Evolutionary and Cell Biology of Plants

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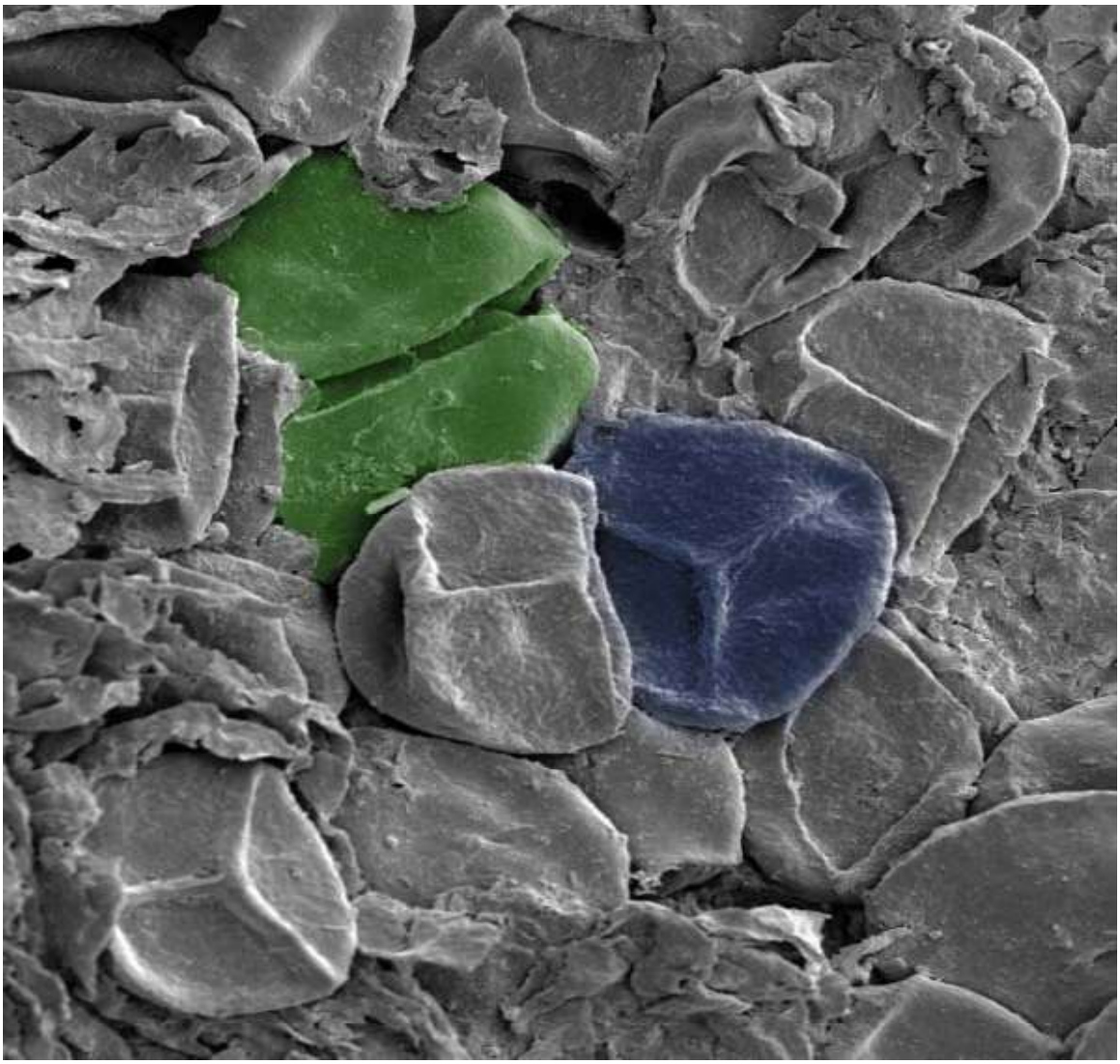
Evolutionary History of Plants



A late Silurian sporangium. **Green**: A spore tetrad. **Blue**: A spore bearing a trilete mark – the *Y*-shaped scar. The spores are about 30-35 μm across

The **evolution of plants** has resulted in increasing levels of complexity, from the earliest algal mats, through bryophytes, lycopods, ferns to the complex gymnosperms and angiosperms of today. While the groups which appeared earlier continue to thrive, especially in the environments in which they evolved, each new grade of organisation has eventually become more "successful" than its predecessors by most measures.

Evidence suggests that an algal scum formed on the land 1,200 million years ago, but it was not until the Ordovician Period, around 450 million years ago, that land plants appeared. These began to diversify in the late Silurian Period, around 420 million years ago, and the fruits of their diversification are displayed in remarkable detail in an early Devonian fossil assemblage from the Rhynie chert. This chert preserved early plants in cellular detail, petrified in volcanic springs. By the middle of the Devonian Period most of the features recognised in plants today are present, including roots, leaves and secondary wood, and by late Devonian times seeds had evolved. Late Devonian plants had thereby reached a degree of sophistication that allowed them to form forests of tall trees. Evolutionary innovation continued after the Devonian period. Most plant groups were relatively unscathed by the Permo-Triassic extinction event, although the structures of communities changed. This may have set the scene for the evolution of flowering plants in the Triassic (~200 million years ago), which exploded in the Cretaceous and Tertiary. The latest major group of plants to evolve were the grasses, which became important in the mid Tertiary, from around 40 million years ago. The grasses, as well as many other groups, evolved new mechanisms of metabolism to survive the low CO_2 and warm, dry conditions of the tropics over the last 10 million years.

Colonisation of land

The Devonian period marks the beginning of extensive land colonization by plants, which through their effects on erosion and sedimentation brought about significant climatic change.

Land plants evolved from chlorophyte algae, perhaps as early as 510 million years ago; their closest living relatives are the charophytes, specifically Charales. Assuming that the Charales' habit has changed little since the divergence of lineages, this means that the land plants evolved from a branched, filamentous, haplontic alga, dwelling in shallow fresh water, perhaps at the edge of seasonally desiccating pools. Co-operative interactions with fungi may have helped early plants adapt to the stresses of the terrestrial realm.

Plants were not the first photosynthesisers on land, though: consideration of weathering rates suggests that organisms were already living on the land 1,200 million years ago, and microbial fossils have been found in freshwater lake deposits from 1,000 million years ago, but the carbon isotope record suggests that they were too scarce to impact the atmospheric composition until around 850 million years ago. These organisms were probably small and simple, forming little more than an "algal scum".

The first evidence of plants on land comes from spores of Mid-Ordovician age (early Llanvirn, ~470 million years ago). These spores, known as cryptospores, were produced either singly (monads), in pairs (diade) or groups of four (tetrads), and their

microstructure resembles that of modern liverwort spores, suggesting they share an equivalent grade of organisation. It could be that atmospheric 'poisoning' prevented eukaryotes from colonising the land prior to this, or it could simply have taken a great time for the necessary complexity to evolve.

Trilete spores similar to those of vascular plants appear soon afterwards, in Upper Ordovician rocks. Depending exactly when the tetrad splits, each of the four spores may bear a "trilete mark", a *Y*-shape, reflecting the points at which each cell was squashed up against its neighbours. However, in order for this to happen, the spore walls must be sturdy and resistant at an early stage. This resistance is closely associated with having a desiccation-resistant outer wall – a trait only of use when spores have to survive out of water. Indeed, even those embryophytes that have returned to the water lack a resistant wall, thus don't bear trilete marks. A close examination of algal spores shows that none have trilete spores, either because their walls are not resistant enough, or in those rare cases where it is, the spores disperse before they are squashed enough to develop the mark, or don't fit into a tetrahedral tetrad.

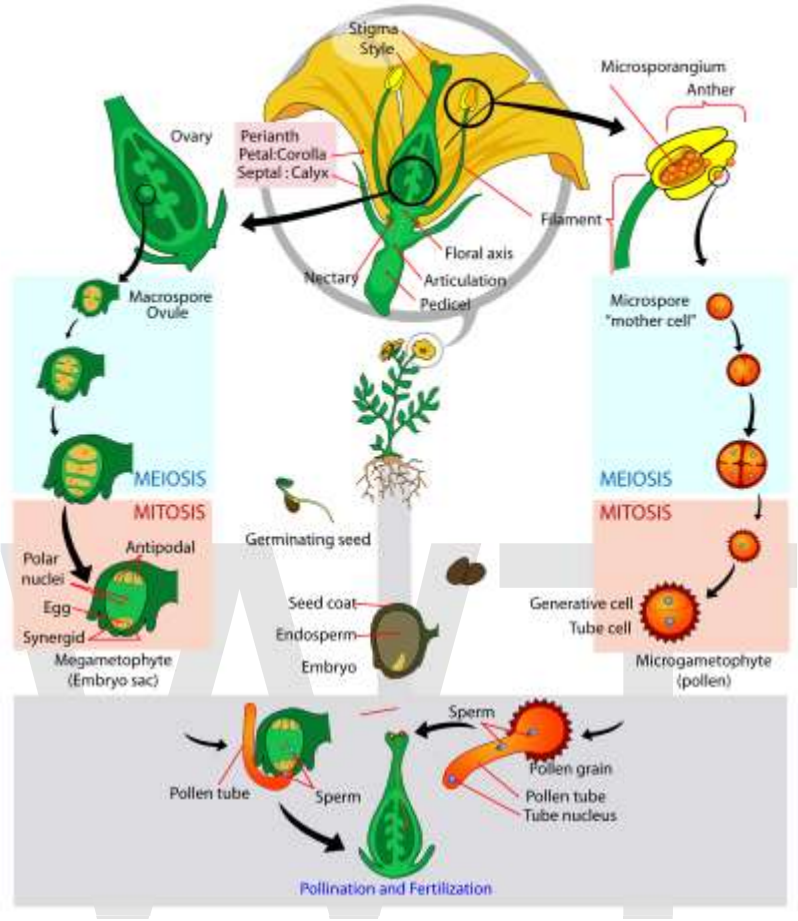
The earliest megafossils of land plants were thalloid organisms, which dwelt in fluvial wetlands and are found to have covered most of an early Silurian flood plain. They could only survive when the land was waterlogged.

Once plants had reached the land, there were two approaches to dealing with desiccation. The bryophytes avoid it or give in to it, restricting their ranges to moist settings, or drying out and putting their metabolism "on hold" until more water arrives. Tracheophytes resist desiccation. They all bear a waterproof outer cuticle layer wherever they are exposed to air (as do some bryophytes), to reduce water loss – but since a total covering would cut them off from CO_2 in the atmosphere, they rapidly evolved stomata – small openings to allow gas exchange. Tracheophytes also developed vascular tissue to aid in the movement of water within the organisms (see below), and moved away from a gametophyte dominated life cycle (see below). Vascular tissue also facilitated upright growth without the support of water and paved the way for the evolution of larger plants on land.

The establishment of a land-based flora permitted the accumulation of oxygen in the atmosphere as never before, as the new hordes of land plants pumped it out as a waste product. When this concentration rose above 13%, it permitted the possibility of wildfire. This is first recorded in the early Silurian fossil record by charcoalified plant fossils. Apart from a controversial gap in the Late Devonian, charcoal is present ever since.

Charcoalification is an important taphonomic mode. Wildfire drives off the volatile compounds, leaving only a shell of pure carbon. This is not a viable food source for herbivores or detritovores, so is prone to preservation; it is also robust, so can withstand pressure and display exquisite, sometimes sub-cellular, detail.

Changing life cycles



Angiosperm life cycle

All multicellular plants have a life cycle comprising two generations or phases. One is termed the **gametophyte**, has a single set of chromosomes (denoted **1N**), and produces gametes (sperm and eggs). The other is termed the **sporophyte**, has paired chromosomes (denoted **2N**), and produces spores. The gametophyte and sporophyte may appear identical – homomorphy – or may be very different – heteromorphy.

The pattern in plant evolution has been a shift from homomorphy to heteromorphy. The algal ancestors to land plants were almost certainly haplobiontic, being haploid for all their life cycles, with a unicellular zygote providing the 2N stage. All land plants (i.e. embryophytes) are diplobiontic – that is, both the haploid and diploid stages are multicellular. Two trends are apparent: bryophytes (liverworts, mosses and hornworts) have developed the gametophyte, with the sporophyte becoming almost entirely dependent on it; vascular plants have developed the sporophyte, with the gametophyte being particularly reduced in the seed plants.

There are two competing theories to explain the appearance of a diplobiontic lifecycle.

The **interpolation theory** (also known as the antithetic or intercalary theory) holds that the sporophyte phase was a fundamentally new invention, caused by the mitotic division of a freshly germinated zygote, continuing until meiosis produces spores. This theory implies that the first sporophytes would bear a very different morphology to the gametophyte, on which they would have been dependent. This seems to fit well with what we know of the bryophytes, in which a vegetative thalloid gametophyte is parasitised by simple sporophytes, which often comprise no more than a sporangium on a stalk. Increasing complexity of the ancestrally simple sporophyte, including the eventual acquisition of photosynthetic cells, would free it from its dependence on a gametophyte, as we see in some hornworts (*Anthoceros*), and eventually result in the sporophyte developing organs and vascular tissue, and becoming the dominant phase, as in the tracheophytes (vascular plants). This theory may be supported by observations that smaller *Cooksonia* individuals must have been supported by a gametophyte generation. The observed appearance of larger axial sizes, with room for photosynthetic tissue and thus self-sustainability, provides a possible route for the development of a self-sufficient sporophyte phase.

The alternative hypothesis is termed the **transformation theory** (or homologous theory). This posits that the sporophyte appeared suddenly by a delay in the occurrence of meiosis after the zygote germinated. Since the same genetic material would be employed, the haploid and diploid phases would look the same. This explains the behaviour of some algae, which produce alternating phases of identical sporophytes and gametophytes. Subsequent adaption to the desiccating land environment, which makes sexual reproduction difficult, would result in the simplification of the sexually active gametophyte, and elaboration of the sporophyte phase to better disperse the waterproof spores. The tissue of sporophytes and gametophytes preserved in the Rhynie chert is of similar complexity, which is taken to support this hypothesis.

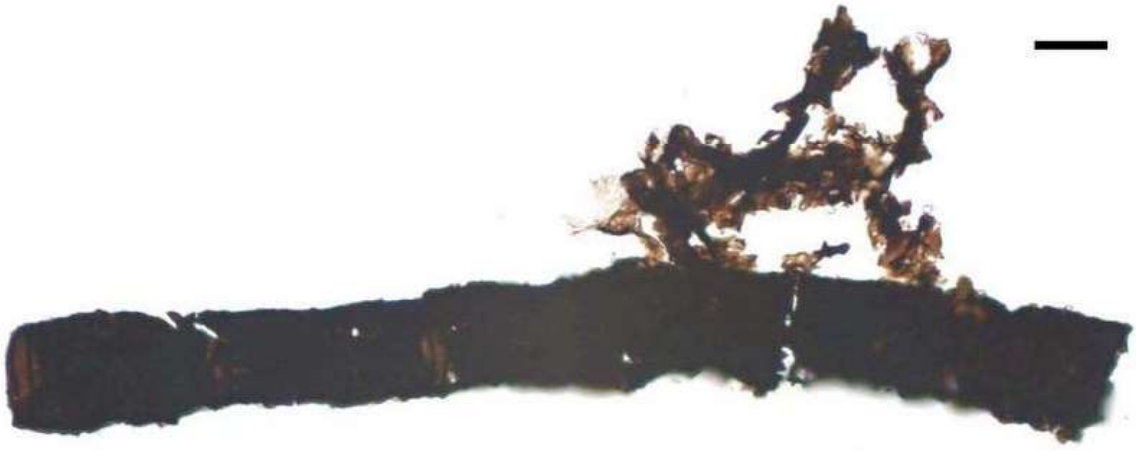
Water transport

In order to photosynthesise, plants must uptake CO_2 from the atmosphere. However, this comes at a price: while stomata are open to allow CO_2 to enter, water can evaporate. Water is lost much faster than CO_2 is absorbed, so plants need to replace it, and have developed systems to transport water from the moist soil to the site of photosynthesis. Early plants sucked water between the walls of their cells, then evolved the ability to control water loss (and CO_2 acquisition) through the use of stomata. Specialised water transport tissues soon evolved in the form of hydroids, tracheids, then secondary xylem, followed by an endodermis and ultimately vessels.

The high CO_2 levels of Silurian-Devonian times, when plants were first colonising land, meant that the need for water was relatively low . As CO_2 was withdrawn from the atmosphere by plants, more water was lost in its capture, and more elegant transport mechanisms evolved. As water transport mechanisms, and waterproof cuticles, evolved, plants could survive without being continually covered by a film of water. This transition from poikilohydry to homoiohydry opened up new potential for colonisation. Plants were

then faced with a balance, between transporting water as efficiently as possible and preventing transporting vessels to implode and cavitate.

During the Silurian, CO_2 was readily available, so little water needed expending to acquire it. By the end of the Carboniferous, when CO_2 levels had lowered to something approaching today's, around 17 times more water was lost per unit of CO_2 uptake. However, even in these "easy" early days, water was at a premium, and had to be transported to parts of the plant from the wet soil to avoid desiccation. This early water transport took advantage of the **cohesion-tension** mechanism inherent in water. Water has a tendency to diffuse to areas that are drier, and this process is accelerated when water can be wicked along a fabric with small spaces. In small passages, such as that between the plant cell walls (or in tracheids), a column of water behaves like rubber – when molecules evaporate from one end, they literally pull the molecules behind them along the channels. Therefore transpiration alone provided the driving force for water transport in early plants. However, without dedicated transport vessels, the cohesion-tension mechanism cannot transport water more than about 2 cm, severely limiting the size of the earliest plants. This process demands a steady supply of water from one end, to maintain the chains; to avoid exhausing it, plants developed a waterproof cuticle. Early cuticle may not have had pores but did not cover the entire plant surface, so that gas exchange could continue. However, dehydration at times was inevitable; early plants cope with this by having a lot of water stored between their cell walls, and when it comes to it sticking out the tough times by putting life "on hold" until more water is supplied.



A banded tube from the late Silurian/early Devonian. The bands are difficult to see on this specimen, as an opaque carbonaceous coating conceals much of the tube.

In order to be free from the constraints of small size and constant moisture that the parenchymatic transport system inflicted, plants needed a more efficient water transport system. During the early Silurian, they developed specialized cells, which were lignified (or bore similar chemical compounds) to avoid implosion; this process coincided with cell death, allowing their innards to be emptied and water to be passed through them. These wider, dead, empty cells were a million times more conductive than the inter-cell

method, giving the potential for transport over longer distances, and higher CO_2 diffusion rates.

The first macrofossils to bear water-transport tubes *in situ* are the early Devonian pretracheophytes *Aglaophyton* and *Horneophyton*, which have structures very similar to the **hydroids** of modern mosses. Plants continued to innovate new ways of reducing the resistance to flow within their cells, thereby increasing the efficiency of their water transport. Bands on the walls of tubes, in fact apparent from the early Silurian onwards, are an early improvisation to aid the easy flow of water. Banded tubes, as well as tubes with pitted ornamentation on their walls, were lignified and, when they form single celled conduits, are considered to be **tracheids**. These, the "next generation" of transport cell design, have a more rigid structure than hydroids, allowing them to cope with higher levels of water pressure. Tracheids may have a single evolutionary origin, possibly within the hornworts, uniting all tracheophytes (but they may have evolved more than once).

Water transport requires regulation, and dynamic control is provided by stomata. By adjusting the amount of gas exchange, they can restrict the amount of water lost through transpiration. This is an important role where water supply is not constant, and indeed stomata appear to have evolved before tracheids, being present in the non-vascular hornworts.

