © University of Newcastle upon Tyne. Image from Centre for Bioscience (Higher Education Academy) ImageBank. http://www.bioscience.heacademy.ac.uk/imagebank/

Molecular phylogenetic evidence indicates strongly that lycopods gave rise to a lineage which then diversified into several groups, including the ferns and other fern-like plants, horsetails and eventually the various seed-plant groups. The horsetails, still represented in today’s biosphere, are particularly interesting. Like lycopods, they produced dominant forests of tall plants. The ability to grow tall was related to the role of silica in supporting the stems, in contrast to today’s tall plants, which are supported by lignin (see Chapter 2, section 2.2.4 and Chapter 6, section 6.5).

In summary then, the invasion of the land that started with bryophytes became a conquest as vascular plants appeared and then diversified. Indeed, the diversification of plant life on land (and its knock-on effects on the evolution of other organisms) known as *the Siluro-Devonian primary radiation*, is regarded as the terrestrial equivalent of the Cambrian explosion of marine life (as discussed in section 1.2).

Examination of fossil assemblages in strata of different ages reveals a succession of plant groups appearing, some of which became abundant for at least several million years. Many of these groups survive today, but there are some notable exceptions. We have already seen that tree lycopods, dominant in Carboniferous forests, became extinct in the Permian. The fossil record also contains a major phylum, the progymnosperms, that arose in the late Devonian and early Carboniferous and flourished for a time. The name is somewhat misleading, because they produced spores rather than seeds[iii] and did not give rise to modern gymnosperms. Nevertheless, the late Carboniferous/early Permian periods saw the emergence of gymnosperm groups which are still represented in extant floras. Indeed, gymnosperms were one of the dominant groups in late Triassic and early Jurassic forests – an indication of the selective advantages of the seed-based mode of reproduction (see Box 1.2).

Box 1.2 Advantages of seeds

Reproduction via seeds provides distinct advantages for life on land. Fertilization does not require water because the sperm does not have to swim to the egg. The one exception to this amongst seed plants is *Ginkgo biloba*,* in which the sperm are motile. The seed that develops following fertilization is effectively an embryo held in a state of quiescence or dormancy, usually provided with a food store and surrounded by a. protective coat.

*Maidenhair tree: the sole extant member of a group of gymnosperms that arose in the Permian and were abundant through to the end of the Triassic. Ginkgo is illustrated in Figure 7.1, Chapter 7.

Today, the gymnosperms are represented by just four groups – the Gnetophyta or Gnetales (see below), the Coniferae, *Gingko* and the cycads (Cycadophyta; Figure 1.4). Except for the conifers, these groups are just relicts in terms of their former abundance and dominance, For example, there are only a few species of cycads, while *Ginkgo biloba* is the sole living representative of a once more diverse group.

1.7 Arrival of the angiosperms

Modern angiosperms share with each other many features that are not represented at all in other groups (see Box 1.3) and on that basis they have been regarded as a single discrete group arising from one ancestral lineage – i.e. they are monophyletic. This view has been extensively confirmed by modern molecular phylogenetic analysis.

Box 1.3 Essential features of angiosperms

The term 'angiosperm' derives from two Greek words: *angeion*, meaning 'vessel' and *sperma*, meaning 'seed'. The angiosperms are those plants whose seeds develop within a surrounding layer of plant tissue, called the carpel, with seeds attached around the margins. This arrangement is easily seen by slicing into a tomato, for example.

Collectively, carpels, together with the style and stigma, are termed the ovary, and these plus associated structures develop into the mature fruit. The enclosed seeds and the presence of carpels distinguish angiosperms from their closest living relatives, the gymnosperms, in which the seed is not enclosed within a fruit but, rather, sits exposed to the environment.

Some defining characteristics of angiosperms include flowers, carpels and the presence of endosperm, a nutritive substance found in seeds, produced via a second fertilisation event. Angiosperms thus exhibit the phenomenon of **double fertilisation** (see Chapter 8, section 8.6.3).

But from where did the angiosperm lineage arise? What is the sister group to the angiosperms? If these questions could be answered, we would be making progress towards solving Darwin's abominable mystery. Prior to the availability of molecular techniques, morphological comparisons had led to the angiosperms being regarded as sister group to the Gnetales, a varied group of gymnosperms represented today by just three families. The most bizarre of these is the family Welwitschioideae, type genus *Welwitschia*, which produce flowers that rest on the ground (see Figure 10.9, Chapter 10).

[iii] The name *gymnosperm* means 'naked seed', in contrast to *angiosperms*, in which seeds are enclosed in a structure called the carpel.