An endodermis probably evolved during the Silu-Devonian, but the first fossil evidence for such a structure is Carboniferous. This structure in the roots covers the water transport tissue and regulates ion exchange (and prevents unwanted pathogens etc. from entering the water transport system). The endodermis can also provide an upwards pressure, forcing water out of the roots when transpiration is not enough of a driver.

Once plants had evolved this level of controlled water transport, they were truly homoiohydric, able to extract water from their environment through root-like organs rather than relying on a film of surface moisture, enabling them to grow to much greater size. As a result of their independence from their surroundings, they lost their ability to survive desiccation – a costly trait to retain.

During the Devonian, maximum xylem diameter increased with time, with the minimum diameter remaining pretty constant. By the middle Devonian, the tracheid diameter of some plant lineages had plateaued. Wider tracheids allow water to be transported faster, but the overall transport rate depends also on the overall cross-sectional area of the xylem bundle itself. The increase in vascular bundle thickness further seems to correlate with the width of plant axes, and plant height; it is also closely related to the appearance of leaves and increased stomatal density, both of which would increase the demand for water.

While wider tracheids with robust walls make it possible to achieve higher water transport pressures, this increases the problem of cavitation. Cavitation occurs when a bubble of air forms within a vessel, breaking the bonds between chains of water molecules and preventing them from pulling more water up with their cohesive tension.

A tracheid, once cavitated, cannot have its embolism removed and return to service (except in a few advanced angiosperms which have developed a mechanism of doing so). Therefore it is well worth plants' while to avoid cavitation occurring. For this reason, pits in tracheid walls have very small diameters, to prevent air entering and allowing bubbles to nucleate. Freeze-thaw cycles are a major cause of cavitation. Damage to a tracheid's wall almost inevitably leads to air leaking in and cavitation, hence the importance of many tracheids working in parallel.

Cavitation is hard to avoid, but once it has occurred plants have a range of mechanisms to contain the damage. Small pits link adjacent conduits to allow fluid to flow between them, but not air – although ironically these pits, which prevent the spread of embolisms, are also a major cause of them. These pitted surfaces further reduce the flow of water through the xylem by as much as 30%. Conifers, by the Jurassic, developed an ingenious improvement, using valve-like structures to isolate cavitated elements. These torus-margo structures have a blob floating in the middle of a donut; when one side depressurises the blob is sucked into the torus and blocks further flow. Other plants simply accept cavitation; for instance, oaks grow a ring of wide vessels at the start of each spring, none of which survive the winter frosts. Maples use root pressure each spring to force sap upwards from the roots, squeezing out any air bubbles.

Growing to height also employed another trait of tracheids – the support offered by their lignified walls. Defunct tracheids were retained to form a strong, woody stem, produced in most instances by a secondary xylem. However, in early plants, tracheids were too mechanically vulnerable, and retained a central position, with a layer of tough sclerenchyma on the outer rim of the stems. Even when tracheids do take a structural role, they are supported by sclerenchymatic tissue.

Tracheids end with walls, which impose a great deal of resistance on flow; vessel members have perforated end walls, and are arranged in series to operate as if they were one continuous vessel. The function of end walls, which were the default state in the Devonian, was probably to avoid embolisms. An embolism is where an air bubble is created in a tracheid. This may happen as a result of freezing, or by gases dissolving out of solution. Once an embolism is formed, it usually cannot be removed; the affected cell cannot pull water up, and is rendered useless.

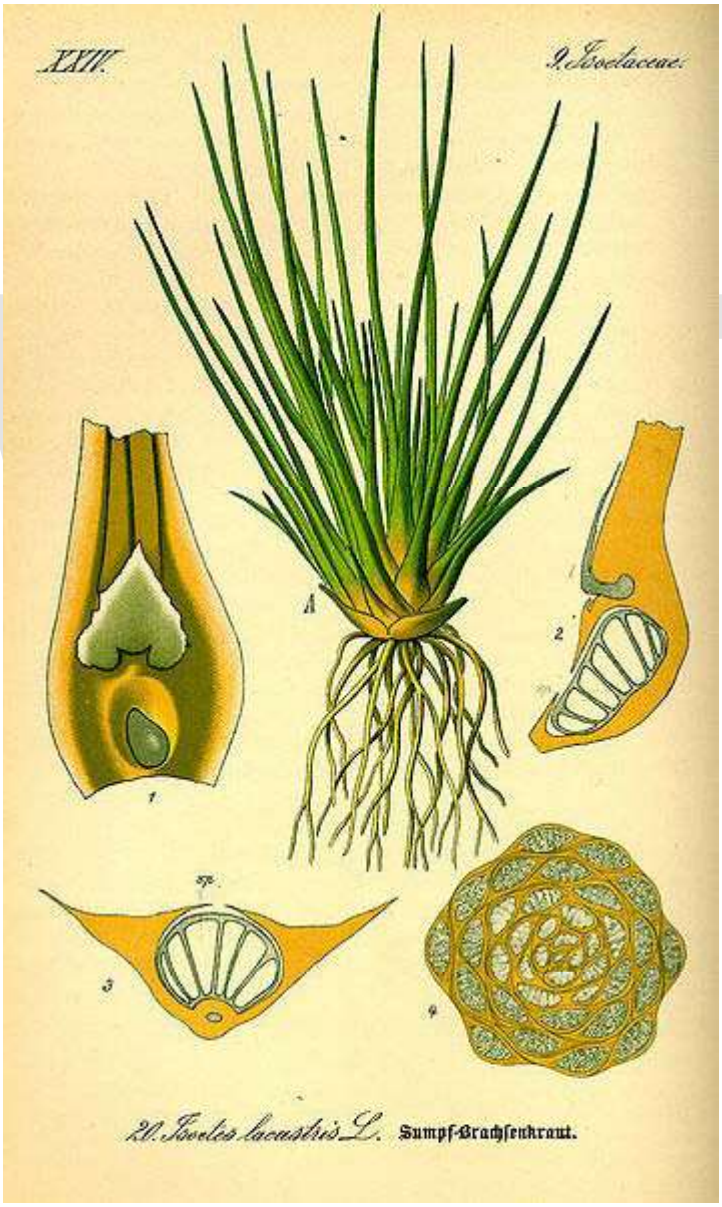
End walls excluded, the tracheids of prevascular plants were able to operate under the same hydraulic conductivity as those of the first vascular plant, *Cooksonia*.

The size of tracheids is limited as they comprise a single cell; this limits their length, which in turn limits their maximum useful diameter to 80 μm. Conductivity grows with the fourth power of diameter, so increased diameter has huge rewards; **vessel elements**, consisting of a number of cells, joined at their ends, overcame this limit and allowed larger tubes to form, reaching diameters of up to 500 μm, and lengths of up to 10 m.

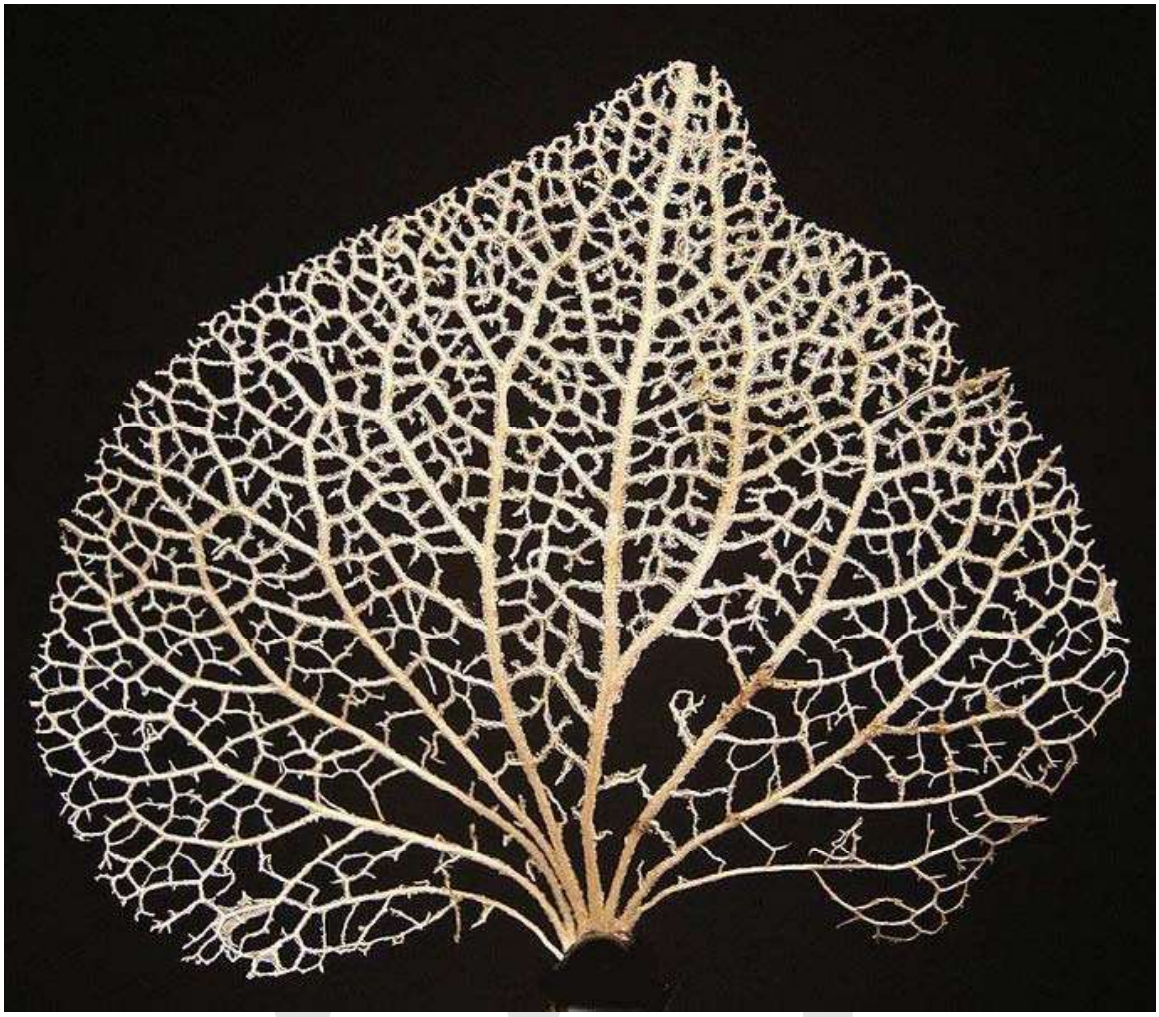
Vessels first evolved during the dry, low CO_2 periods of the late Permian, in the horsetails, ferns and Selaginellales independently, and later appeared in the mid

Cretaceous in angiosperms and gnetophytes. Vessels allow the same cross-sectional area of wood to transport around a hundred times more water than tracheids! This allowed plants to fill more of their stems with structural fibres, and also opened a new niche to vines, which could transport water without being as thick as the tree they grew on. Despite these advantages, tracheid-based wood is a lot lighter, thus cheaper to make, as vessels need to be much more reinforced to avoid cavitation.

Evolution of leaves



The lycopod *Isoetes* bears microphylls with a single vascular trace.



The branching pattern of megaphyll veins may belie their origin as webbed, dichotomising branches.

Leaves today are, in almost all instances, an adaptation to increase the amount of sunlight that can be captured for photosynthesis. Leaves certainly evolved more than once, and probably originated as spiny outgrowths to protect early plants from herbivory.

The rhyniophytes of the Rhynie chert comprised nothing more than slender, unornamented axes. The early to middle Devonian trimerophytes, therefore, are the first evidence we have of anything that could be considered leafy. This group of vascular plants are recognisable by their masses of terminal sporangia, which adorn the ends of axes which may bifurcate or trifurcate. Some organisms, such as *Psilophyton*, bore enations. These are small, spiny outgrowths of the stem, lacking their own vascular supply.

Around the same time, the zosterophyllophytes were becoming important. This group is recognisable by their kidney-shaped sporangia, which grew on short lateral branches close to the main axes. They sometimes branched in a distinctive H-shape. The majority

of this group bore pronounced spines on their axes. However, none of these had a vascular trace, and the first evidence of vascularised enations occurs in the Rhynie genus *Asteroxylon*. The spines of *Asteroxylon* had a primitive vasuclar supply – at the very least, leaf traces could be seen departing from the central protostele towards each individual "leaf". A fossil known as *Baragwanathia* appears in the fossil record slightly earlier, in the late Silurian. In this organism, these leaf traces continue into the leaf to form their mid-vein. One theory, the "enation theory", holds that the leaves developed by outgrowths of the protostele connecting with existing enations, but it is also possible that microphylls evolved by a branching axis forming "webbing".

Asteroxylon and *Baragwanathia* are widely regarded as primitive lycopods. The lycopods are still extant today, familiar as the quillwort *Isoetes* and the club mosses. Lycopods bear distinctive microphylls – leaves with a single vascular trace. Microphylls could grow to some size – the Lepidodendrales boasted microphylls over a meter in length – but almost all just bear the one vascular bundle. (An exception is the branching *Selaginella*).

The more familiar leaves, megaphylls, are thought to have separate origins – indeed, they appeared four times independently, in the ferns, horsetails, progymnosperms, and seed plants. They appear to have originated from dichotomising branches, which first overlapped (or "overtopped") one another, and eventually developed "webbing" and evolved into gradually more leaf-like structures. So megaphylls, by this "teleome theory", are composed of a group of webbed branches – hence the "leaf gap" left where the leaf's vascular bundle leaves that of the main branch resembles two axes splitting. In each of the four groups to evolve megaphylls, their leaves first evolved during the late Devonian to early Carboniferous, diversifying rapidly until the designs settled down in the mid Carboniferous.

The cessation of further diversification can be attributed to developmental constraints, but why did it take so long for leaves to evolve in the first place? Plants had been on the land for at least 50 million years before megaphylls became significant. However, small, rare mesophylls are known from the early Devonian genus *Eophyllophyton* – so development could not have been a barrier to their appearance. The best explanation so far incorporates observations that atmospheric CO_2 was declining rapidly during this time – falling by around 90% during the Devonian. This corresponded with an increase in stomatal density by 100 times. Stomata allow water to evaporate from leaves, which causes them to curve. It appears that the low stomatal density in the early Devonian meant that evaporation was limited, and leaves would overheat if they grew to any size. The stomatal density could not increase, as the primitive steles and limited root systems would not be able to supply water quickly enough to match the rate of transpiration.

Clearly, leaves are not always beneficial, as illustrated by the frequent occurrence of secondary loss of leaves, famously exemplified by cacti and the "whisk fern" *Psilotum*.

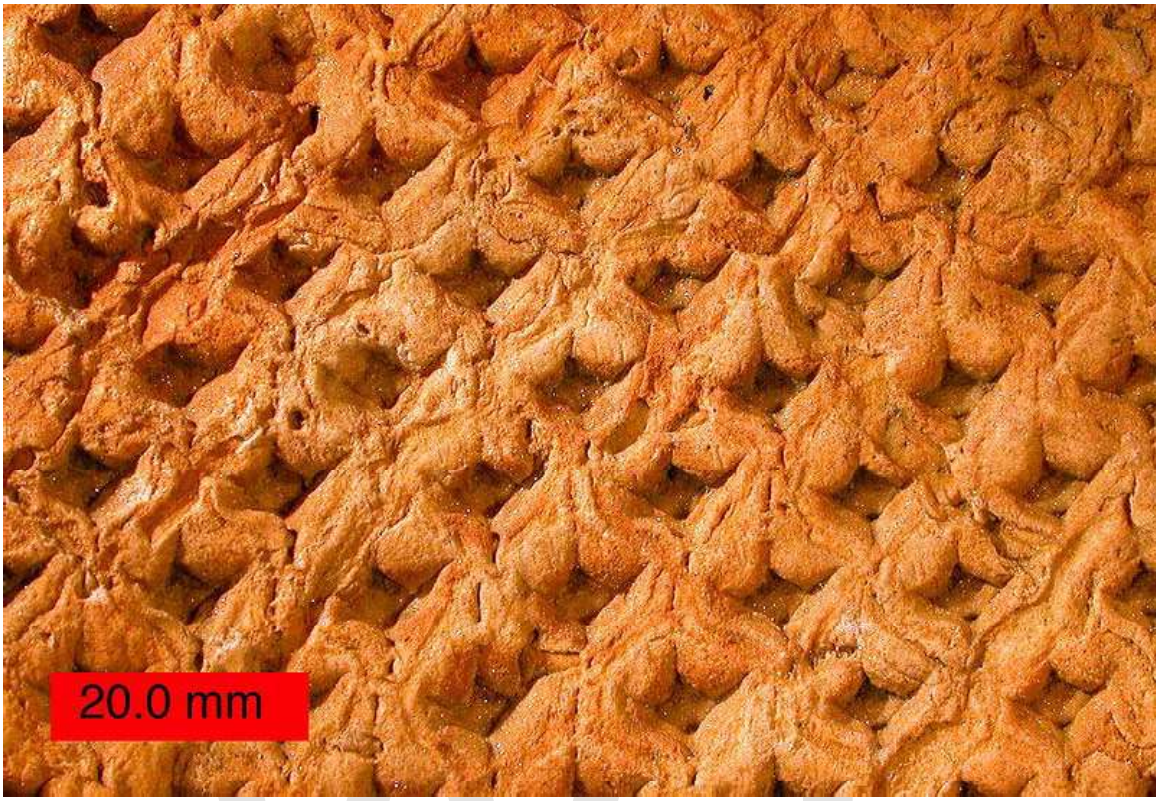
Secondary evolution can also disguise the true evolutionary origin of some leaves. Some genera of ferns display complex leaves which are attached to the pseudostele by an outgrowth of the vascular bundle, leaving no leaf gap. Further, horsetail (*Equisetum*)

leaves bear only a single vein, and appear for all the world to be microphyllous; however, in the light of the fossil record and molecular evidence, we conclude that their forbears bore leaves with complex venation, and the current state is a result of secondary simplification.

Deciduous trees deal with another disadvantage to having leaves. The popular belief that plants shed their leaves when the days get too short is misguided; evergreens prospered in the Arctic circle during the most recent greenhouse earth. The generally accepted reason for shedding leaves during winter is to cope with the weather – the force of wind and weight of snow are much more comfortably weathered without leaves to increase surface area. Seasonal leaf loss has evolved independently several times and is exhibited in the ginkgoales, pinophyta and angiosperms. Leaf loss may also have arisen as a response to pressure from insects; it may have been less costly to lose leaves entirely during the winter or dry season than to continue investing resources in their repair.

Evolution of trees

The trunk of early tree fern *Psaronius*, showing internal structure. The top of the plant would have been to the left of the image



External mold of *Lepidodendron* trunk showing leaf scars from the Upper Carboniferous of Ohio

The early Devonian landscape was devoid of vegetation taller than waist height. Without the evolution of a robust vascular system, taller heights could not be attained. There was, however, a constant evolutionary pressure to attain greater height. The most obvious advantage is the harvesting of more sunlight for photosynthesis – by overshadowing competitors – but a further advantage is present in spore distribution, as spores (and, later, seeds) can be blown greater distances if they start higher. This may be demonstrated by *Prototaxites*, thought to be a late Silurian fungus reaching eight metres in height.

In order to attain arborescence, early plants needed to develop woody tissue that would act as both support and water transport. To understand wood, we must know a little of vascular behaviour. The stele of plants undergoing "secondary growth" is surrounded by the vascular cambium, a ring of cells which produces more xylem (on the inside) and phloem (on the outside). Since xylem cells comprise dead, lignified tissue, subsequent rings of xylem are added to those already present, forming wood.

The first plants to develop this secondary growth, and a woody habit, were apparently the ferns, and as early as the middle Devonian one species, *Wattieza*, had already reached heights of 8 m and a tree-like habit.

Other clades did not take long to develop a tree-like stature; the late Devonian *Archaeopteris*, a precursor to gymnosperms which evolved from the trimerophytes,

reached 30 m in height. These progymnosperms were the first plants to develop true wood, grown from a bifacial cambium, of which the first appearance is in the mid Devonian *Rellimia*. True wood is only thought to have evolved once, giving rise to the concept of a "lignophyte" clade.

These *Archaeopteris* forests were soon supplemented by lycopods, in the form of lepidodendrales, which topped 50m in height and 2m across at the base. These lycopods rose to dominate late Devonian and Carboniferous coal deposits. Lepidodendrales differ from modern trees in exhibiting determinate growth: after building up a reserve of nutrients at a low height, the plants would "bolt" to a genetically determined height, branch at that level, spread their spores and die. They consisted of "cheap" wood to allow their rapid growth, with at least half of their stems comprising a pith-filled cavity. Their wood was also generated by a unifacial vascular cambium – it did not produce new phloem, meaning that the trunks could not grow wider over time.

The horsetail *Calamites* was next on the scene, appearing in the Carboniferous. Unlike the modern horsetail *Equisetum*, *Calamites* had a unifacial vascular cambium, allowing them to develop wood and grow to heights in excess of 10 m. They also branched multiple times.

While the form of early trees was similar to that of today's, the groups containing all modern trees had yet to evolve.

The dominant groups today are the gymnosperms, which include the coniferous trees, and the angiosperms, which contain all fruiting and flowering trees. It was long thought that the angiosperms arose from within the gymnosperms, but recent molecular evidence suggests that their living representatives form two distinct groups. It must be noted that the molecular data has yet to be fully reconciled with morphological data, but it is becoming accepted that the morphological support for paraphyly is not especially strong. This would lead to the conclusion that both groups arose from within the pteridosperms, probably as early as the Permian.

The angiosperms and their ancestors played a very small role until they diversified during the Cretaceous. They started out as small, damp-loving organisms in the understory, and have been diversifying ever since the mid-Cretaceous, to become the dominant member of non-boreal forests today.

Evolution of roots

The roots (bottom image) of lepidodendrales are thought to be functionally equivalent to the stems (top), as the similar appearance of "leaf scars" and "root scars" on these specimens from different species demonstrates.

Roots are important to plants for two main reasons: Firstly, they provide anchorage to the substrate; more importantly, they provide a source of water and nutrients from the soil. Roots allowed plants to grow taller and faster.

The onset of roots also had effects on a global scale. By disturbing the soil, and promoting its acidification (by taking up nutrients such as nitrate and phosphate), they enabled it to weather more deeply, promoting the draw-down of CO_2 with huge implications for climate. These effects may have been so profound they led to a mass extinction.

But how and when did roots evolve in the first place? While there are traces of root-like impressions in fossil soils in the late Silurian, body fossils show the earliest plants to be devoid of roots. Many had tendrils which sprawled along or beneath the ground, with upright axes or thalli dotted here and there, and some even had non-photosynthetic subterranean branches which lacked stomata. The distinction between root and specialised branch is developmental; true roots follow a different developmental trajectory to stems. Further, roots differ in their branching pattern, and in possession of a root cap. So while Silu-Devonian plants such as *Rhynia* and *Horneophyton* possessed the physiological equivalent of roots, roots – defined as organs differentiated from stems – did not arrive until later. Unfortunately, roots are rarely preserved in the fossil record, and our understanding of their evolutionary origin is sparse.

Rhizoids – small structures performing the same role as roots, usually a cell in diameter – probably evolved very early, perhaps even before plants colonised the land; they are recognised in the Characeae, an algal sister group to land plants. That said, rhizoids probably evolved more than once; the rhizines of lichens, for example, perform a similar role. Even some animals (*Lamellibrachia*) have root-like structures!

More advanced structures are common in the Rhynie chert, and many other fossils of comparable early Devonian age bear structures that look like, and acted like, roots. The rhyniophytes bore fine rhizoids, and the trimerophytes and herbaceous lycopods of the chert bore root-like structure penetrating a few centimetres into the soil. However, none of these fossils display all the features borne by modern roots. Roots and root-like structures became increasingly more common and deeper penetrating during the Devonian period, with lycopod trees forming roots around 20 cm long during the Eifelian and Givetian. These were joined by progymnosperms, which rooted up to about a metre deep, during the ensuing Frasnian stage. True gymnosperms and zygopterid ferns also formed shallow rooting systems during the Famennian period.

The rhizomorphs of the lycopods provide a slightly approach to rooting. They were equivalent to stems, with organs equivalent to leaves performing the role of rootlets. A similar construction is observed in the extant lycopod *Isoetes*, and this appears to be evidence that roots evolved independently at least twice, in the lycophytes and other plants.

A vascular system is indispensable to a rooted plants, as non-photosynthesising roots need a supply of sugars, and a vascular system is required to transport water and nutrients from the roots to the rest of the plant. These plants are little more advanced than their Silurian forbears, without a dedicated root system; however, the flat-lying axes can be clearly seen to have growths similar to the rhizoids of bryophytes today.

By the mid-to-late Devonian, most groups of plants had independently developed a rooting system of some nature. As roots became larger, they could support larger trees, and the soil was weathered to a greater depth. This deeper weathering had effects not only on the aforementioned drawdown of CO_2, but also opened up new habitats for colonisation by fungi and animals.

Roots today have developed to the physical limits. They penetrate many metres of soil to tap the water table. The narrowest roots are a mere 40 μm in diameter, and could not physically transport water if they were any narrower. The earliest fossil roots recovered, by contrast, narrowed from 3 mm to under 700 μm in diameter; of course, taphonomy is the ultimate control of what thickness we can see.

Arbuscular mycorrhizae

The efficiency of many plants' roots is increased via a symbiotic relationship with a fungal partner. The most common are arbuscular mycorrhizae (AM), literally "tree-like fungal roots". These comprise fungi which invade some root cells, filling the cell membrane with their hyphae. They feed on the plant's sugars, but return nutrients generated or extracted from the soil (especially phosphate), which the plant would otherwise have no access to.

This symbiosis appears to have evolved early in plant history. AM are found in all plant groups, and 80% of extant vascular plants, suggesting an early ancestry; a "plant"-fungus symbiosis may even have been the step that enabled them to colonise the land, and indeed AM are abundant in the Rhynie chert; the association occurred even before there were true roots to colonise, and is has even been suggested that roots evolved in order to provide a more comfortable habitat for mycorrhizal fungi.

Evolution of seeds

The fossil seed *Trigonocarpus*

Early land plants reproduced in the fashion of ferns: spores germinated into small gametophytes, which produced sperm. These would swim across moist soils to find the female organs (archegonia) on the same or another gametophyte, where they would fuse with an ovule to produce an embryo, which would germinate into a sporophyte.

This mode of reproduction restricted early plants to damp environments, moist enough that the sperm could swim to their destination. Therefore, early land plants were constrained to the lowlands, near shores and streams. The development of heterospory freed them from this constraint.

Heterosporic organisms, as their name suggests, bear spores of two sizes – microspores and megaspores. These would germinate to form microgametophytes and megagametophytes, respectively. This system paved the way for seeds: taken to the extreme, the megasporangia could bear only a single megaspore tetrad, and to complete the transition to true seeds, three of the megaspores in the original tetrad could be aborted, leaving one megaspore per megasporangium.

The transition to seeds continued with this megaspore being "boxed in" to its sporangium while it germinates. Then, the megagametophyte is contained within a waterproof integument, which forms the bulk of the seed. The microgametophyte – a pollen grain which has germinated from a microspore – is employed for dispersal, only releasing its desiccation-prone sperm when it reaches a receptive megagametophyte.

Lycopods go a fair way down the path to seeds without ever crossing the threshold. Fossil lycopod megaspores reaching 1 cm in diameter, and surrounded by vegetative tissue, are known – these even germinate into a megagametophyte *in situ*. However, they fall short of being seeds, since the nucellus, an inner spore-covering layer, does not completely enclose the spore. A very small slit remains, meaning that the seed is still exposed to the atmosphere. This has two consequences – firstly, it means it is not fully resistant to desiccation, and secondly, sperm do not have to "burrow" to access the archegonia of the megaspore.

The first "spermatophytes" (literally:seed plants) – that is, the first plants to bear true seeds – are called **pteridosperms**: literally, "seed ferns", so called because their foliage consisted of fern-like fronds, although they were not closely related to ferns. The oldest fossil evidence of seed plants is of Late Devonian age and they appear to have evolved out of an earlier group known as the progymnosperms. These early seed plants ranged from trees to small, rambling shrubs; like most early progymnosperms, they were woody plants with fern-like foliage. They all bore ovules, but no cones, fruit or similar. While it is difficult to track the early evolution of seeds, we can trace the lineage of the seed ferns from the simple trimerophytes through homosporous Aneurophytes.

This seed model is shared by basically all gymnosperms (literally: "naked seeds"), most of which encase their seeds in a woody or fleshy (the yew, for example) cone, but none of which fully enclose their seeds. The angiosperms ("vessel seeds") are the only group to fully enclose the seed, in a carpel.

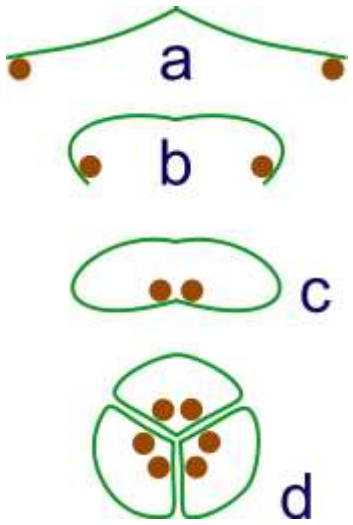
Fully enclosed seeds opened up a new pathway for plants to follow: that of seed dormancy. The embryo, completely isolated from the external atmosphere and hence protected from desiccation, could survive some years of drought before germinating. Gymnosperm seeds from the late Carboniferous have been found to contain embryos, suggesting a lengthy gap between fertilisation and germination. This period is associated with the entry into a greenhouse earth period, with an associated increase in aridity. This suggests that dormancy arose as a response to drier climatic conditions, where it became advantageous to wait for a moist period before germinating. This evolutionary breakthrough appears to have opened a floodgate: previously inhospitable areas, such as dry mountain slopes, could now be tolerated, as were soon covered by trees.

Seeds offered further advantages to their bearers: they increased the success rate of fertilised gametophytes, and because a nutrient store could be "packaged" in with the embryo, the seeds could germinate rapidly in inhospitable environments, reaching a size where it could fend for itself more quickly. For example, without an endosperm, seedlings growing in arid environments would not have the reserves to grow roots deep

enough to reach the water table before they expired. Likewise, seeds germinating in a gloomy understory require an additional reserve of energy to quickly grow high enough to capture sufficient light for self-sustenance. A combination of these advantages gave seed plants the ecological edge over the previously dominant genus *Archaeopteris*, this increasing the biodiversity of early forests.

Evolution of flowers

The pollen bearing organs of the early "flower" *Crossotheca*



The evolution of syncarps.
a: sporangia borne at tips of leaf
b: Leaf curls up to protect sporangia
c: leaf curls to form enclosed roll
d: grouping of three rolls into a syncarp

Flowers are modified leaves possessed only by the group known as the angiosperms, which are relatively late to appear in the fossil record. Colourful and/or pungent structures surround the cones of plants such as cycads and gnetales, making a strict definition of the term "flower" elusive.

The flowering plants have long been assumed to have evolved from within the *gymnosperms*; according to the traditional morphological view, they are closely allied to the gnetales. However, as noted above, recent molecular evidence is at odds to this hypothesis, and further suggests that gnetales are more closely related to some gymnosperm groups than angiosperms, and that extant gymnosperms form a distinct clade to the angiosperms, the two clades diverging some 300 million years ago.

The relationship of stem groups to the angiosperms is of utmost importance in determining the evolution of flowers; stem groups provide an insight into the state of earlier "forks" on the path to the current state. If we identify an unrelated group as a stem group, then we will gain an incorrect image of the lineages' history. The traditional view that flowers arose by modification of a structure similar to that of the gnetales, for example, no longer bears weight in the light of the molecular data.

Convergence increases our chances of misidentifying stem groups. Since the protection of the megagametophyte is evolutionarily desirable, it would be unsurprising if many separate groups stumbled upon protective encasements independently. Distinguishing ancestry in such a situation, especially where we usually only have fossils to go on, is tricky – to say the least.

In flowers, this protection is offered by the carpel, an organ believed to represent an adapted leaf, recruited into a protective role, shielding the ovules. These ovules are further protected by a double-walled integument.

Penetration of these protective layers needs something more that a free-floating microgametophyte. Angiosperms have pollen grains comprising just three cells. One cell is responsible for drilling down through the integuments, and creating a conduit for the two sperm cells to flow down. The megagametophyte has just seven cells; of these, one fuses with a sperm cell, forming the nucleus of the egg itself, and another other joins with the other sperm, and dedicates itself to forming a nutrient-rich endosperm. The other cells take auxiliary roles. This process of "double fertilisation" is unique and common to all angiosperms.

The inflorescences of the Bennettitales are strikingly similar to flowers

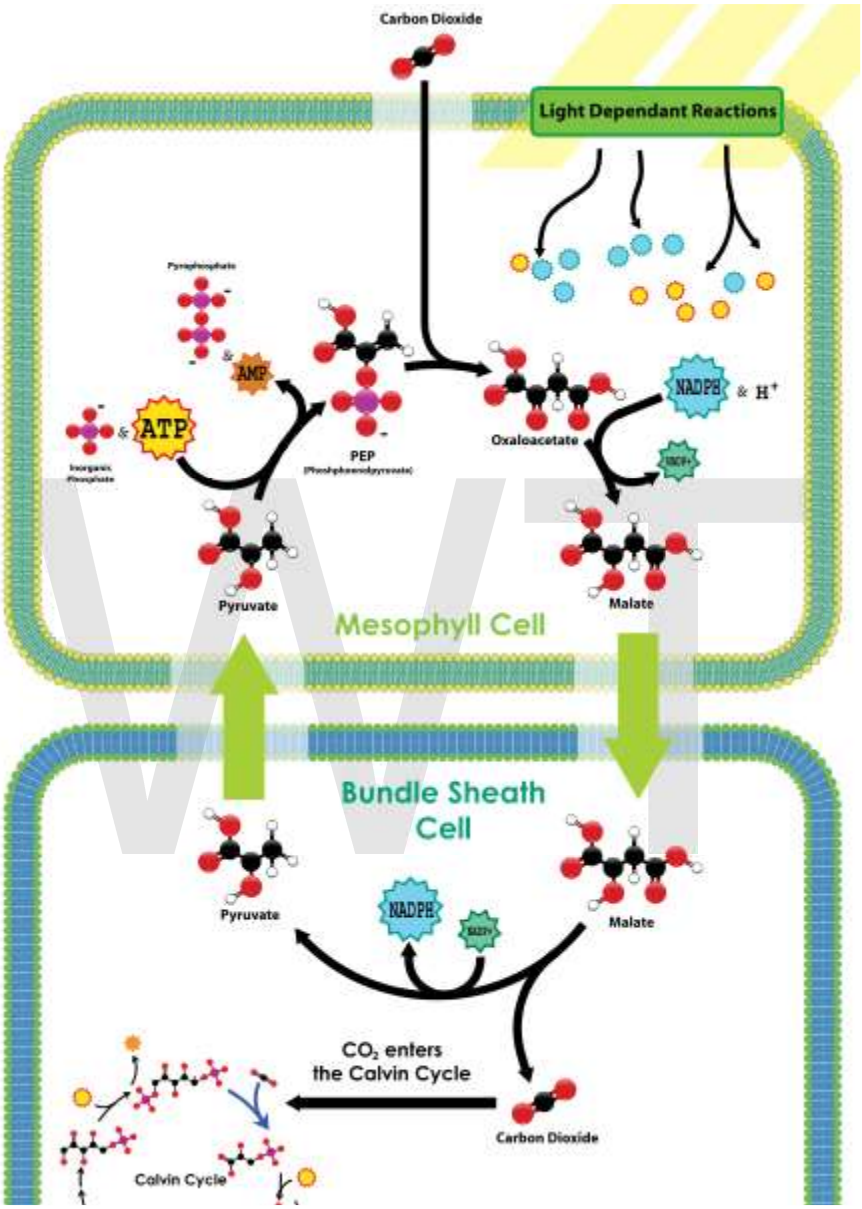
In the fossil record, there are three intriguing groups which bore flower-like structures. The first is the Permian pteridosperm *Glossopteris*, which already bore recurved leaves resembling carpels. The Triassic *Caytonia* is more flower-like still, with enclosed ovules – but only a single integument. Further, details of their pollen and stamens set them apart from true flowering plants.

The Bennettitales bore remarkably flower-like organs, protected by whorls of bracts which may have played a similar role to the petals and sepals of true flowers; however, these flower-like structures evolved independently, as the Bennettitales are more closely related to cycads and ginkgos than to the angiosperms.

However, no true flowers are found in any groups save those extant today. Most morphological and molecular analyses place *Amborella*, the nymphaeales and Austrobaileyaceae in a basal clade dubbed "ANA". This clade appear to have diverged in the early Cretaceous, around 130 million years ago – around the same time as the earliest fossil angiosperm, and just after the first angiosperm-like pollen, 136 million years ago. The magnoliids diverged soon after, and a rapid radiation had produced eudicots and monocots by 125 million years ago. By the end of the Cretaceous 65.5 million years ago, over 50% of today's angiosperm orders had evolved, and the clade accounted for 70% of global species. It was around this time that flowering trees became dominant over conifers

The features of the basal "ANA" groups suggest that angiosperms originated in dark, damp, frequently disturbed areas. It appears that the angiosperms remained constrained to such habitats throughout the Cretaceous – occupying the niche of small herbs early in the successional series. This may have restricted their initial significance, but given them the flexibility that accounted for the rapidity of their later diversifications in other habitats.

Advances in metabolism



The C_4 carbon concentrating mechanism

The most recent major innovation by the plants is the development of the C_4 metabolic pathway.

Photosynthesis is not quite as simple as adding water to CO_2 to produce sugars and oxygen. A complex chemical pathway is involved, facilitated along the way by a range of enzymes and co-enzymes. The enzyme RuBisCO is responsible for "fixing" CO_2 – that

is, it attaches it to a carbon-based molecule to form a sugar, which can be used by the plant, releasing an oxygen molecule along the way. However, the enzyme is notoriously inefficient, and just as effectively will also fix oxygen instead of CO_2 in a process called photorespiration. This is energetically costly as the plant has to use energy to turn the products of photorepsiration back into a form that can react with CO_2.

Concentrating carbon

To work around this inefficiency, C_4 plants evolved carbon concentrating mechanisms. These work by increasing the concentration of CO_2 around RuBisCO, thereby increasing the amount of photosynthesis and decreasing photorespiration. The process of concentrating CO_2 around RuBisCO requires more energy than allowing gases to diffuse, but under certain conditions – i.e. warm temperatures (>25°C), low CO_2 concentrations, or high oxygen concentrations – pays off in terms of the decreased loss of sugars through photorespiration.

One, C_4 metabolism, employs a so-called Kranz anatomy. This transports CO_2 through an outer mesophyll layer, via a range of organic molecules, to the central bundle sheath cells, where the CO_2 is released. In this way, CO_2 is concentrated near the site of RuBisCO operation. Because RuBisCO is operating in an environment with much more CO_2 than it otherwise would be, it performs more efficiently.