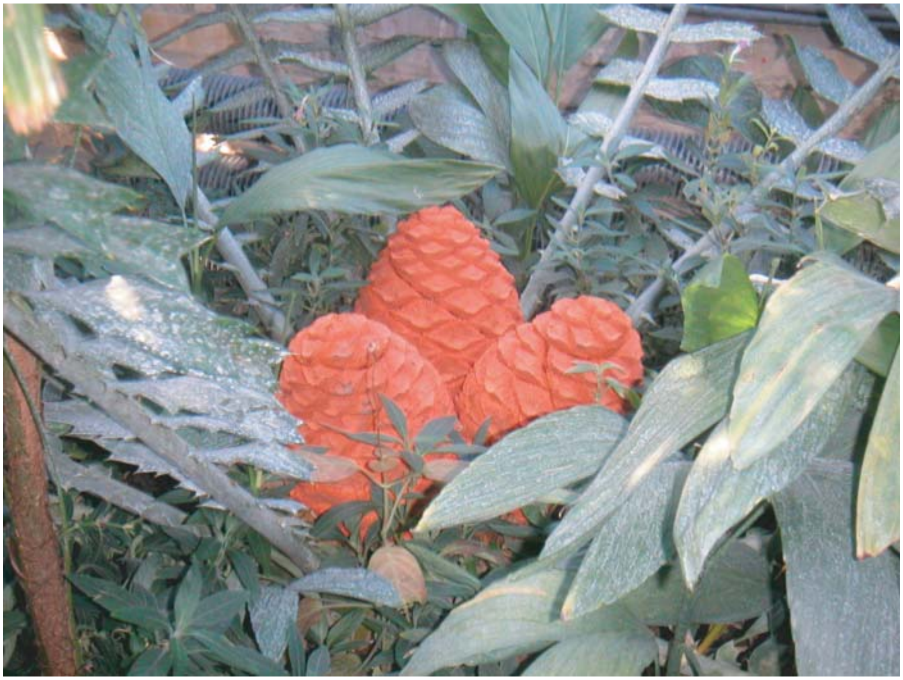

Figure 1.4 The cycad *Encephalatos ferox*, native to coastal habitats in Mozambique. Photo: MJH. Used with the permission of Oxford Botanic Gardens.

Angiosperms and Gnetales were together known as the anthophytes, but it is now clear from molecular phylogenetic analyses that the anthophyte hypothesis is untenable. However, that is not to say such analyses have solved the problem. When molecular phylogenetic analysis first became available, it was widely thought that its careful application to seed plants would sooner or later lead to an understanding of angiosperm origins. However, this has not proved to be the case. Indeed, some plant scientists believe that the mystery is as deep now as it was in Darwin's day. The problem is that different analyses tell different stories, depending on which genes are used in the analysis and whether DNA or protein sequences form the basis for comparison. Thus, the distinguished evolutionary botanist, James Doyle, at Davis, California, wrote in 2008: '*Much of what we thought we knew 10 years ago about seed plant phylogeny . . . has been thrown into doubt by molecular analyses.*'

Doyle also wrote that: '*Resolution of these problems requires integration of molecular, morphological and fossil data in a phylogenetic framework.*'

The data from fossils include analyses of flowers and flower-like structures in presumed angiosperms such as *Archaefructus* from the early Cretaceous (currently the earliest known fossils of angiosperm-like flowers date from this period) and in seed ferns. The molecular data include molecular clock estimates that put the origins of angiosperms no earlier than the Jurassic period. This integration of approaches suggests that the divergence from the gymnosperms (and more specifically from the cycads) of the lineage that led to angiosperms happened probably as early as the Carboniferous. In other words, the last common ancestor between the two groups of extant seed plants – gymnosperms and angiosperms – was alive in the Carboniferous period. This divergence established the lineage known variously as the angiophytes or the pan-angiosperms. However, as pointed out by Doyle in a personal communication to JAB, the early members of this lineage: '. . . *need not have looked any more like modern angiosperms than pelycosaurs (in an early Permian branch from the mammalian stem lineage) look like mammals.*'

So, although the gymnosperms (and in particular the cycads) are the nearest living relatives to modern angiosperms, the actual sister-group to angiosperms is to be found among extinct groups within the pan-angiosperms, namely the seed ferns (Figure 1.5). Current

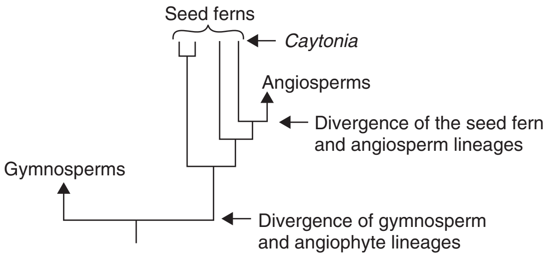
Seed ferns
Caytonia
Angiosperms
Divergence of the seed fern and angiosperm lineages
Gymnosperms
Divergence of gymnosperm and angiophyte lineages

Figure 1.5 Diagram illustrating a simplified version of current views on ancestry of the angiosperms. The divergence between the gymnosperm and the angiophyte (or pan-angiosperm) lineages took place in the Carboniferous period, while the final separation between seed-ferns and true angiosperms occurred in the late Jurassic or early Cretaceous period (also see Figure 1.7). The seed ferns themselves became extinct in the late Cretaceous. For the sake of clarity, the diversification of gymnosperms (which occurred mainly in the Permian period) is not shown.

views are that the most likely seed-fern group to fulfil this role is that containing the genus *Caytonia*.

In summary then, angiosperms arose as the crown group of the pan-angiosperms in the late Jurassic (based on molecular clock data), or slightly later in the early Cretaceous (based on fossil evidence). Their evolution as a distinct group, after divergence from seed-ferns, involved the adaptation and development of pre-existing structures to form, among other things, the characteristic angiosperm flower. The double fertilization involved in endosperm formation (see Chapter 4, section 4.3 and Chapter 8, section 8.6.3) also evolved at this time (double fertilization also occurs in the Gnetales, mentioned above, but in that group it leads to the formation of two embryos).

Until relatively recently, the Nymphaeales (including present-day water lilies and probably also the fossil *Archaefructus*) were regarded as the most primitive angiosperms. However, based on extensive phylogenetic analysis, *Amborella trichopoda* (Figure 1.6), a semi-climbing shrub only found in the rain forests of New Caledonia, is now regarded as sister to all extant angiosperms and is therefore at the base of the angiosperm phylogenetic tree (Figure 1.7)[iv].