A second method, CAM photosynthesis, temporally separates photosynthesis from the action of RuBisCO. RuBisCO only operates during the day, when stomata are sealed and CO_2 is provided by the breakdown of the chemical malate. More CO_2 is then harvested from the atmosphere when stomata open, during the cool, moist nights, reducing water loss.

Evolutionary record

These two pathways, with the same effect on RuBisCO, evolved a number of times independently – indeed, C_4 alone arose in 18 different plant families. The C_4 construction is most famously used by a subset of grasses, while CAM is employed by many succulents and cacti. The trait appears to have emerged during the Oligocene, around 25 to 32 million years ago; however, they did not become ecologically significant until the Miocene, -1 million years ago. Remarkably, some charcoalified fossils preserve tissue organised into the Kranz anatomy, with intact bundle sheath cells, allowing the presence C_4 metabolism to be identified without doubt at this time. In deducing their distribution and significance, we resort to the use of isotopic markers. C_3 plants preferentially use the lighter of two isotopes of carbon in the atmosphere, ^{12}C, which is more readily involved in the chemical pathways involved in its fixation. Because C_4 metabolism involves a further chemical step, this effect is accentuated. Plant material can be analysed to deduce the ratio of the heavier ^{13}C to ^{12}C. This ratio is denoted $\delta^{13}C$. C_3 plants are on average around 14‰ (parts per thousand) lighter than the atmospheric ratio, while C_4 plants are about 28‰ lighter. The $\delta^{13}C$ of CAM plants depends on the percentage of carbon fixed at

night relative to what is fixed in the day, being closer to C_3 plants if they fix most carbon in the day and closer to C_4 plants if they fix all their carbon at night.

It's troublesome procuring original fossil material in sufficient quantity to analyse the grass itself, but fortunately we have a good proxy: horses. Horses were globally widespread in the period of interest, and browsed almost exclusively on grasses. There's an old phrase in isotope palæontology, "you are what you eat (plus a little bit)" – this refers to the fact that organisms reflect the isotopic composition of whatever they eat, plus a small adjustment factor. There is a good record of horse teeth throughout the globe, and their $\delta^{13}C$ has been measured. The record shows a sharp negative inflection around -1 million years ago, during the Messinian, and this is interpreted as the rise of C_4 plants on a global scale.

When is C_4 an advantage?

While C_4 enhances the efficiency of RuBisCO, the concentration of carbon is highly energy intensive. This means that C_4 plants only have an advantage over C_3 organisms in certain conditions: namely, high temperatures and low rainfall. C_4 plants also need high levels of sunlight in order to thrive. Models suggest that without wildfires removing shade-casting trees and shrubs, there would be no space for C_4 plants. But wildfires have occurred for 400 million years – why did C_4 take so long to arise, and then appear independently so many times? The Carboniferous period (~300 million years ago) had notoriously high oxygen levels – almost enough to allow spontaneous combustion – and very low CO_2, but there is no C_4 isotopic signature to be found. And there doesn't seem to be a sudden trigger for the Miocene rise.

During the Micoene, the atmosphere and climate was relatively stable. If anything, CO_2 increased gradually from 14 to 9 million years ago before settling down to concentrations similar to the Holocene. This suggests that it did not have a key role in invoking C_4 evolution. Grasses themselves (the group which would give rise to the most occurrences of C_4) had probably been around for 60 million years or more, so had had plenty of time to evolve C_4, which in any case is present in a diverse range of groups and thus evolved independently. There is a strong signal of climate change in South Asia; increasing aridity – hence increasing fire frequency and intensity – may have led to an increase in the importance of grasslands. However, this is difficult to reconcile with the North American record. It is possible that the signal is entirely biological, forced by the fire-(and elephant?)- driven acceleration of grass evolution – which, both by increasing weathering and incorporating more carbon into sediments, reduced atmospheric CO_2 levels. Finally, there is evidence that the onset of C_4 from 9 to 7 million years ago is a biased signal, which only holds true for North America, from where most samples originate; emerging evidence suggests that grasslands evolved to a dominant state at least 15Ma earlier in South America.

Evolutionary trends

The process of evolution works slightly differently in plants than animals. Differences in plant physiology and reproduction mean that while the same evolutionary principles of natural selection apply, the finer nuances of their effect are radically different.

One major difference is the ability of plants to reproduce clonally, and the totipotent nature of their cells, allowing them to reproduce asexually much more easily than most animals. They are also capable of polyploidy – where more than two chromosome sets are inherited from parents. This allows relatively fast bursts of evolution to occur. The long periods of dormancy that seed plants can employ also makes them less vulnerable to extinction, as they can "sit out" the tough periods and wait until more clement times to leap back to life.

The effect of these differences is most profoundly seen during extinction events. These events, which wiped out between 6 and 62% of terrestrial animal families, had "negligible" effect on plant families. However, the ecosystem structure is significantly rearranged, with the abundances and distributions of different groups of plants changing profoundly. These effects are perhaps due to the higher diversity within families, as extinction – which *was* common at the species level – was very selective. For example, wind-pollinated species survived better than insect-pollinated taxa, and specialised species generally lost out. In general, the surviving taxa were rare before the extinction, suggesting that they were generalists who were poor competitors when times were easy, but prospered when specialised groups went extinct and left ecological niches vacant.

Chapter 2

Plant Evolutionary Developmental Biology

Evolutionary developmental biology (evo-devo) refers to the study of developmental programs and patterns from an evolutionary perspective. It seeks to understand the various influences shaping the form and nature of life on the planet. Evo-devo arose as a separate branch of science only in the last decade. Most of the synthesis in evo-devo has been in the field of animal evolution, one reason being the presence of elegant model systems like *Drosophila melanogaster*, *C. elegans*, zebrafish and *Xenopus laevis*. However, in the past couple of decades, a wealth of information on plant morphology, coupled with modern molecular techniques has helped shed light on the conserved and unique developmental patterns in the plant kingdom also.

Historical perspective

Before 1900

Johann Wolfgang von Goethe

The origin of the term "morphology" is generally attributed to Johann Wolfgang von Goethe (1749–1832). He was of the opinion that there is an underlying fundamental organisation *(Bauplan)* in the diversity of flowering plants. In his book titled The Metamorphosis of Plants, he proposed that the *Bauplan* enabled us to predict the forms of plants that had not yet been discovered. Goethe also was the first to make the perceptive suggestion that flowers consist of modified leaves.

In the middle centuries, several basic foundations of our current understanding of plant morphology were laid down. Nehemiah Grew, Marcello Malpighi, Robert Hooke, Antonie van Leeuwenhoek, Wilhelm von Nageli were just some of the people who helped build knowledge on plant morphology at various levels of organisation. It was the taxonomical classification of Carolus Linnaeus in the eighteenth century though, that generated a firm base for the knowledge to stand on and expand. The introduction of the concept of Darwinism in contemporary scientific discourse also had had an effect on the thinking on plant forms and their evolution.

Wilhelm Hofmeister, one of the most brilliant botanists of his times, was the one to diverge away from the idealist way of pursuing botany. Over the course of his life, he brought an interdisciplinary outlook into botanical thinking. He came up with biophysical explanations on phenomena like phototaxis and geotaxis, and also discovered the alternation of generations in the plant life cycle.

1900 to the present

Arabidopsis thaliana. This flowering plant has been a model system for most of plant molecular studies

The past century witnessed a rapid progress in the study of plant anatomy. The focus shifted from the population level to more reductionist levels. While the first half of the century saw expansion in developmental knowledge at the tissue and the organ level, in the latter half, especially since the 1990s, there has also been a strong impetus on gaining molecular information.

Edward Charles Jeffrey was one of the early evo devo researchers of the 20th century. He performed a comparative analyses of the vasculatures of living and fossil Gymnosperms and came to the conclusion that the storage parenchyma has been derived from tracheids.

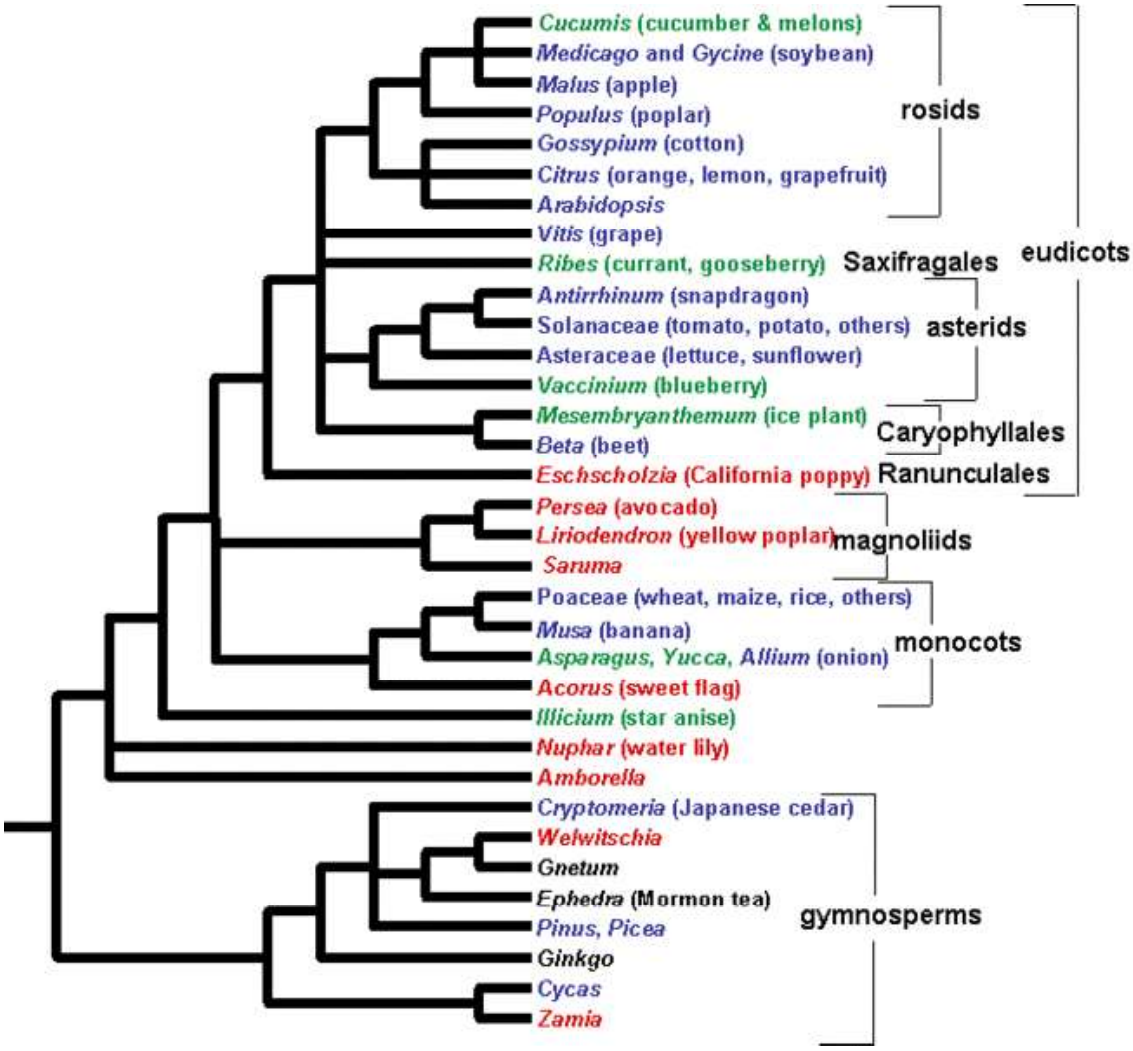
His research focussed primarily on plant anatomy in the context of phylogeny. This tradition of evolutionary analyses of plant architectures was further advanced by Katherine Esau, best known for her book *The Plant Anatomy*. Her work focussed on the origin and development of various tissues in different plants. Working with Vernon Cheadle, she also explained the evolutionary specialization of the phloem tissue with respect to its function.

In the meantime, by the beginning of the latter half of 1900s, *Arabidopsis thaliana* had begun to be used in some developmental studies. The first collection of *Arabidopsis thaliana* mutants were made around 1945. However it formally became established as a model organism only in 1998.

The recent spurt in information on various plant-related processes has largely been a result of the revolution in molecular biology. Powerful techniques like mutagenesis and complementation were made possible in *Arabidopsis thaliana* via generation of T-DNA containing mutant lines, recombinant plasmids, techniques like Transposon Tagging etc. Availability of complete physical and genetic maps, RNAi vectors, and rapid transformation protocols are some of the technologies that have significantly altered the scope of the field. Recently, there has also been a massive increase in the genome and EST sequences of various non-model species, which, coupled with the Bioinformatics tools existing today, generate interesting opportunities in the field of plant evo devo research.

Cusset provided a detailed in-depth analysis of the history of plant morphology, including plant development and evolution, from its beginnings to the end of the 20th century.

Organisms, databases and tools



The sampling of the Floral Genome Project

The most important model systems in plant development have been arabidopsis and maize. Maize has traditionally been the favorite of plant geneticists, while extensive resources in almost every area of plant physiology and development are available for *Arabidopsis thaliana*. Apart from these, rice, *Antirrhinum majus*, *Brassica*, tomato are also being used in a variety of studies. The genomes of *Arabidopsis thaliana* and rice have been completely sequenced, while the others are in process. It must be emphasized here that the information from these "model" organisms form the basis of our developmental knowledge. While *Brassica* has been used primarily because of its convenient location in the phylogenetic tree in the mustard family, *Antirrhinum majus* is a convenient system for studying leaf architecture. Rice has been traditionally used for studying responses to hormones like abscissic acid and gibberelin as well as responses to stress. However, recently, not just the domesticated rice strain, but also the wild strains have been studied for their underlying genetic architectures.

Some people have objected against extending the results of model organisms to the plant world. One argument is that the effect of gene knockouts in lab conditions wouldn't truly reflect even the same plant's response in the natural world. Also, these supposedly *crucial* genes might not be responsible for the evolutionary origin of that character. For these reasons, a comparative study of plant traits has been proposed as the way to go now.

Since the past few years, researchers have indeed begun looking at non-model, "non-conventional" organisms using modern genetic tools. One example of this is the Floral Genome Project, which envisages to study the evolution of the current patterns in the genetic architecture of the flower through comparative genetic analyses, with a focus on EST sequences. Like the FGP, there are several such ongoing projects that aim to find out conserved and diverse patterns in evolution of the plant shape. Expressed sequence tag (EST) sequences of quite a few non-model plants like Sugarcane, Apple, Lotus, Barley, Cycas, Coffee, to name a few, are available freely online. The Cycad Genomics Project, for example, aims to understand the differences in structure and function of genes between gymnosperms and angiosperms through sampling in the order Cycadales. In the process, it intends to make available information for the study of evolution of structures like seeds, cones and evolution of life cycle patterns. Presently the most important sequenced genomes from an evo-devo point of view include those of *A.thaliana (a flowering plant)*, Poplar *(a woody plant)*, *Physcomitrella patens (a bryophyte)*, Maize *(extensive genetic information)*, and *Chlamydomonas reinhardtii (a green alga)*. The impact of such a vast amount of information on understanding common underlying developmental mechanisms can easily be realised.

Apart from EST and genome sequences, several other tools like PCR, Yeast two hybrid system, microarrays, RNA Interference, SAGE, QTL mapping etc. permit the rapid study of plant developmental patterns. Recently, cross-species hybridization has begun to be employed on microarray chips, to study the conservation and divergence in mRNA expression patterns between closely related species. Techniques for analyzing this kind of data have also progressed over the past decade. We now have better models for molecular evolution, more refined analysis algorithms and better computing power as a result of advances in computer sciences.

Evolution of plant morphology

Overview of plant evolution

Evidence suggests that an algal scum formed on the land 1,200 million years ago, but it was not until the Ordovician period, around 500 million years ago, that land plants appeared. These begun to diversify in the late Silurian period, around 420 million years ago, and the fruits of their diversification are displayed in remarkable detail in an early Devonian fossil assemblage known as the Rhynie chert. This chert preserved early plants in cellular detail, petrified in volcanic springs. By the middle of the Devonian period most of the features recognised in plants today are present, including roots, leaves and seeds. By the late Devonian, plants had reached a degree of sophistication that allowed them to form forests of tall trees. Evolutionary innovation continued after the Devonian

period. Most plant groups were relatively unscathed by the Permo-Triassic extinction event, although the structures of communities changed. This may have set the scene for the evolution of flowering plants in the Triassic (~200 million years ago), which exploded the Cretaceous and Tertiary. The latest major group of plants to evolve were the grasses, which became important in the mid Tertiary, from around 40 million years ago. The grasses, as well as many other groups, evolved new mechanisms of metabolism to survive the low CO_2 and warm, dry conditions of the tropics over the last 10 million years.

Evolution of meristems

The meristematic cells give rise to various organs of the plant, and keep the plant growing. The Shoot Apical Meristem (SAM) gives rise to organs like the leaves and flowers. The cells of the apical meristems - SAM and RAM (Root Apical Meristem)- divide rapidly and are considered to be indeterminate, in that they do not possess any defined end fate. In that sense, the meristematic cells are frequently compared to the stem cells in animals, that have an analogous behavior and function.

Diversity in meristem architectures

Is the mechanism of being *indeterminate* conserved in the SAM's of the plant world? The SAM contains a population of stem cells that also produce the lateral meristems while the stem elongates. It turns out that the mechanism of regulation of the stem cell number might indeed be evolutionarily conserved. The *CLAVATA* gene *CLV2* responsible for maintaining the stem cell population in *Arabidopsis thaliana* is very closely related to the Maize gene *FASCIATED EAR 2*(*FEA2*) also involved in the same function. Similarly, in Rice, the *FON1-FON2* system seems to bear a close relationship with the CLV signaling system in *Arabidopsis thaliana*. These studies suggest that the regulation of stem cell number, identity and differentiation might be an evolutionarily conserved mechanism in monocots, if not in angiosperms. Rice also contains another genetic system distinct from *FON1-FON2*, that is involved in regulating stem cell number. This example underlines the innovation that goes about in the living world all the time.

Role of the KNOX-family genes

Note the long spur of the above flower. Spurs attract pollinators and confer pollinator specificity. *(Flower:Linaria dalmatica)*

Complex leaves of *C. hirsuta* are a result of KNOX gene expression

Genetic screens have identified genes belonging to the KNOX family in this function. These genes essentially maintain the stem cells in an undifferentiated state. The KNOX family has undergone quite a bit of evolutionary diversification, while keeping the overall mechanism more or less similar. Members of the KNOX family have been found in plants as diverse as Arabidopsis thaliana, rice, barley and tomato. KNOX-like genes are also present in some algae, mosses, ferns and gymnosperms. Misexpression of these genes leads to formation of interesting morphological features. For example, among members of *Antirrhinae*, only the species of genus *Antirrhinum* lack a structure called spur in the floral region. A spur is considered an evolutionary innovation because it defines pollinator specificity and attraction. Researchers carried out transposon mutagenesis in *Antirrhinum majus*, and saw that some insertions led to formation of spurs

that were very similar to the other members of *Antirrhinae*, indicating that the loss of spur in wild *Antirrhinum majus* populations could probably be an evolutionary innovation.

The KNOX family has also been implicated in leaf shape evolution. One study looked at the pattern of KNOX gene expression in *A. thaliana*, that has simple leaves and *Cardamine hirsuta*, a plant having complex leaves. In *A. thaliana*, the KNOX genes are completely turned off in leaves, but in *C.hirsuta*, the expression continued, generating complex leaves. Also, it has been proposed that the mechanism of KNOX gene action is conserved across all vascular plants, because there is a tight correlation between KNOX expression and a complex leaf morphology.

Evolution of the meristem architecture

The meristem architectures do differ between angiosperms, gymnosperms and pteridophytes. The gymnosperm vegetative meristem lacks organization into distinct tunica and corpus layers. They possess large cells called Central Mother Cells in the meristem. In angiosperms, the outermost layer of cells divides anticlinally to generate the new cells, while in gymnosperms, the plane of division in the meristem differs for different cells. However, the apical cells do contain organelles like large vacuoles and starch grains, like the angiosperm meristematic cells.

Pteridophytes, like fern, on the other hand, do not possess a multicellular apical meristem. They possess a tetrahedral apical cell, which goes on to form the plant body. Any somatic mutation in this cell can lead to hereditary transmission of that mutation. The earliest meristem-like organization is seen in an algal organism from group *Charales* that has a single dividing cell at the tip, much like the pteridophytes, yet simpler. One can thus see a clear pattern in evolution of the meristematic tissue, from pteridophytes to angiosperms. Pteridophytes, with a single meristematic cell; gymnosperms with a multicellular, but less defined organization and finally, angiosperms, with the highest degree of organization. The genetic innovations that contributed to this evolution are yet not clearly known.

Evolution of leaves

Origins of the leaf

Leaf lamina. The leaf architecture probably arose multiple times in the plant lineage

Leaves are the primary photosynthetic organs of a plant. Based on their structure, they are classified into two types - microphylls, that lack complex venation patterns and megaphylls, that are large and with a complex venation. It has been proposed that these structures arose independently. Megaphylls, according to the Telome hypothesis, have evolved from plants that showed a three dimensional branching architecture, through three transformations—**plantation**, which involved formation of a planar architecture, **webbing**, or formation of the outgrowths between the planar branches and **fusion**, where these webbed outgrowths fused to form a proper leaf lamina. Studies have revealed that these three steps happened multiple times in the evolution of today's leaves.

It has been proposed that the before the evolution of leaves, plants had the photosynthetic apparatus on the stems. Today's megaphyll leaves probably became commonplace some 360mya, about 40my after the simple leafless plants had colonized the land in the early Devonian period. This spread has been linked to the fall in the atmospheric carbon

dioxide concentrations in the Late Paleozoic era associated with a rise in density of stomata on leaf surface. This must have allowed for better transpiration rates and gas exchange. Large leaves with less stomata would have gotten heated up in the sun's heat, but an increased stomatal density allowed for a better-cooled leaf, thus making its spread feasible.

Factors influencing leaf architectures

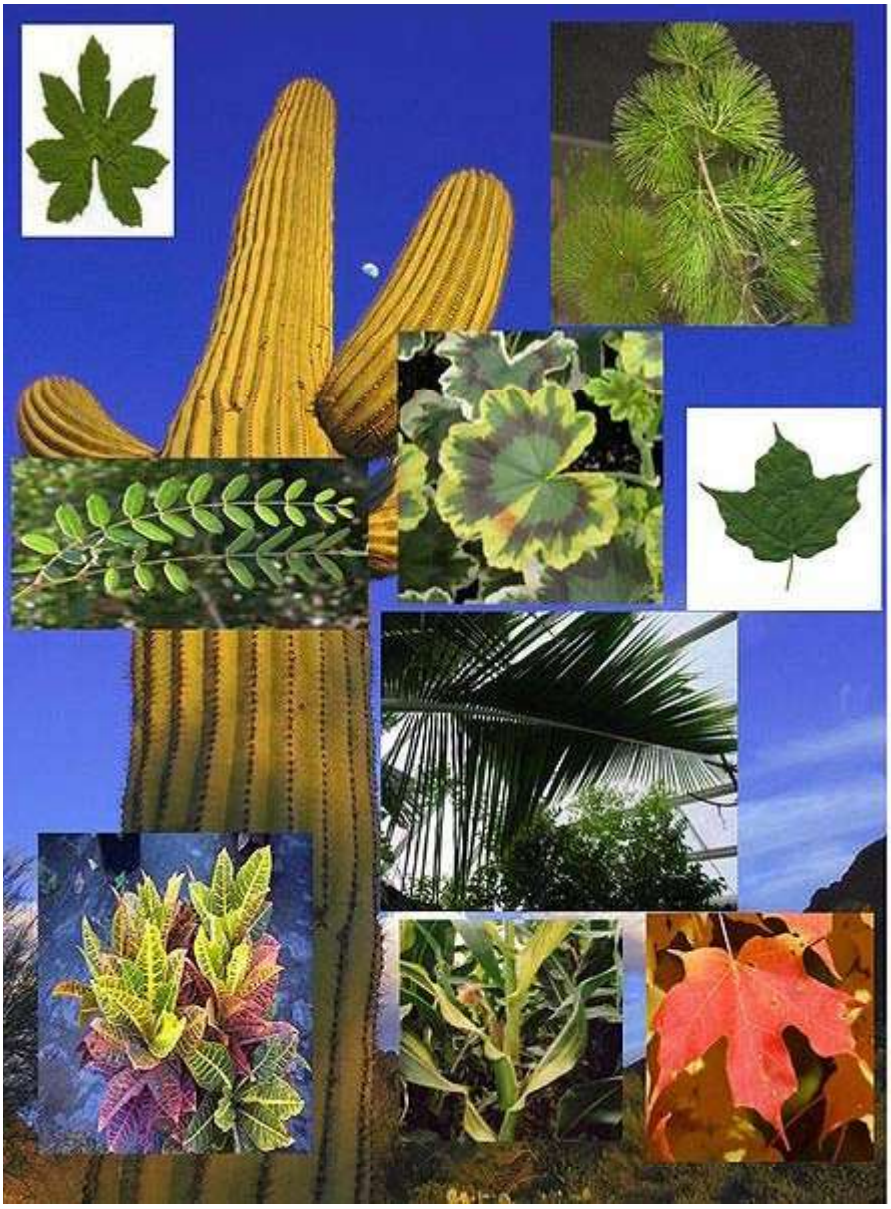
Spiny leaves of *Aciphylla squarrosa*. It is thought that these leaves evolved as an adaptation against the now extinct Moas

Various physical and physiological forces like light intensity, humidity, temperature, wind speeds etc. are thought to have influenced evolution of leaf shape and size. It is observed that high trees rarely have large leaves, owing to the obstruction they generate for winds. This obstruction can eventually lead to the tearing of leaves, if they are large. Similarly, trees that grow in temperate or taiga regions have pointed leaves, presumably to prevent nucleation of ice onto the leaf surface and reduce water loss due to transpiration. Herbivory, not only by large mammals, but also small insects has been implicated as a driving force in leaf evolution, an example being plants of the genus *Aciphylla*, that are commonly found in New Zealand. The now extinct Moas fed upon these plants, and its seen that the leaves have spines on their bodies, which probably

functioned to discourage the moas from feeding on them. Other members of *Aciphylla* that did not co-exist with the moas, do not have these spines.

Genetic evidences for leaf evolution

At the genetic level, developmental studies have shown that repression of the KNOX genes is required for initiation of the leaf primordium. This is brought about by *ARP* genes, which encode transcription factors. Genes of this type have been found in many plants studied till now, and the mechanism i.e. repression of KNOX genes in leaf primordia, seems to be quite conserved. Interestingly, expression of KNOX genes in leaves produces complex leaves. It is speculated that the *ARP* function arose quite early in vascular plant evolution, because members of the primitive group Lycophytes also have a functionally similar gene Other players that have a conserved role in defining leaf primordia are the phytohormone auxin, gibberelin and cytokinin.



The diversity of leaves

One interesting feature of a plant is its phyllotaxy. The arrangement of leaves on the plant body is such that the plant can maximally harvest light under the given constraints, and hence, one might expect the trait to be genetically robust. However, it may not be so. In maize, a mutation in only one gene called *abphyl (ABNORMAL PHYLLOTAXY)* was enough to change the phyllotaxy of the leaves. It implies that sometimes, mutational tweaking of a single locus on the genome is enough to generate diversity. The *abphyl* gene was later on shown to encode a cytokinin response regulator protein.

Once the leaf primordial cells are established from the SAM cells, the new axes for leaf growth are defined, one important (and more studied) among them being the abaxial-adaxial (lower-upper surface) axes. The genes involved in defining this, and the other

axes seem to be more or less conserved among higher plants. Proteins of the *HD-ZIPIII* family have been implicated in defining the adaxial identity. These proteins deviate some cells in the leaf primordium from the default abaxial state, and make them adaxial. It is believed that in early plants with leaves, the leaves just had one type of surface - the abaxial one. This is the underside of today's leaves. The definition of the adaxial identity occurred some 200 million years after the abaxial identity was established. One can thus imagine the early leaves as an intermediate stage in evolution of today's leaves, having just arisen from spiny stem-like outgrowths of their leafless ancestors, covered with stomata all over, and not optimized as much for light harvesting.

How the infinite variety of plant leaves is generated is a subject of intense research. Some common themes have emerged. One of the most significant is the involvement of KNOX genes in generating compound leaves, as in tomato. But this again is not universal. For example, pea uses a different mechanism for doing the same thing. Mutations in genes affecting leaf curvature can also change leaf form, by changing the leaf from flat, to a crinky shape, like the shape of cabbage leaves. There also exist different morphogen gradients in a developing leaf which define the leaf's axis. Changes in these morphogen gradients may also affect the leaf form. Another very important class of regulators of leaf development are the microRNAs, whose role in this process has just begun to be documented. The coming years should see a rapid development in comparative studies on leaf development, with many EST sequences involved in the process coming online.

Evolution of flowers

The pollen bearing organs of the early flower *Crossotheca*

A flower is, arguably, one of the most beautiful products of evolution. Flower-like structures first appear in the fossil records some ~130 mya, in the Cretaceous era.

The flowering plants have long been assumed to have evolved from within the gymnosperms; according to the traditional morphological view, they are closely allied to the gnetales. However, recent molecular evidence is at odds to this hypothesis, and further suggests that gnetales are more closely related to some gymnosperm groups than angiosperms, and that gymnosperms form a distinct clade to the angiosperms,. Molecular clock analysis predicts the divergence of flowering plants (anthophytes) and gymnosperms to ~300 mya

The main function of a flower is reproduction, which, before the evolution of the flower and angiosperms, was the job of microsporophylls and megasporophylls. A flower can be considered a powerful evolutionary innovation, because its presence allowed the plant world to access new means and mechanisms for reproduction.

Origins of the flower

Amborella trichopoda : Amborellaceae is considered the sister family of all flowering plants *(magnified image)*

The family Amborellaceae is regarded as the sister family of all living flowering plants. That means members of this family were most likely the first flowering plants.

It seems that on the level of the organ, the leaf may be the ancestor of the flower, or at least some floral organs. When we mutate some crucial genes involved in flower development, we end up with a cluster of leaf-like structures. Thus, sometime in history, the developmental program leading to formation of a leaf must have been altered to generate a flower. There probably also exists an overall robust framework within which the floral diversity has been generated. A example of that is a gene called *LEAFY (LFY)*, which is involved in flower development in *Arabidopsis thaliana*. The homologs of this

gene are found in angiosperms as diverse as tomato, snapdragon, pea, maize and even gymnosperms. Interestingly, expression of *Arabidopsis thaliana* LFY in distant plants like poplar and citrus also results in flower-production in these plants. The *LFY* gene regulates the expression of some gene belonging to the MADS-box family. These genes, in turn, act as direct controllers of flower development.

Evolution of the MADS-box family

The members of the MADS-box family of transcription factors play a very important and evolutionarily conserved role in flower development. According to the ABC Model of flower development, three zones - A,B and C - are generated within the developing flower primordium, by the action of some transcription factors, that are members of the MADS-box family. Among these, the functions of the B and C domain genes have been evolutionarily more conserved than the A domain gene. Many of these genes have arisen through gene duplications of ancestral members of this family. Quite a few of them show redundant functions.

The evolution of the MADS-box family has been extensively studied. These genes are present even in pteridophytes, but the spread and diversity is many times higher in angiosperms. There appears to be quite a bit of pattern into how this family has evolved. Consider the evolution of the C-region gene *AGAMOUS (AG)*. It is expressed in today's flowers in the stamens, and the carpel, which are reproductive organs. It's ancestor in gymnosperms also has the same expression pattern. Here, it is expressed in the strobili, an organ that produces pollens or ovules. Similarly, the B-genes' *(AP3 and PI)* ancestors are expressed only in the male organs in gymnosperms. Their descendants in the modern angiosperms also are expressed only in the stamens, the male reproductive organ. Thus, the same, then-existing components were used by the plants in a novel manner to generate the first flower. This is a recurring pattern in evolution.

Factors influencing floral diversity

The various shapes and colors of flowers

How is the enormous diversity in the shape, color and sizes of flowers established? There is enormous variation in the developmental program in different plants. For example, monocots possess structures like lodicules and palea, that were believed to be analogous to the dicot petals and carpels respectively.It turns out that this is true, and the variation is due to slight changes in the MADS-box genes and their expression pattern in the monocots. Another example is that of a plant called *Linaria vulgaris*, which has two kinds of flower symmetries-radial and bilateral. These symmetries are due to epigenetic changes in just one gene called *CYCLOIDEA*.

Large number of petals in roses has probably been a result of human selection

Arabidopsis thaliana has a gene called *AGAMOUS* that plays an important role in defining how many petals and sepals and other organs are generated. Mutations in this gene give rise to the floral meristem obtaining an indeterminate fate, and many floral organs keep on getting produced. We have flowers like roses, carnations and morning glory, for example, that have very dense floral organs. These flowers have been selected by horticulturists since long for increased number of petals. Researchers have found that the morphology of these flowers is because of strong mutations in the *AGAMOUS* homolog in these plants, which leads to them making a large number of petals and sepals. Several studies on diverse plants like petunia, tomato, Impatiens, maize etc. have suggested that the enormous diversity of flowers is a result of small changes in genes controlling their development.

Some of these changes also cause changes in expression patterns of the developmental genes, resulting in different phenotypes. The Floral Genome Project looked at the EST data from various tissues of many flowering plants. The researchers confirmed that the ABC Model of flower development is not conserved across all angiosperms. Sometimes expression domains change, as in the case of many monocots, and also in some basal angiosperms like *Amborella*. Different models of flower development like the *The fading boundaries model*, or the *Overlapping-boundaries model* which propose non-rigid domains of expression, may explain these architectures. There is a possibility that from the basal to the modern angiosperms, the domains of floral architecture have gotten more and more fixed through evolution.

Flowering time

Another floral feature that has been a subject of natural selection is flowering time. Some plants flower early in their life cycle, others require a period of vernalization before flowering. This decision is based on factors like temperature, light intensity, presence of pollinators and other environmental signals. We know that genes like *CONSTANS (CO)*, *Flowering Locus C* (*FLC*) and *FRIGIDA* regulate integration of environmental signals into the pathway for flower development. Variations in these loci have been associated with flowering time variations between plants. For example, *Arabidopsis thaliana* ecotypes that grow in the cold, temperate regions require prolonged vernalization before they flower, while the tropical varieties, and the most common lab strains, don't. We now know that this variation is due to mutations in the *FLC* and *FRIGIDA* genes, rendering them non-functional.

Quite a few players in this process are conserved across all the plants studied. Sometimes though, despite genetic conservation, the mechanism of action turns out to be different. For example, rice is a short-day plant, while *Arabidopsis thaliana* is a long-day plant. Now, in both plants, the proteins *CO* and *FLOWERING LOCUS T (FT)* are present. But in *Arabidopsis thaliana*, *CO* enhances *FT* production, while in rice, the *CO* homolog represses *FT* production, resulting in completely opposite downstream effects.

Theories of flower evolution

There are many theories that propose how flowers evolved. Some of them are described below.

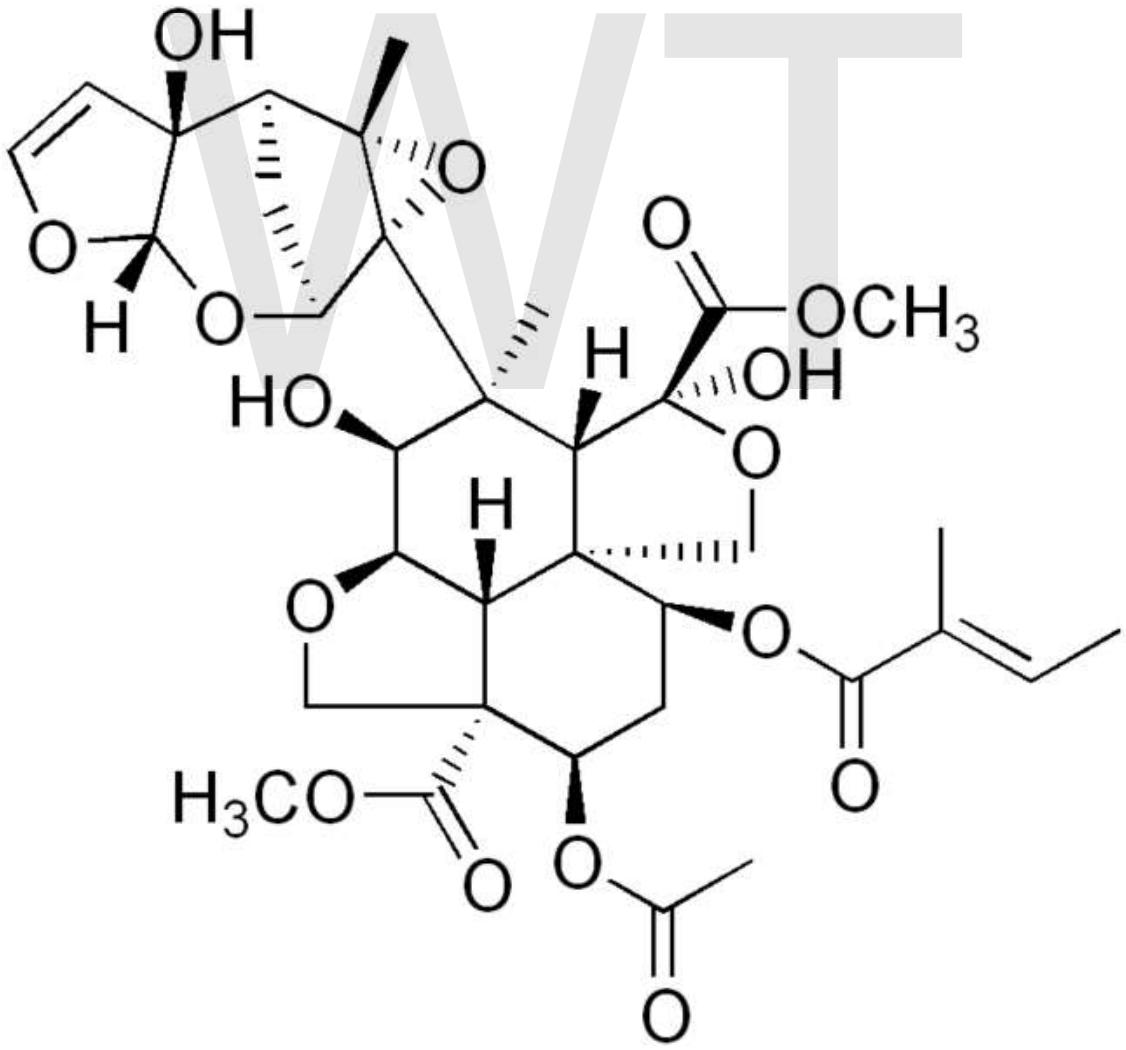
The *Anthophyte Theory* was based upon the observation that a gymnospermic group Gnetales has a flower-like ovule. It has partially developed vessels as found in the angiosperms, and the megasporangium is covered by three envelopes, like the ovary structure of angiosperm flowers. However, many other lines of evidence show that Gnetales is not related to angiosperms.