Thus *Amborella* is at the base of the very diverse taxon, extant angiosperms. Early divergence brought into existence two other primitive groups, the Nymphaeales (water lilies) and Austrobaileyales; these groups, together with *Amborella* are often known as the **ANITA grade**, based on the genera *Amborella, Nymphaea, Illicium, Trimenia* and *Austrobaileya*. All other angiosperm groups are often termed the mesangiospermae. The more primitive mesangiosperms include the magnolids (see Figure 1.7), but the most obvious indications of the extensive radiation of the angiosperms are the **monocots** (monocotyledones) and **eudicots** (eudicotyledones). The latter term, meaning effectively ‘good dicots’ or ‘true dicots’ distinguishes these from the ‘paleodicots’ represented by the ANITA grade and by the magnolids and other more primitive groups.

The ecology of *Amborella* and of other primitive angiosperms suggests that the group first arose in shady, damp or wet and possibly disturbed habitats. The subsequent radiation of the angiosperms occurred mainly between 100 and 65 million years ago, and a large proportion of currently living groups had appeared by the end of the Cretaceous period.

This very rapid radiation is certainly worthy of comment. It was one of the features that caught Darwin’s attention; flowering plants seemed to him to appear from nowhere (although we are now beginning to understand something of their origins). The rapidity and extent of the angiosperm radiation is indeed astonishing, such that we are justified in speaking of a ‘*Cretaceous explosion*’. This radiation has seen angiosperms progress from being a relatively minor component of the biosphere to becoming the major vascular plant group, totalling between 250,000 and 300,000 species[v], occupying the widest possible range of ecological niches and dominating the vegetation in many terrestrial and some aquatic ecosystems. In attaining such dominance they ousted the gymnosperms from their previously dominant position, and although angiosperm distribution over the Earth has changed with the changing form and climate of the planet, they have remained the dominant plant group for the past 65 million years.

In morphology and growth form, the angiosperms vary between the tiny *Wolffia*, a genus in the duckweed family (Figure 1.8a) to very large and long-lived trees (Figure 1.8b) (although, admittedly, the tallest, the largest

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[iv] Interestingly *Amborella* (or any plant similar to it) has not been found in fossil form – an indication of the incompleteness of the fossil record.

[v] The current estimate for the total number of species of all land plants is 370,000 (see Chapter 12, section 12.2.1).

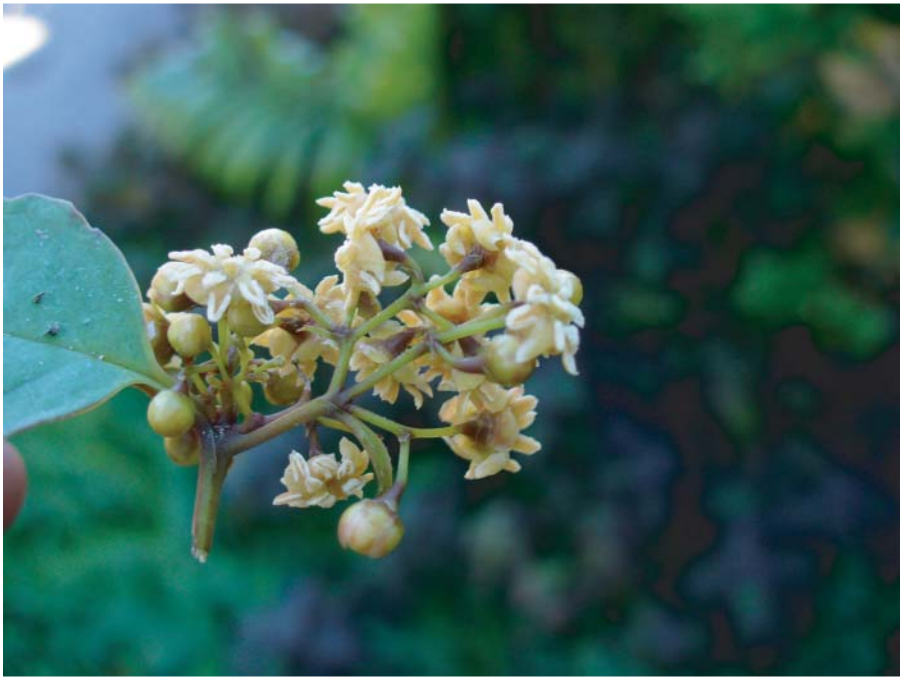


Figure 1.6 Flowers of *Amborella trichopoda*, the most primitive living angiosperm. Photo: Scott Zona, Florida International University.

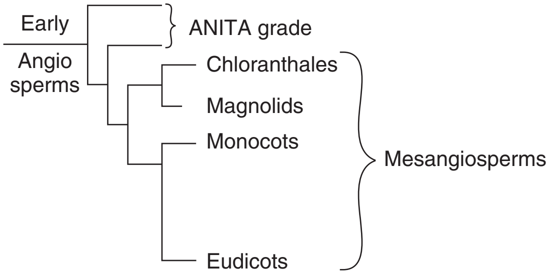


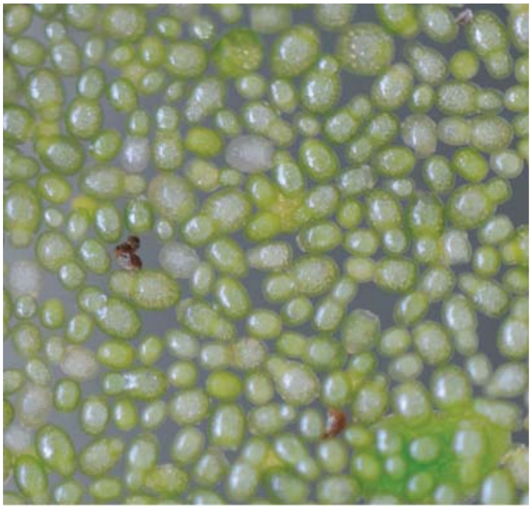
Figure 1.7 Simplified diagram illustrating the divergence of angiosperm groups.

and the longest-lived trees are all gymnosperms: see Chapter 6, section 6.6). As a group, angiosperms exhibit a wide range of interactions with other members of the biosphere, of which arguably the most famous is that many species rely on insects for pollination. Indeed, several authorities regard co-existence and co-evolution with insect pollinators as being one of the factors that contributed to the angiosperm rise to dominance. This relationship is discussed further in Chapter 8, section 8.6.2, but at this point we discuss a more basic aspect of reproduction.