The *Mostly Male Theory* has a more genetic basis. Proponents of this theory point out that the gymnosperms have two very similar copies of the gene *LFY* while angiosperms just one. Molecular clock analysis has shown that the other *LFY* paralog was lost in

angiosperms around the same time as flower fossils become abundant, suggesting that this event might have led to floral evolution. According to this theory, loss of one of the *LFY* paralog led to flowers that were more male, with the ovules being expressed ectopically. These ovules initially performed the function of attracting pollinators, but sometime later, may have been integrated into the core flower.

One theory also suggests that humans have been one of the reasons for the diversity of flowers. This theory suggests that since the early settlers found flowers beautiful, they may have started selecting for them artificially. The flowers may have evolved to exploit the ecological niche being opened because of humans finding them attractive. The validity of this theory, however, is debatable, not least because flowers started diversifying long before they came into contact with humans.

Evolution of secondary metabolism



Structure of Azadirachtin, a terpenoid produced by the Neem plant, which helps ward off microbes and insects. Many secondary metabolites have complex structures

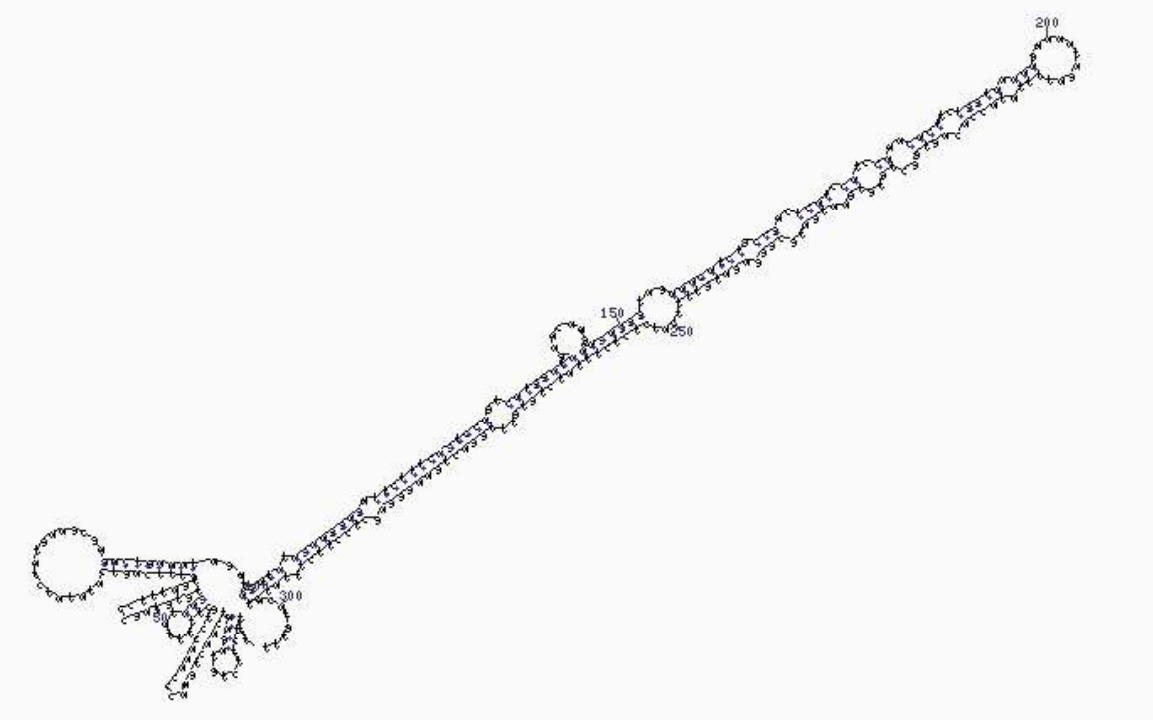
Although we know many secondary metabolites produced by plants, the extent of the same is still unfathomable. Secondary metabolites are essentially low molecular weight compounds, sometimes having complex structures. They function in processes as diverse as immunity, anti-herbivory, pollinator attraction, communication between plants, maintaining symbiotic associations with soil flora, enhancing the rate of fertilization etc., and hence are significant from the evo-devo perspective. The structural and functional diversity of these secondary metabolites across the plant kingdom is so huge that it is estimated that hundreds of thousands of enzymes might be involved in this process in the entire of the plant kingdom, with about 15–25% of the coding genome coding for these enzymes. Despite this, every species has its unique arsenal of secondary metabolites. Many of these metabolites are of enormous medical significance to humans.

What is the purpose of having so many secondary metabolites being produced, with a significant chunk of the metabolome devoted to this activity? It is hypothesized that most of these chemicals help in generating immunity, and in consequence, the diversity of these metabolites is a result of a constant war between plants and their parasites. There is evidence that this may be true in many cases. The big question here is the reproductive cost involved in maintaining such an impressive inventory. Various models have been suggested that probe into this aspect of the question, but a consensus on the extent of the cost is lacking. We still cannot predict whether a plant with more secondary metabolites would be better off than other plants in its vicinity.

Secondary metabolite production seems to have arisen quite early during evolution. Even bacteria possess the ability to make these compounds. But they assume more significant roles in life from fungi onwards to plants. In plants they seem to have spread out using different mechanisms like gene duplications, evolution of novel genes etc. Furthermore, studies have shown that diversity in some of these compounds may be positively selected for.

Although the role of novel gene evolution in the evolution of secondary metabolism cannot be denied, there are several examples where new metabolites have been formed by small changes in the reaction. For example, cyanogen glycosides have been proposed to have evolved multiple times in different plant lineages. There are several such instances of convergent evolution. For example, we now know that enzymes for synthesis of limonene – a terpene – are more similar between angiosperms and gymnosperms than to their own terpene synthesis enzymes. This suggests independent evolution of the limonene biosynthetic pathway in these two lineages.

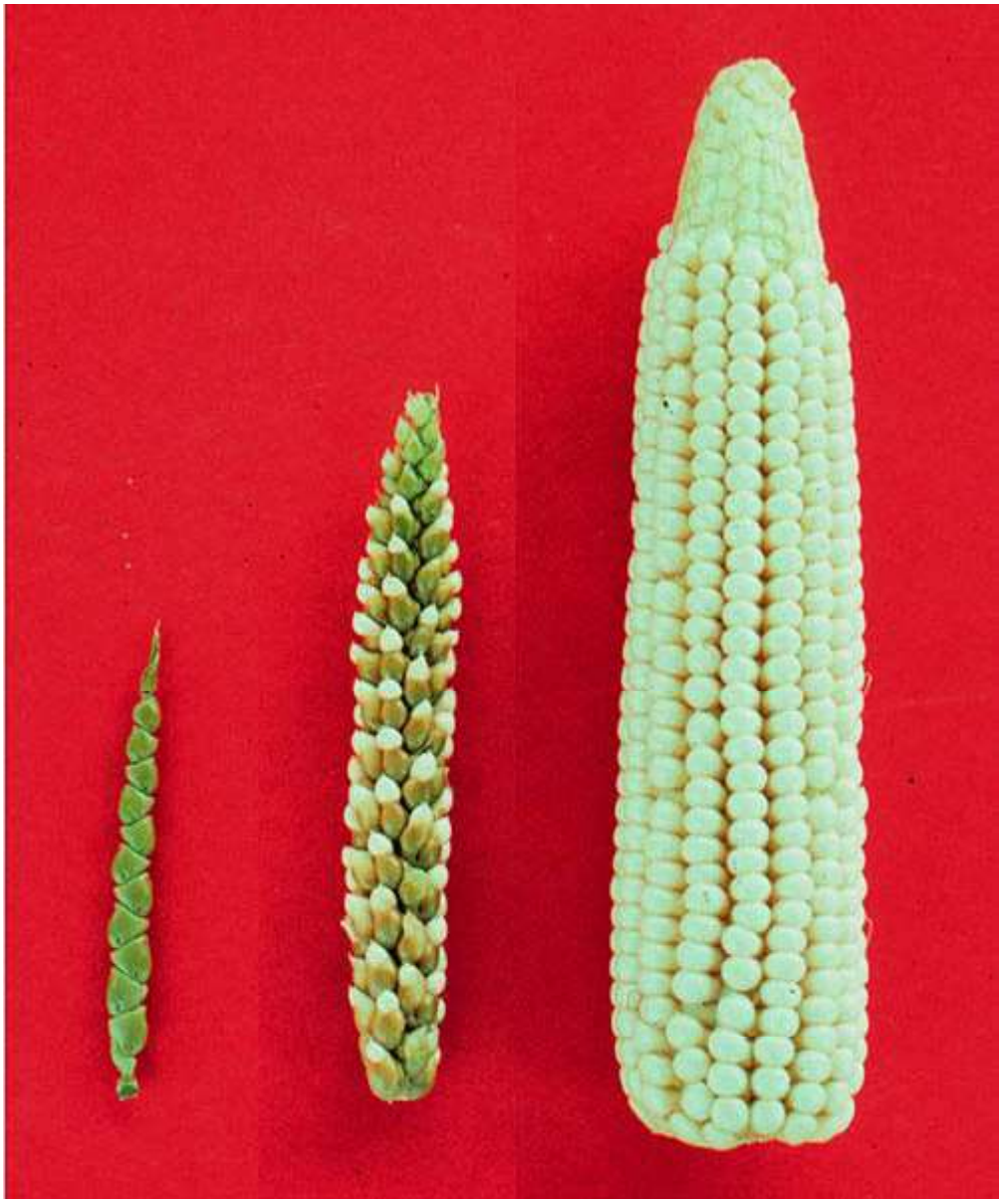
Mechanisms and players in evolution



The stem-loop secondary structure of a pre-microRNA from Brassica oleracea

While environmental factors are significantly responsible for evolutionary change, they act merely as agents for natural selection. Change is inherently brought about via phenomena at the genetic level - mutations, chromosomal rearrangements and epigenetic changes. While the general types of mutations hold true across the living world, in plants, some other mechanisms have been implicated as highly significant.

Polyploidy is a very common feature in plants. It is believed that at least half *(and probably all)* plants are or have been polyploids. Polyploidy leads to genome doubling, thus generating functional redundancy in most genes. The duplicated genes may attain new function, either by changes in expression pattern or changes in activity. Polyploidy and gene duplication are believed to be among the most powerful forces in evolution of plant form. It is not know though, why genome doubling is such a frequent process in plants. One probable reason is the production of large amounts of secondary metabolites in plant cells. Some of them might interfere in the normal process of chromosomal segregation, leading to polypoidy.



Extreme left: teosinte, Extreme right: maize, middle: maize-teosinte hybrid

In recent times, plants have been shown to possess significant microRNA families, which are conserved across many plant lineages. In comparison to animals, while the number of plant miRNA families are lesser than animals, the size of each family is much larger. The miRNA genes are also much more spread out in the genome than those in animals, where we find them clustered. It has been proposed that these miRNA families have expanded by duplications of chromosomal regions. Many miRNA genes involved in regulation of plant development have been found to be quite conserved between plants studied.

Domestication of plants like maize, rice, barley, wheat etc. has also been a significant driving force in their evolution. Some studies have tried to look at the origins of the maize plant and it turns out that maize is a domesticated derivative of a wild plant from

Mexico called teosinte. Teosinte belongs to the genus *Zea*, just as maize, but bears very small inflorescence, 5-10 hard cobs and a highly branched and spread out stem.

Cauliflower : *Brassica oleracea var botrytis*

Interestingly, crosses between a particular teosinte variety and maize yields fertile offsprings that are intermediate in phenotype between maize and teosinte. QTL analysis has also revealed some loci that when mutated in maize yield a teosinte-like stem or teosinte-like cobs. Molecular clock analysis of these genes estimates their origins to some 9000 years ago, well in accordance with other records of maize domestication. It is believed that a small group of farmers must have selected some maize-like natural mutant of teosinte some 9000 years ago in Mexico, and subjected it to continuous selection to yield the maize plant as we know today.

Another interesting case is that of cauliflower. The edible cauliflower is a domesticated version of the wild plant *Brassica oleracea*, which does not possess the dense undifferentiated inflorescence called the curd, that cauliflower possesses.

Cauliflower possesses a single mutation in a gene called *CAL*, controlling meristem differentiation into inflorescence. This causes the cells at the floral meristem to gain an undifferentiated identity, and instead of growing into a flower, they grow into a lump of undifferentiated cells. This mutation has been selected through domestication at least since the Greek empire.

Chapter 3

Alternation of Generations

Alternation of generations (also known as **alternation of phases** or **metagenesis**) is a term primarily used in describing the life cycle of plants (taken here to mean the Archaeplastida). A multicellular diploid sporophyte, with N paired chromosomes (i.e. 2N in total), alternates with a multicellular haploid gametophyte, with N unpaired chromosomes. A mature sporophyte produces spores by meiosis, a process which results in a reduction of the number of chromosomes by a half. Spores germinate and grow into a gametophyte. At maturity, the gametophyte produces gametes by mitosis. Two gametes (originating from different organisms of the same species or from the same organism) fuse to produce a zygote, which develops into a diploid sporophyte. This cycle, from sporophyte to sporophyte (or equally from gametophyte to gametophyte), is the way in which all land plants and many algae undergo sexual reproduction.

All animals develop differently. A mature animal is diploid and so is, in one sense, equivalent to a sporophyte. However, an animal *directly* produces haploid gametes by meiosis. No haploid spores capable of dividing are produced, so neither is a haploid gametophyte. There is no alternation between diploid and haploid forms.

Other organisms, such as fungi, can have life cycles in which different kinds of organism alternate. The term 'alternation of generations' has also been applied to these cases.

Life cycles, such as those of plants, with alternating haploid and diploid phases can be referred to as **diplohaplontic** (the equivalent terms **haplodiplontic**, **diplobiontic** or **dibiontic** are also in use). Life cycles, such as those of animals, in which there is only a diploid phase are referred to as **diplontic**. (Life cycles in which there is only a haploid phase are referred to as **haplontic**.)

Definition

The discussion of 'alternation of generations' above treats the alternation of a multicellular diploid form with a multicellular haploid form as the defining characteristic, regardless of whether these forms are free-living or not. In some species, such as the alga *Ulva lactuca*, the diploid and haploid forms are indeed both free-living independent organisms, essentially identical in appearance. The free-swimming gametes form a

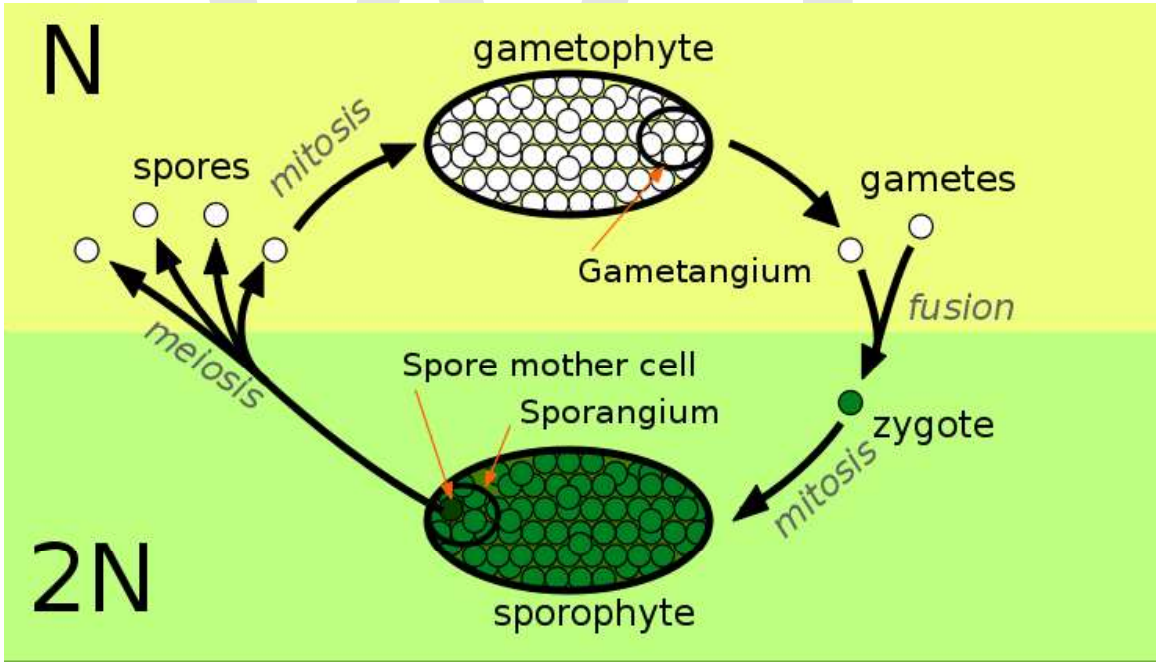
zygote which germinates into a diploid sporophyte; the free-swimming spores germinate into a haploid gametophyte. Alternation of *generations* is an appropriate term.

However, in other species, either the sporophyte or the gametophyte is very much reduced and is incapable of free-living. For example, in seed plants, the gametophyte 'generation' develops totally within the sporophyte which protects and nurtures it, with the sole exception of pollen grains, which are the 'male' gametophytes, but which have been reduced to only three cells. Here the notion of two generations is less obvious; as Bateman & Dimichele say "[s]porophyte and gametophyte effectively function as a single organism". The alternative term 'alternation of phases' may then be more appropriate.

Alternation of generations in plants

Fundamental elements

The diagram below shows the fundamental elements of the alternation of generations in plants. It is vital to have a good understanding of these fundamentals before considering the many variations found in different groups of plants. Starting from the right of the diagram, the processes involved are as follows:



- Two single-celled haploid gametes, each containing N unpaired chromosomes, fuse to form a single-celled diploid zygote, which now contains N paired chromosomes, i.e. 2N chromosomes in total.
- The single-celled diploid zygote germinates, dividing by the normal process (mitosis), which maintains the number of chromosomes at 2N. The result is a multi-cellular diploid organism, called the *sporophyte* (because at maturity it produces spores).

- When it reaches maturity, the sporophyte produces one or more **sporangia** (singular sporangium) which are the organs which produce diploid spore mother cells (sporocytes). These divide by a special process (meiosis) which reduces the number of chromosomes by a half. This results in four single-celled haploid spores, each containing N unpaired chromosomes.
- The single-celled haploid spore germinates, dividing by the normal process (mitosis), which maintains the number of chromosomes at N. The result is a multi-cellular haploid organism, called the *gametophyte* (because at maturity it produces gametes).
- When it reaches maturity, the gametophyte produces one or more **gametangia** (singular gametangium) which are the organs which produce haploid gametes. At least one kind of gamete possesses some mechanism for reaching another gamete in order to fuse with it.

The 'alternation of generations' in the life cycle is thus between a diploid (2N) generation of sporophytes and a haploid (N) generation of gametophytes.

Gametophyte of the fern *Onoclea sensibilis* (the flat thallus at the bottom of the picture) with a descendant sporophyte beginning to grow from it (the small frond at the top of the picture).

The situation is quite different in all animals, where the fundamental process is that a diploid (2N) individual *directly* produces haploid (N) gametes by meiosis. Spores (i.e. haploid cells which are able to undergo mitosis) are not produced, so neither is a haploid multi-cellular organism. The single-celled gametes are the only entities which are haploid.

Variations

The diagram shown above is a good representation of the life cycle of some multi-cellular algae (e.g. the genus *Cladophora*) which have sporophytes and gametophytes of very similar, if not identical, appearance, and which do not have different kinds of spores or gametes.

However, there are many possible variations on the fundamental elements of a life cycle which has alternation of generations. Each variation may occur separately or in combination, resulting in a bewildering variety of life cycles. The terms used by botanists in describing these life cycles can be equally bewildering. As Bateman and Dimichele say "[...] the alternation of generations has become a terminological morass; often, one term represents several concepts or one concept is represented by several terms."

Possible variations are:

- *Relative importance of the sporophyte and the gametophyte.*
    - *Equal* (**homomorphy** or **isomorphy**).
      Filamentous algae of the genus *Cladophora*, which are predominantly found in fresh water, have diploid sporophytes and haploid gametophytes which are externally indistinguishable. No living land plant has equally dominant sporophytes and gametophytes, although some theories of the evolution of alternation of generations suggest that ancestral land plants did.
    - *Unequal* (**heteromorphy** or **anisomorphy**).

Gametophyte of *Mnium hornum*, a moss.

- *Dominant gametophyte* (**gametophytic**).
  In liverworts, mosses and hornworts, the dominant form is the haploid gametophyte. The diploid sporophyte is not capable of an independent existence, gaining most of its nutrition from the parent gametophyte, and having no chlorophyll when mature.

Sporophyte of *Blechnum discolor*, a fern.

- *Dominant sporophyte* (**sporophytic**).
  In ferns, both the sporophyte and the gametophyte are capable of living independently, but the dominant form is the diploid sporophyte. The haploid gametophyte is much smaller and simpler in structure. In seed plants, the gametophyte is even more reduced (at the minimum to only three cells), gaining all its nutrition from the sporophyte. The extreme reduction in the size of the gametophyte and its retention within the sporophyte means that when applied to seed plants the term 'alternation of generations' is somewhat misleading: "[s]porophyte and gametophyte effectively function as a single organism". Some authors have preferred the term 'alternation of phases'.

- *Differentiation of the gametes.*
  - *Both gametes the same* (**isogamy**).
    Like other species of *Cladophora*, *C. callicoma* has flagellated gametes which are identical in appearance and ability to move.
  - *Gametes of two distinct sizes* (**anisogamy**).
    - *Both of similiar motility*.
      Species of *Ulva*, the sea lettuce, have gametes which all have two flagella and so are motile. However they are of two sizes: larger 'female' gametes and smaller 'male' gametes.
    - *One large and sessile, one small and motile* (**oogamy**). The larger sessile megagametes are eggs (ova), and smaller motile

microgametes are sperm (spermatazoa, spermatozoids). The degree of motility of the sperm may be very limited (as in the case of flowering plants) but all are able to move towards the sessile eggs. When (as is almost always the case) the sperm and eggs are produced in different kinds of gametangia, these are called **antheridia** (singular antheridium) and **archegonia** (singular archegonium) respectively.

Gametophyte of *Pellia epiphylla* with sporophytes growing from the remains of archegonia.

- *Antheridia and archegonia occur on the same gametophyte,* which is then called **monoicous**. (Many sources, particularly those concerned with bryophyes, use the term 'monoecious' for this situation and 'dioecious' for the opposite. Here 'monoecious' and 'dioecious' are used only for sporophytes.)
  The liverwort *Pellia epiphylla* has the gametophyte as the dominant generation. It is monoicous: the small reddish antheridia are scattered along the midrib while the archegonia grow nearer the tip of divisions of the plant.
- *Antheridia and archegonia occur on different gametophytes,* which are then called **dioicous**.
  The moss *Mnium hornum* has the gametophyte as the dominant generation. It is dioicous: male plants produce

only antheridia in terminal rosettes, female plants produce only archegonia in the form of stalked capsules. Seed plants are also dioicous; however, the extreme reduction of the gametophyte, particularly the microgametophyte, means that the antheridia and archegonia are microscopic.

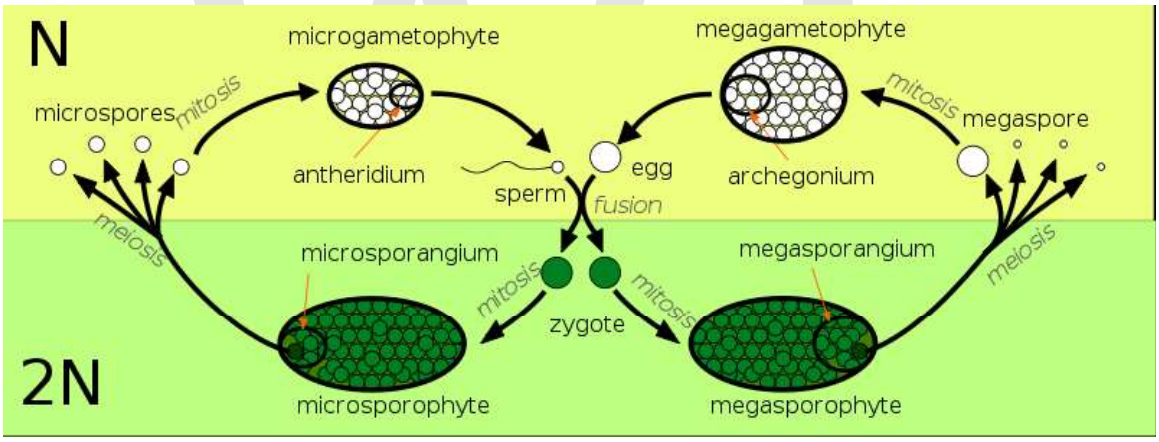
- *Differentiation of the spores.*
  - *All spores the same size* (**homospory** or isospory).
    Horsetails (species of *Equisetum*) have spores which are all of the same size.
  - *Spores of two distinct sizes* (**heterospory** or anisospory): larger **megaspores** and smaller **microspores**. When the two kinds of spore are produced in different kinds of sporangia, these are called **megasporangia** and **microsporangia**. A megaspore often (but not always) develops at the expense of the other three cells resulting from meiosis, which abort.
    - *Megasporangia and microsporangia occur on the same sporophyte,* which is then called **monoecious**.
      Most flowering plants fall into this category. Thus the flower of a lily contains six stamens (the microsporangia) which produce microspores which develop into pollen grains (the microgametophytes), and three fused carpels (the megasporangia) which produce megaspores which develop into ovules (the megagametophytes). In other plants, such as hazel, some flowers have only stamens, others only carpels, but the same plant (i.e. sporophyte) has both kinds of flower and so is monoecious.

Flowers of European Holly, a dioecious species: male above, female below (leaves cut to show flowers more clearly)

- *Megasporangia and microsporangia occur on different sporophytes,* which are then called **dioecious**.
  An individual tree of the European holly (*Ilex aquifolium*) produces either 'male' flowers which have only functional stamens (microsporangia) producing microspores which develop into pollen grains (microgametophytes) or 'female' flowers which have only functional carpels (megasporangia) producing megaspores which develop into ovules (megagametophytes).

There are some correlations between these variations, but they are just that, correlations, and not absolute. For example, in flowering plants, microspores ultimately produce microgametes (sperm) and megaspores ultimately produce megagametes (eggs). However, in pteridophytes there are groups with undifferentiated spores but differentiated gametophytes. For example, the fern *Ceratopteris thalictrioides* has spores of only one kind, which vary continuously in size. Smaller spores tend to produce gametophytes which have only sperm-producing antheridia.

A complex life cycle



The diagram shows the alternation of generations in a species which is heteromorphic, sporophytic, oogametic, dioicous, heterosporic and dioecious. A seed plant example is a willow tree (genus *Salix*). Starting in the centre of the diagram, the processes involved are:

- An immobile egg, typically remaining in the archegonium, fuses with a mobile sperm, released from an antheridium. The resulting zygote is either 'male' or 'female'.
- A 'male' zygote develops by mitosis into a microsporophyte, which at maturity produces one or more microsporangia. Microspores develop within the microsporangium by meiosis.
  In a willow (like all seed plants) the zygote first develops into a seed within the ovule (megasporangium). Later the seed is shed and grows into a mature tree. A 'male' willow tree (a microsporophyte) produces flowers with only stamens, the anthers of which are the microsporangia.

- Microspores germinate producing microgametophytes; at maturity one or more antheridia are produced. Sperm develop within the antheridia.
  In a willow, microspores are not liberated from the anther (the microsporangium), but develop into pollen grains (microgametophytes) within it. The whole pollen grain is moved (typically by an insect) to an ovule (megagametophyte), where a sperm is produced which moves down a pollen tube to reach the egg.
- A 'female' zygote develops by mitosis into a megasporophyte, which at maturity produces one or more megasporangia. Megaspores develop within the megasporangium; typically one of the four spores produced by meiosis gains bulk at the expense of the remaining three, which disappear.
  'Female' willow trees (megasporophytes) produce flowers with only carpels (the megasporangia).
- Megaspores germinate producing megagametophytes; at maturity one or more archegonia are produced. Eggs develop within the archegonia.
  In a willow, megaspores develop into ovules (megagametophytes) within the carpels (megasporangia). An archegonium develops within the ovule and produces an egg. All of this happens within the carpel (the megasporangium). The whole of the gametophytic 'generation' remains within the protection of the sporophyte except for pollen grains (which have been reduced to just three cells).

Life cycles of different plant groups

The term 'plants' is taken here to mean the Archaeplastida, i.e. the glaucophytes, red and green algae and land plants.

Alternation of generations occurs in almost all multicellular red and green algae, both freshwater forms (such as *Cladophora*) and seaweeds (such as *Ulva*). In most, the generations are homomorphic (isomorphic) and free-living. Some species of red algae have a complex triphasic alternation of generations, in which there is a gametophyte phase and two distinct sporophyte phases.

Land plants all have heteromorphic (anisomorphic) alternation of generations, in which the sporophyte and gametophyte are distinctly different. All bryophytes, i.e. liverworts, mosses and hornworts, have the gametophyte generation as the most conspicuous. As an illustration, consider a monoicous moss. Antheridia and archegonia develop on the mature plant (the gametophyte). In the presence of water, the biflagellate sperm from the antheridia swim to the archegonia and fertilisation occurs, leading to the production of a diploid sporophyte. The sporophyte grows up from the archegonium. Its body comprises a long stalk topped by a capsule within which spore-producing cells undergo meiosis to form haploid spores. Most mosses rely on the wind to disperse these spores.

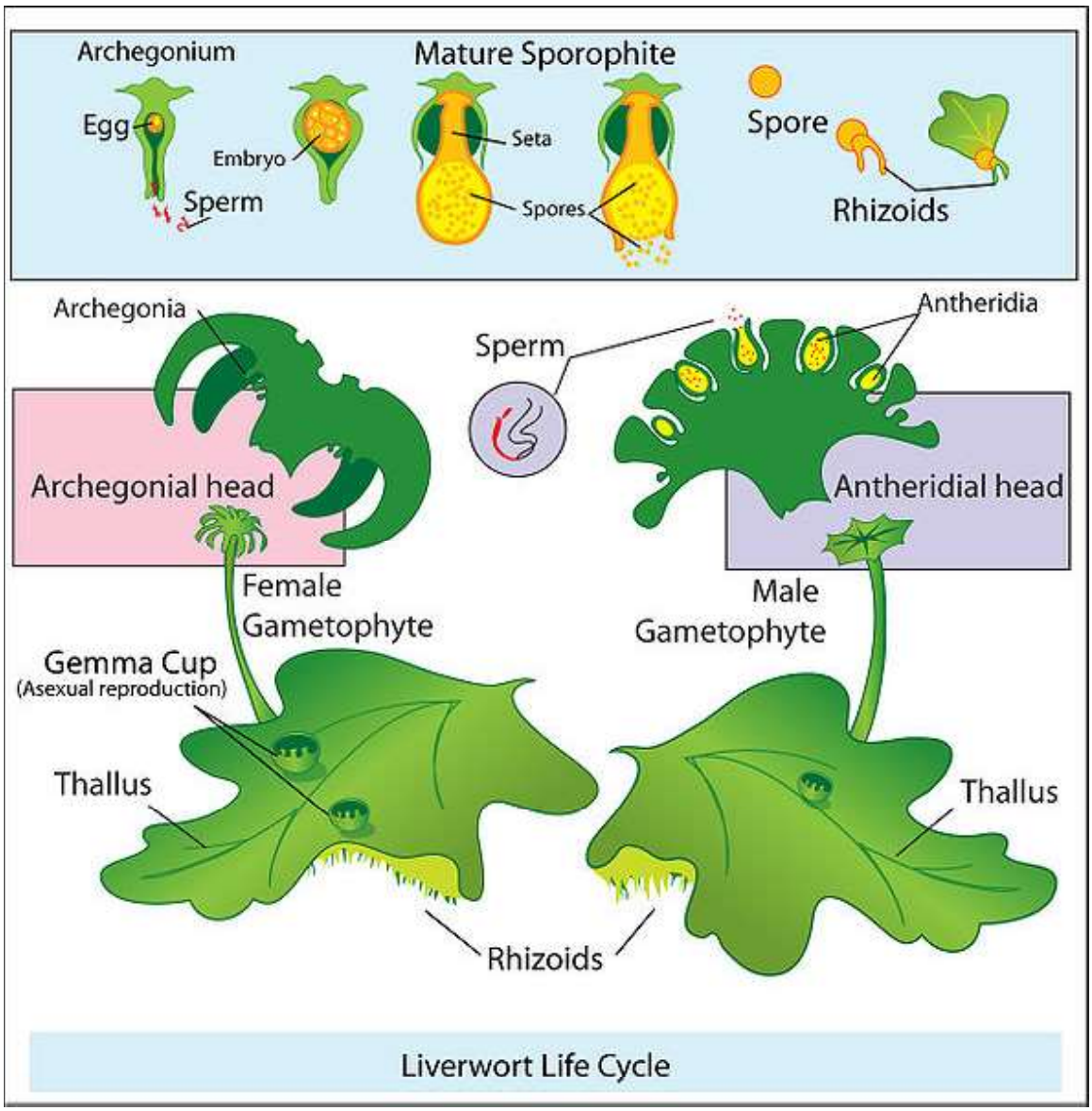
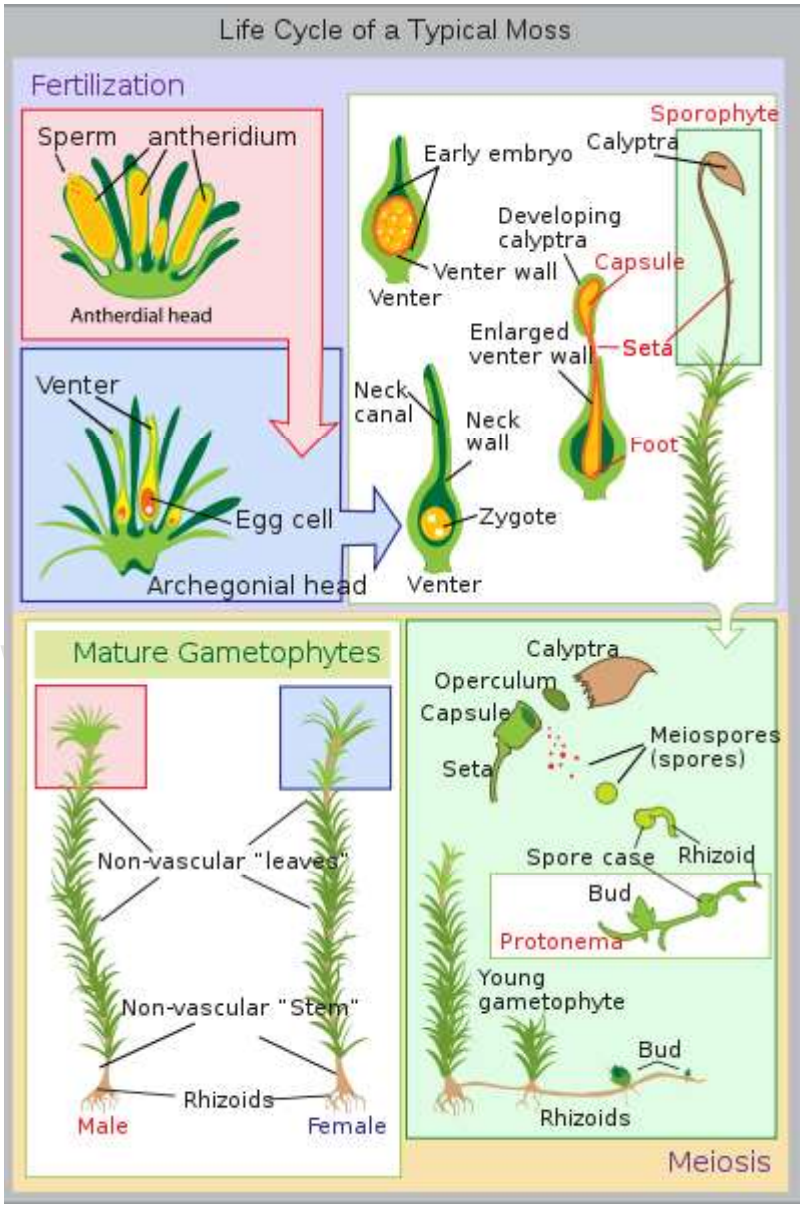
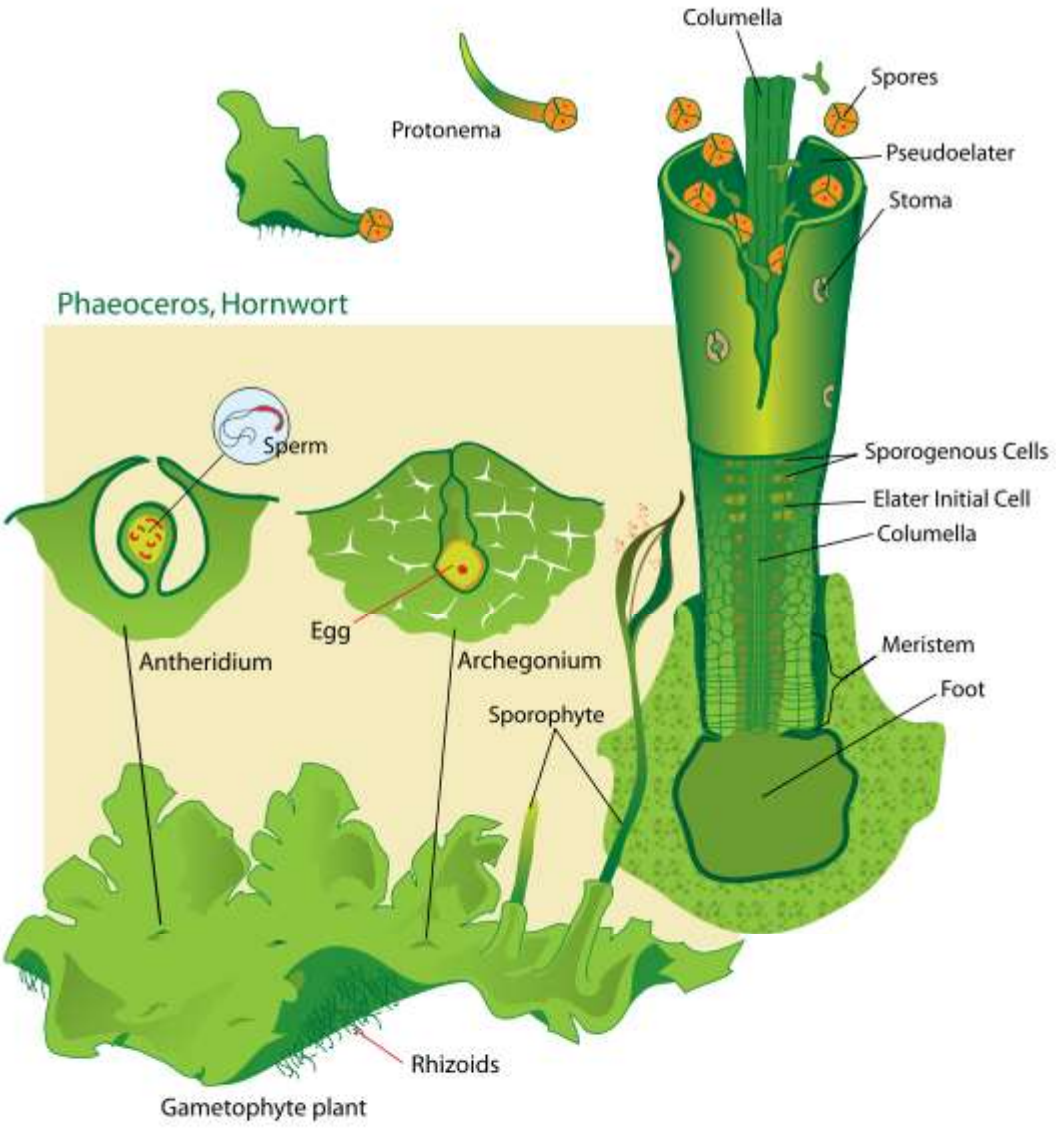


Diagram of alternation of generations in liverworts.