1.8 Sex and the alternation of generations

The evolutionary history of the angiosperms, traced back to the origins of life, incorporates another story, namely the evolution of reproductive mechanisms. Indeed, the angiosperms are named for one aspect of their reproduction, namely the enclosure of the developing seeds in the carpel. It is therefore appropriate at this point to consider another facet of sexual reproduction – namely the need to alternate between the haploid and the diploid states.

In section 1.1 we noted the emergence of eukaryotic cells as cells with defined sub-cellular organelles, including a nucleus that harbours the genetic material, DNA. Among the 60 or so differences between prokaryotes and eukaryotes is the existence in the latter of complex cell division mechanisms to ensure the segregation of the genetic material between daughter nuclei and, hence, between daughter cells. One of these cell division



(a)

(b)

Figure 1.8 (a) Individual plants of *Wolffia arrhiza* (Watermeal) are 1–2 mm wide, and it is the smallest vascular plant. Photo: Aaron Woods. (b) Oak trees (*Quercus robur*) coming into leaf in spring. Fallow deer grazing under the trees. Photograph taken by JAB at Ripley, Yorkshire, UK.

mechanisms is meiosis, the division that produces haploid cells from diploid cells (i.e. halves the number of genome copies in a cell). This is an absolute requirement for sexual reproduction, without which the number of copies of the genome per cell would double with each generation.

Although it is not entirely clear, it is likely that both mitosis and meiosis evolved before the endosymbiont engulfment that produced the first true eukaryote. The acquisition of these activities was part of a process known as *eukaryogenesis*. What is clear is that sex is a eukaryotic activity. Prokaryotes cannot undertake a reduction division and therefore cannot indulge in sexual reproduction.[vi] The evolutionary significance of sex is enormous. Not only can genetic variation be generated by mutation and horizontal gene transfer, but also by the mixing of the genetic variation of the two sexual parents.

Sexual reproduction has been incorporated into eukaryotic lifestyles in a number of different ways but all inevitably involve an alternation between a haploid and a diploid phase. Eukaryotic organisms exhibit several basic types of sexual life cycles, differing in the ploidy of adult organisms and in the site of meiosis. The simplest sexual lifestyle we can envisage would involve the meiotic reduction division occurring immediately after the sexual fusion of two haploid cells. This is seen in the single-celled green alga *Chlamydomonas*, in which the haploid vegetative cells produce haploid gametes. Gametes fuse to form diploid zygotes, which undergo meiosis to form new haploid vegetative cells. The zygote is therefore the only diploid cell in the lifecycle.

Animals provide a complete contrast. They exist as diploid organisms and the only haploid cells are the gametes; meiosis occurs during gametogenesis. The fusion of the two gametes restores the diploid state, thus initiating the next generation. Admittedly there are many variants of this basic pattern. For example, some animals have larval stages and attainment of the adult form may involve quite a dramatic metamorphosis. At the other end of the scale, in reptiles, birds and mammals, the young that hatch from the egg, or that are born, grow and develop 'seamlessly' into the adult. Nevertheless, among all this variety of animal life progressions, it remains true that the only haploid cells are the gametes.

Land plants (and some non-vascular aquatic plants), however, have adopted a completely different pattern, in which there are two different multicellular generations, one haploid and one diploid. In other words, there is an *alternation of generations* (also known as a diplobiontic life cycle; see Figure 1.9). The diploid phase is the **sporophyte** or spore-producing generation. Spores are produced by meiosis; the haploid spores germinate, undergo cell

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[vi] Prokaryotes are able to exchange genetic material in processes such as conjugation, but these processes are not equivalent to sexual reproduction.

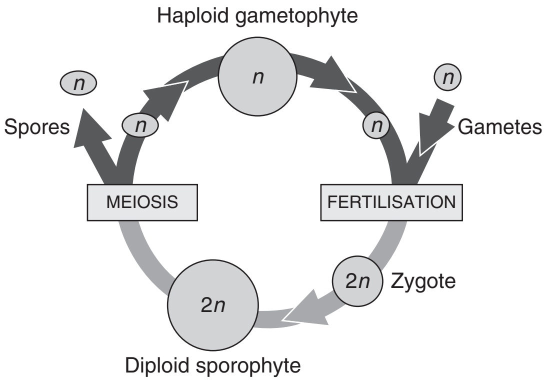

Figure 1.9 Diagram illustrating the basic features of alternation of generations. Note that some organisms are *heterosporous* (the spores germinate to form single-sex gametophytes); male gametophytes release sperm, while female gametophytes carry the egg cells. In *homosporous* organisms, the spores germinate to give only one type of gametophyte, which produces both sperm and egg cells.

division, differentiation and morphogenesis to produce the mature **gametophytes**. As the name implies, the gametophyte phase produces gametes which fuse to produce a diploid zygote which is the start of the new sporophyte generation. In land plants, the two generations differ in appearance; the two generations are *heteromorphic*. However, in the green alga *Ulva* which exhibits alternation of generations, the two life cycle phases look the same; they are thus *isomorphic*.

In the simpler land plants, bryophytes, the dominant generation (the one that we see and recognize as a moss or liverwort) is the gametophyte generation. The sporophyte generation is short-lived and generally dependent on the gametophyte. In vascular plants, by contrast, the sporophyte is the dominant generation. Gametophytes of vascular plants are much smaller than their sporophytes and are either free-living or retained within sporophytic tissues. For example, in ferns, spores develop within clusters of sporangia on the sporophyte plant; spores germinate to produce gametophytes that are, like the sporophyte, photosynthetic and free-living but small and inconspicuous. The gametophytes produce sperm cells and egg cells within specialized structures called antheridia and archegonia; the gametes fuse to produce a diploid zygote which develops into the new sporophyte.

In gymnosperms and angiosperms, it is again the sporophyte generation that we recognize as the plant. The gametophytes are very small, non-free-living and non-photosynthetic. The female gametophyte is retained on the parent plant within an ovule. Pollen grains are immature male gametophytes which produce sperm cells. The details of sperm cell generation and of fertilization mechanisms differ between gymnosperms and angiosperms but, essentially, fertilization of the egg cell occurs within the female gametophyte, as do the early stages of growth of the new sporophyte generation, namely embryogenesis and seed development. These processes in angiosperms are discussed more fully in Chapters 4 and 8.

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CHAPTER 2

Introduction to Plant Cells

The previous chapter described the emergence in evolution of the angiosperms, the flowering plants. Much of the rest of this book deals with angiosperm function at the levels of organ and whole organism; we discuss the integration of growth and development, the angiosperm life cycle and the inter-organism interactions involved in various angiosperm lifestyles.

However, in order to understand the plant as a functioning organism, it is necessary to have some knowledge of plant biology at the cellular and sub-cellular levels. Therefore, in this chapter and the next, we provide introductions to plant cells and to the major molecular activities in which the cells participate.

2.1 Plant cells

There is a sense in which there is no such thing as a ‘typical’ plant cell. Cell structure varies extensively according to the function of the cell in question. Nevertheless, it is helpful at this point to consider the main features of plant cells before looking at those features in more detail in subsequent sections. The features are illustrated diagrammatically in Figure 2.1.

First, plant cells are characterized by being contained within a *cell wall* (section 2.2), composed mostly of polysaccharides and whose structure varies according to cell age and function. Inside the cell wall is the cell’s outer membrane, the *plasma membrane* (section 2.3). In older cells, the next most obvious feature is the cell *vacuole* (section 2.8), a large aqueous space bounded by another membrane, the *tonoplast*. The vacuole’s main functions are storage of particular solutes and the sequestration of hydrolytic enzymes. In vacuolated cells, the *cytosol* or *cytoplasm* is confined to a narrow zone between the vacuole and the plasma membrane (Figure 2.1) but, in non-vacuolated cells, the cytosol occupies much of the space bounded by the plasma membrane.

Within the cytosol, three membrane-bound organelles are very apparent. The first is the *nucleus* (section 2.7), a feature of all eukaryotic cells (although some cells, such as red blood cells in mammals and phloem sieve tubes in plants, lose their nuclei during cell differentiation). Most of the genetic material, DNA, is located in the form of chromosomes within the nucleus, and all of the biochemical activities associated with gene expression and DNA replication occur there (sections 2.7 and 2.13.3 and Chapter 3, section 3.1.2).

The other two obvious organelles are the *chloroplasts/plastids* (section 2.5) and the *mitochondria* (section 2.6). The former are primarily associated with photosynthesis and starch storage and the latter with energy conservation (as ATP and NADH) during respiration, although chloroplasts actually carry out a much wider range of biochemical reactions. *Microbodies* (Section 2.10) are often located in the vicinity of chloroplasts and mitochondria. These organelles, which are sometimes known as *peroxisomes*, participate in photorespiration (Chapter 7, section 7.5). A particular class of microbody, the *glyoxysome*, is involved in the mobilization of the lipid reserves during germination of fat-storing seeds (Chapter 4, section 4.11.2).

The cytosol is permeated by an extensive endomembrane system, the *endoplasmic reticulum* or ER (section 2.9), which is involved in transport within and out of cells, in the sequestration of calcium ions and in the synthesis of (among other things) proteins that are destined for export. The ER is continuous with the outer envelope of the nucleus and also interacts with the *Golgi apparatus*, also known as *Golgi bodies* or *dictyosomes* (section 2.9). The Golgi bodies appear as stacks of flattened sacs with vesicles located around them. These flattened sacs are

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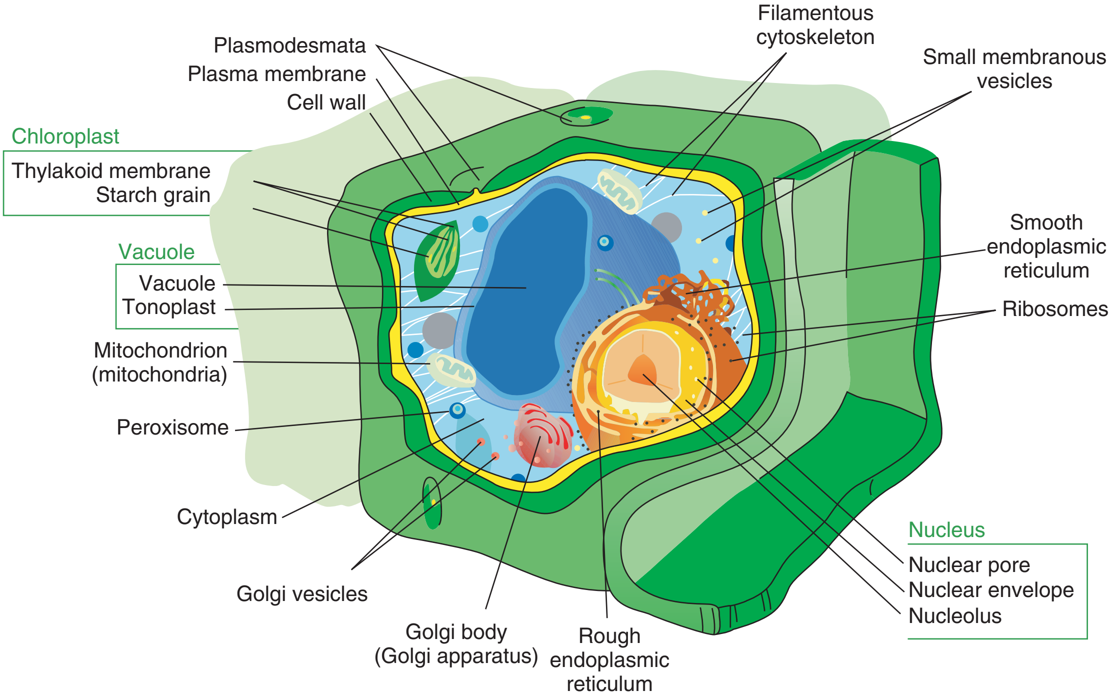
Plasmodesmata
Plasma membrane
Cell wall
Chloroplast
Thylakoid membrane
Starch grain
Vacuole
Vacuole
Tonoplast
Mitochondrion (mitochondria)
Peroxisome
Cytoplasm
Golgi vesicles
Golgi body (Golgi apparatus)
Rough endoplasmic reticulum
Filamentous cytoskeleton
Small membranous vesicles
Smooth endoplasmic reticulum
Ribosomes
Nucleus
Nuclear pore
Nuclear envelope
Nucleolus

Figure 2.1 Diagram of a plant cell.
Author: Mariana Ruiz. http://en.wikipedia.org/wiki/File:Plant_cell_structure_svg.svg

the sites of synthesis of cell wall polysaccharides; 'shuttle vesicles' are budded off from the Golgi bodies for transport of the polysaccharides to the cell wall. Vesicles budded off the ER and carrying proteins for export merge with the Golgi bodies, which then transfer the proteins to shuttle vesicles for further movement.

The cytosol also contains millions of *ribosomes* (section 2.11), some of them located on the surface of the ER (regions of ER with associated ribosomes are known as 'rough ER'). These particles, consisting of RNA and protein, are the sites of protein synthesis. Finally, there is the *cytoskeleton* (section 2.12), a network of microtubules (made of the protein tubulin) and actin filaments (consisting, as the name implies of the protein actin). Among other things, the cytoskeleton is involved in the organization of the plane of cell division, the orientation of cellulose microfibrils (section 2.2.2), the channelling of Golgi vesicles to the plasma membrane (section 2.2.2) and the organization and orientation of chromosomes during cell division (section 2.13).

2.2 Cell walls

2.2.1 General structural features

As was noted in Box 1.1 in Chapter 1, *cellulose* (Figure 2.2) is the most abundant organic compound in the world, because it is a major component of the cell walls of nearly all photosynthetic eukaryotes. In primary cell walls (see section 2.2.3), it makes up between 15 and 30 per cent of the dry mass of the wall. In secondary but unlignified walls (see below), the proportion is even greater.

However, cellulose is only one of several different types of molecule that make up the cell wall: the cellulose, organized as microfibrils (see below) is embedded in a matrix of other polysaccharides. In order to understand this, it is necessary to go back to the earliest phase in the deposition of the plant cell, namely synthesis of the new wall immediately after cell division.

The first cell wall that separates the two daughter cells after cell division (see section 2.13) is known as the *cell plate*. It can be seen in more mature cells as the *middle*

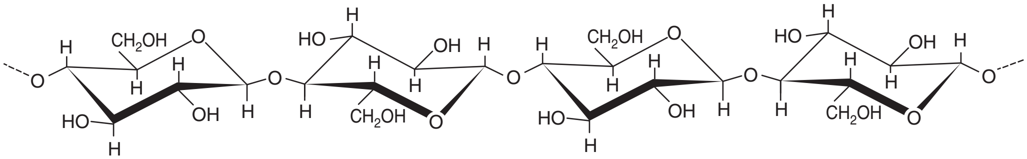

Figure 2.2 Structure of part of a cellulose molecule. Cellulose is a polymer of glucose units joined by β1→4 linkages. In the cell wall, the polymers are aligned in large parallel arrays called microfibrils.

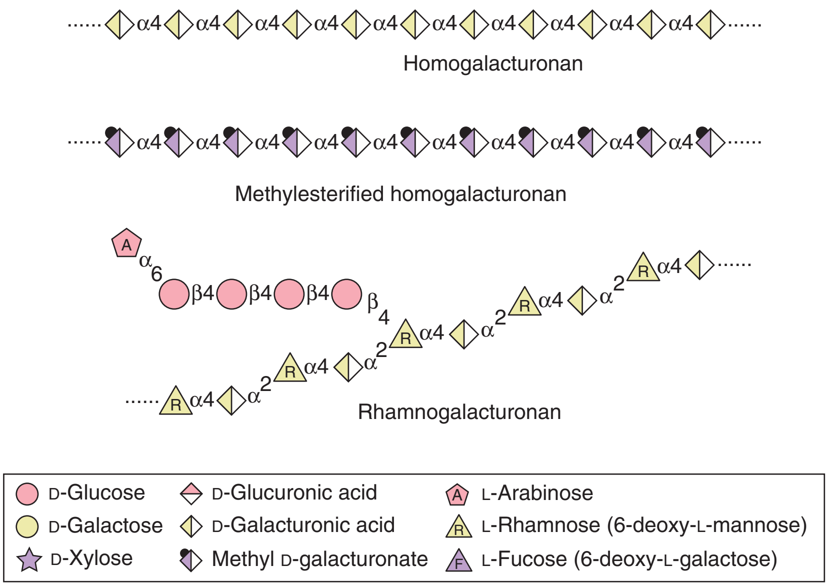
α4
Homogalacturonan
Methylesterified homogalacturonan
A
α6
β4
β4
R
α2
Rhamnogalacturonan
D-Glucose
D-Galactose
D-Xylose
D-Glucuronic acid
D-Galacturonic acid
Methyl D-galacturonate
L-Arabinose
L-Rhamnose (6-deoxy-L-mannose)
L-Fucose (6-deoxy-L-galactose)

Figure 2.3 Diagram showing structures of the main pectic polysaccharides.
Based on Figure 1 of Fry, SC *et al*. (2011)The Biochemist 33, 14–19. http://www.biochemist.org/bio/03302/0014/033020014.pdf

lamella between adjacent cells. The cell plate/middle lamella consists almost entirely of a group of polysaccharides known collectively as *pectins* (Figure 2.3). These are gel-forming polysaccharides (as is well-known by anyone who makes jam) made up of acidic sugars (especially galacturonic acid) and neutral sugars such as arabinose, galactose and rhamnose. Some of the polysaccharides are relatively simple, such as polygalacturonic acid, which is a polymer of α-D-galacturonic acid joined by 1→4 glycosidic linkages. It is often methyl-esterified (i.e. in the form of methyl-galacturonic acid), as shown in Figure 2.3.

Some molecules incorporate an occasional rhamnose residue which kinks the chain at that point. However, the bulk of pectic polysaccharides are more complex, as is typified by the rhamnogalacturonans – large polymers whose ‘backbone’ consists of alternating galacturonic acid and rhamnose residues. Particular regions of these molecules carry complex oligo/polysaccharide side-chains, which are joined to the backbone via the rhamnose units. The most abundant side chains are branched *arabinans* (oligosaccharides consisting of arabinose units), *galactans* and *arabinogalactans*. In the latter, the backbone of the side chain consists of galactose units, some of which themselves carry a short side chain of a single arabinose unit. The presence of side chains limits the extent to which individual polysaccharide chains can align with each other, and thus extensive branching makes for a very open structure. Conversely, adjacent pectin molecules may be cross-linked by Ca^{2+} ions bridging between two carboxyl groups. This bridging is inhibited if the carboxyl groups are esterified with a methyl group (see above).

Onto this middle lamella the primary wall is deposited, with cellulose now embedded into the background matrix of firstly pectins (as described above) and then hemicelluloses (see below and Figure 2.4). Cellulose is a β-glucan, a polymer of β-D-glucose units joined via 1→4 glycosidic linkages (Figure 2.2). The linear cellulose molecules, each consisting of several thousand individual glucose units, can hydrogen-bond with each other to make microfibrils comprising many molecules lying parallel to each other. These are the main strengthening components of the unlignified wall.

There are on average, 36 cellulose molecules at any one place in a microfibril, all lying in the same 'chemical orientation'. However, there are many more than 36 cellulose chains in a typical microfibril. Chains do not all start and finish in the same place, but instead overlap with each other. Thus an individual microfibril may contain several thousand individual cellulose chains and may be several hundred μm in length.

The presence of cellulose and its organization into microfibrils is a very important feature of plant cell walls, contributing very significantly to plant cell form and function. Indeed, Canadian plant scientists Luc Duchesne and Doug Larson suggest that: '*The presence of cellulose microfibrils in cell walls may be one of the most critical factors in the evolution of modern plant life.*'

As well as hydrogen-bonding with each other, cellulose molecules can form hydrogen bonds and other non-covalent linkages with the polymers that form the *hemicellulose* component of the matrix in which the microfibrils are embedded. The major polysaccharides of the hemicellulose fraction, some of which are shown in Figure 2.4, are *xylans* (polymers of xylose), *xyloglucans* (with a backbone of β-D-glucose units joined via 1→4 glycosidic linkages and carrying individual xylose molecules as side groups), *arabinoxylans* (polymers of xylose with arabinose side chains) *glucomannans* (mixed polymers of glucose and mannose units), *galactomannans* (β-D-mannose unit joined via 1→4 glycosidic linkages and carrying individual galactose units as side-groups) and mixed-linkage glucans (β-D glucose units joined by either 1→3 or 1→4 linkages). These polymers coat the cellulose microfibrils (Figure 2.5) and hydrogen-bond both with themselves and with the cellulose.

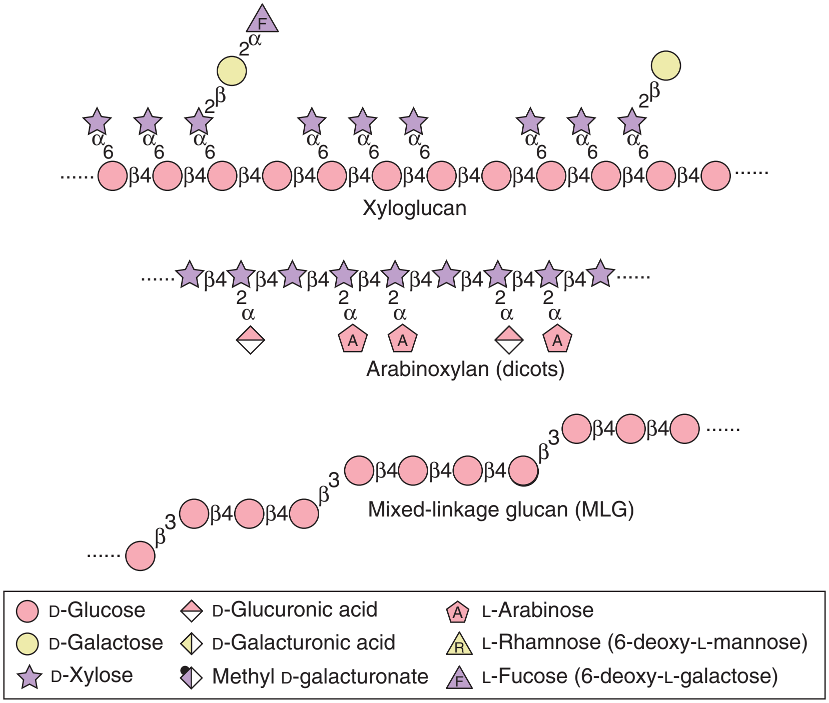

Figure 2.4 Diagram showing structures of some of the commoner polysaccharides in the hemicellulose fraction.
Based on Figure 1 of Fry, SC *et al*. (2011) The Biochemist 33, 14-19. http://www.biochemist.org/bio/03302/0014/033020014.pdf

In addition to polysaccharides, the cell wall also contains proteins. The protein component changes as the wall is built up from the middle lamella, but in general these proteins are rich in proline or hydroxyproline or glycine. Some of the hydroxyproline-rich proteins are glycosylated, i.e. they carry carbohydrate side-chains, consisting in this instance of arabinose and galactose. They are thus known as *arabinogalactan proteins* or AGPs.

The specific roles of all these proteins at different stages of plant cell development have not been established. However, it is likely that the hydoxyproline-rich **extensin** (which was the first to be discovered) is involved in cross-linking cellulose microfibrils after cell wall extension and that **expansins** actually participate in the expansion process (see section 2.2.3).

As well as these structural proteins, the cell wall also contains *enzymes*. They are mostly hydrolases of various types, possibly involved in defence and in the recycling or scavenging of nutrients. The enzymes involved in polymerization of lignin precursors are also located in the wall.

It must be noted that the proportion of these different polymers varies during the development of an individual cell (as already noted) and between species. Thus, the grasses, including the economically important cereals, have low proportions of pectic polysaccharides and of xyloglucans. The latter are replaced by *mixed-linkage glucans* (polymers of β-D-glucose units joined via either 1→4 or 1→3 glycosidic linkages).

The cell wall is often referred to as 'rigid'. However, this is not true of the primary cell wall. Cell walls are hydrated dynamic structures (the matrix component contains up to 75 per cent water).

As Stephen Fry and his colleagues at Edinburgh University put it so clearly:

> *'Although it is true that they are often strong (resisting breakage) and may be inextensible (resisting stretching and thus limiting cell expansion), most primary walls are highly flexible ... The phrase "rigid cell wall" should be expunged except in discussions of secondary walls.'*

2.2.2 Cell wall synthesis

Discussion of cell wall expansion is deferred until the next chapter. Here we briefly consider the synthesis of the cell wall polysaccharides. Cell wall polysaccharides are no exception to the general rule that the donors for building up polymers of monosaccharides are nucleotide-sugars, as shown in these general equations:

1. $\text{M-1-P} + \text{NTP} \rightarrow \text{NDP-M} + \text{PP}_i$
2. $\text{M}_n + \text{NDP-M} \rightarrow \text{M}_{n+1} + \text{NDP}$

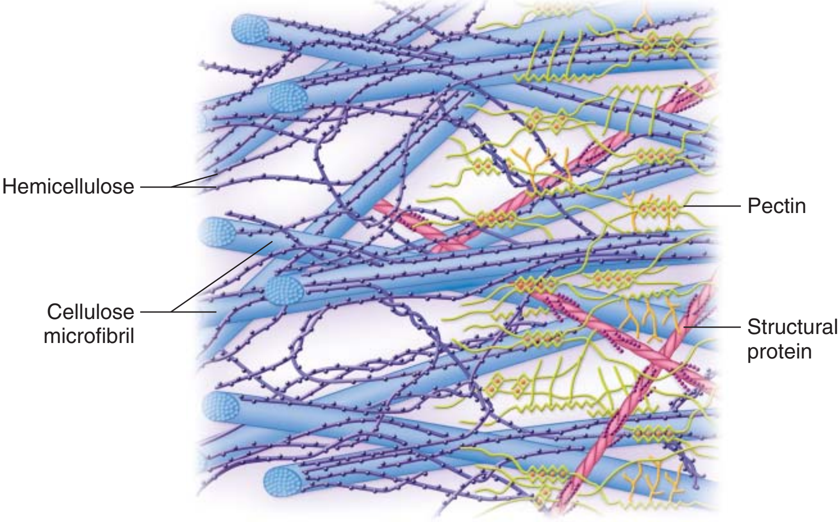

Figure 2.5 Cellulose in the cell wall. Cellulose microfibrils are embedded in a matrix of pectic polysaccharides and cross-linked (mainly via H-bonds) in a network of hemicelluloses polymers and of the arabino-galactan proteins, the extensins (the latter are shown in purple). From Buchanan, B. *et al*. (2002) Biochemistry and Molecular Biology of Plants. ASPB, Rockville, MD, p 81. http://www.aspb.org/publications/biotext/