Moss life cycle diagram



Hornwort life cycle diagram

In ferns and their allies, including clubmosses and horsetails, the conspicuous plant observed in the field is the diploid sporophyte. The haploid spores develop in sori on the underside of the fronds and are dispersed by the wind (or in some cases, by floating on water). If conditions are right, a spore will germinate and grow into a rather inconspicuous plant body called a prothallus. The haploid prothallus does not resemble the sporophyte, and as such ferns and their allies have a heteromorphic alternation of generations. The prothallus is short-lived, but carries out sexual reproduction, producing the diploid zygote that then grows out of the prothallus as the sporophyte.

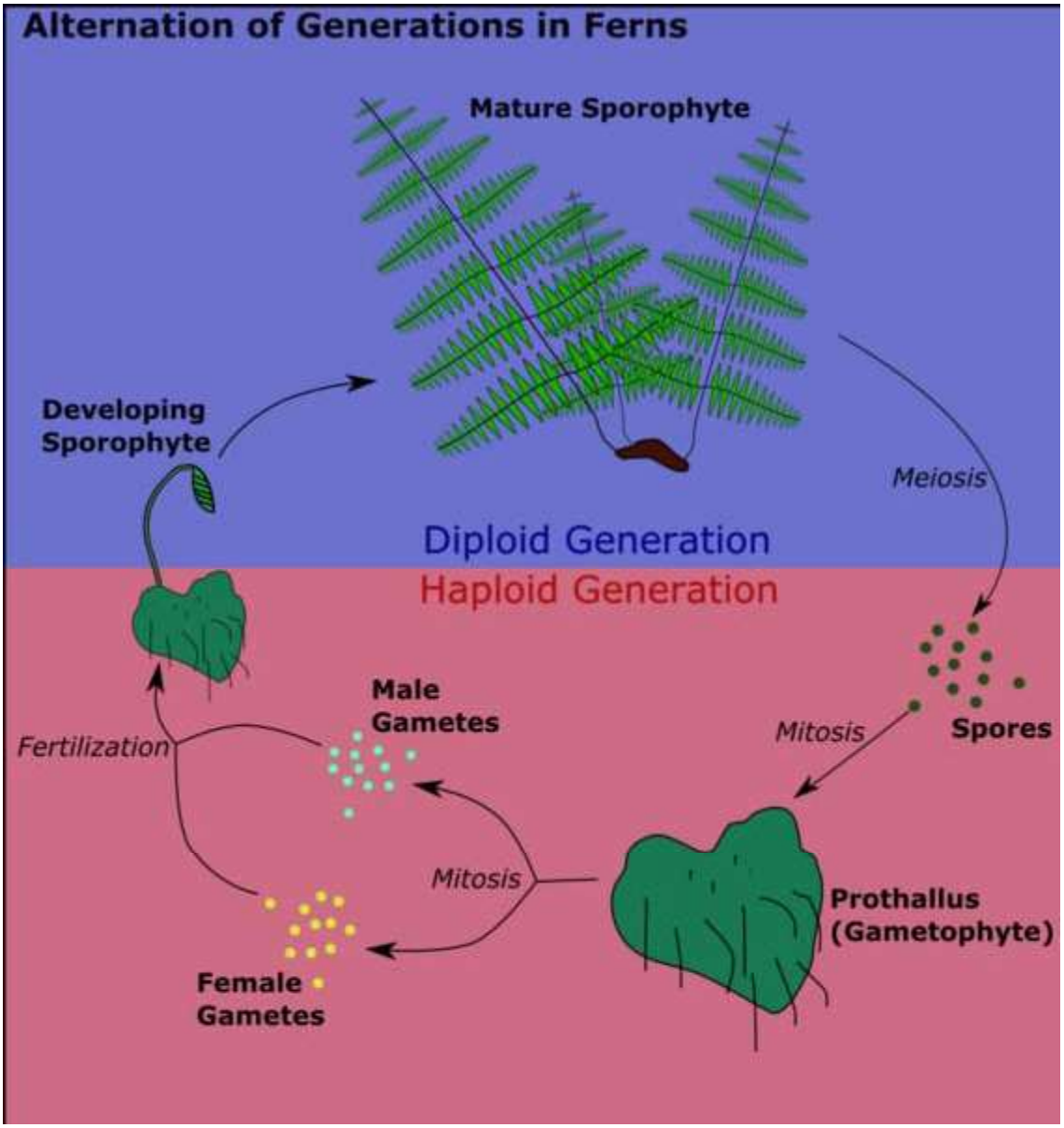


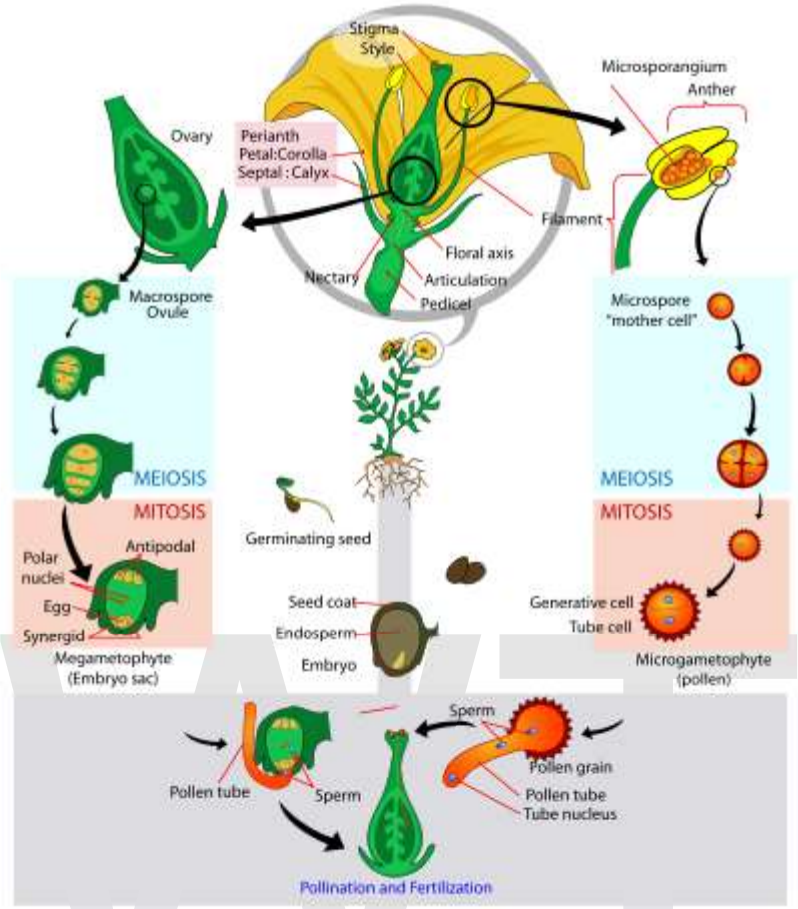
Diagram of alternation of generations in ferns.

A gametophyte (prothallus) of *Dicksonia sp.*

A sporophyte of *Dicksonia antarctica*.

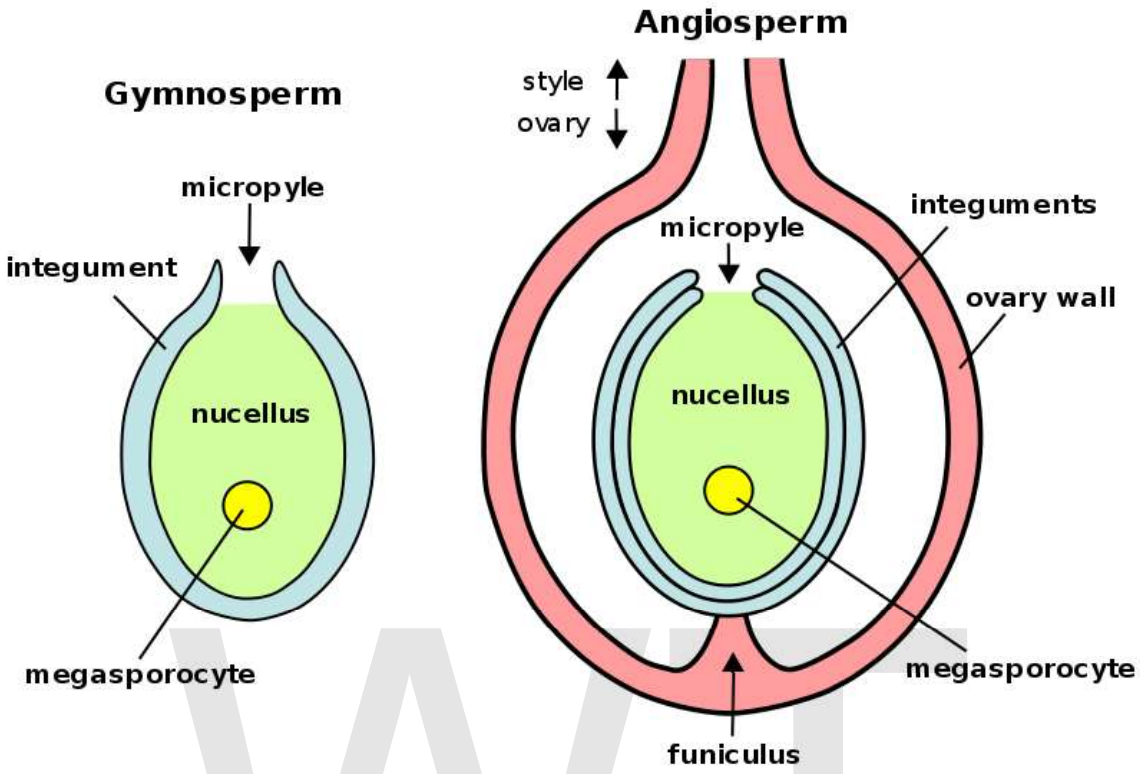
The underside of a *Dicksonia antarctica* frond showing the sori, or spore-producing structures.

In the spermatophytes, the seed plants, the sporophyte is the dominant multicellular phase; the gametophytes are strongly reduced in size and very different in morphology. The entire gametophyte generation, with the sole exception of pollen grains (microgametophytes), is contained within the sporophyte. The life cycle of a dioecious flowering plant (angiosperm), the willow, has been outlined in some detail in an earlier section (A complex life cycle). The life cycle of a gymnosperm is similar. However, flowering plants have in addition a phenomenon called 'double fertilization'. Two sperm nuclei from a pollen grain (the microgametophyte), rather than a single sperm, enter the archegonium of the megagametophyte; one fuses with the egg nucleus to form the zygote, the other fuses with two other nuclei of the gametophyte to form 'endosperm', which nourishes the developing embryo.

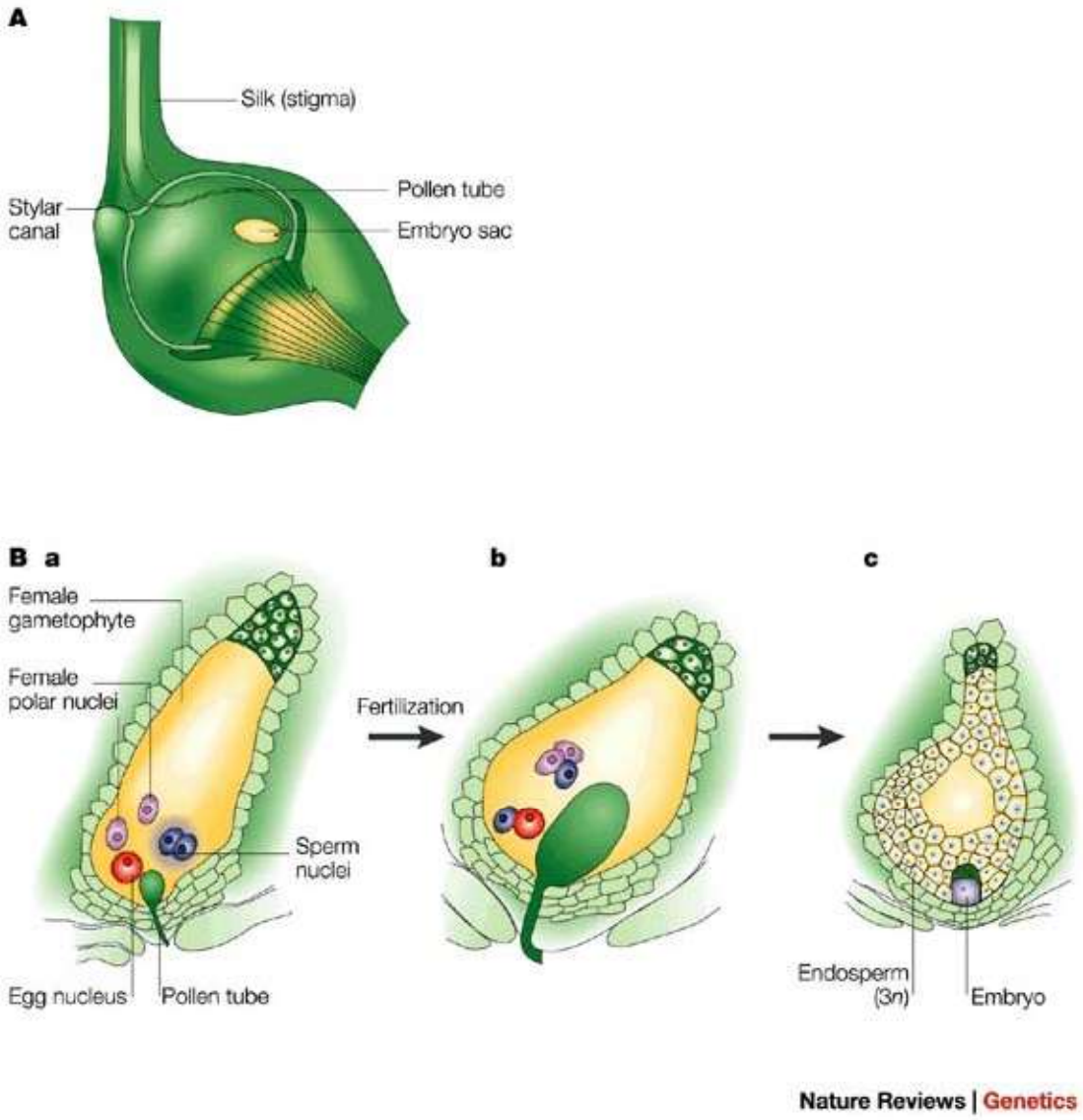


Angiosperm life cycle

Tip of tulip stamen showing pollen (microgametophytes)



Plant ovules (megagametophytes): Gymnosperm ovule on left, angiosperm ovule (inside ovary) on right



Double fertilization

Other groups of organism

Some organisms currently classified in the Chromalveolata, and thus not plants in the sense used here, exhibit alternation of generations. Kelp are an example of a brown alga with a heteromorphic alternation of generations. Species from the genus *Laminaria* have a large sporophytic thallus that produces haploid spores which germinate to produce free-living microscopic male and female gametophytes. Foraminifera undergo a heteromorphic alternation of generations between haploid ***gamont*** and diploid ***agamont*** forms. The single-celled haploid organism is typically much larger than the diploid organism.

Fungal mycelia are typically haploid. When mycelia of different mating types meet, they produce two multinucleate ball-shaped cells, which join via a "mating bridge". Nuclei move from one mycelium into the other, forming a ***heterokaryon*** (meaning "different nuclei"). This process is called ***plasmogamy***. Actual fusion to form diploid nuclei is called ***karyogamy***, and may not occur until sporangia are formed. Karogamy produces a diploid zygote, which is a short-lived sporophyte that soon undergoes meiosis to form haploid spores. When the spores germinate, they develop into new mycelia.

The life cycle of slime molds is very similar to that of fungi. Haploid spores germinate to form swarm cells or ***myxamoebae***. These fuse in a process referred to as *plasmogamy* and *karyogamy* to form a diploid zygote. The zygote develops into a plasmodium, and the mature plasmodium produces, depending on the species, one to many fruiting bodies containing haploid spores.

In some animals, there is an alternation between parthenogenic and sexually reproductive phases (**heterogamy**). Although in some ways similar to alternation of generations, the genetics of heterogamy is significantly different.

Chapter 4

Plant Reproduction

Plant reproduction is the production of new individuals or offspring in plants, which can be accomplished by sexual or asexual means. Sexual reproduction produces offspring by the fusion of gametes, resulting in offspring genetically different from the parent or parents. Asexual reproduction produces new individuals without the fusion of gametes, genetically identical to the parent plants and each other, except when mutations occur. In seed plants, the offspring can be packaged in a protective seed, which is used as an agent of dispersal.

Asexual reproduction

Plants have two main types of asexual reproduction in which new plants are produced that are genetically identical clones of the parent individual. "Vegetative" reproduction involves a vegetative piece of the original plant (budding, tillering, etc.) and is distinguished from "apomixis", which is a "replacement" for sexual reproduction, and in some cases involves seeds. Apomixis occurs in many plant species and also in some non-plant organisms.

Natural vegetative reproduction is mostly a process found in herbaceous and woody perennial plants, and typically involves structural modifications of the stem or roots and in a few species leaves. Most plant species that employ vegetative reproduction, do so as a means to perennialize the plants, allowing them to survive from one season to the next and often facilitating their expansion in size. A plant that persists in a location through vegetative reproduction of individuals constitutes a clonal colony, a single ramet, or apparent individual, of a clonal colony is genetically identical to all others in the same colony. The distance that a plant can move during vegetative reproduction is limited, though some plants can produce ramets from branching rhizomes or stolons that cover a wide area, often in only a few growing seasons. In a sense, this process is not one of "reproduction" but one of survival and expansion of biomass of the individual. When an individual organism increases in size via cell multiplication and remains intact, the process is called "vegetative growth". However, in vegetative reproduction, the new plants that result are new individuals in almost every respect except genetic. A major disadvantage to vegetative reproduction, is the transmission of pathogens from parent to

daughter plants; it is uncommon for pathogens to be transmitted from the plant to its seeds, though there are occasions when it occurs.

Seeds generated by apomixis are a means of asexual reproduction, involving the formation and dispersal of seeds that do not originate from the fertilization of the embryos. Hawkweed (*Hieracium*), dandelion (*Taraxacum*), some Citrus (*Citrus*) and Kentucky blue grass (*Poa pratensis*) all use this form of asexual reproduction. Pseudogamy occurs in some plants that have apomictic seeds, where pollination is often needed to initiate embryo growth, though the pollen contributes no genetic material to the developing offspring. Other forms of apomixis occur in plants also, including the generation of a plantlet in replacement of a seed or the generation of bulbils instead of flowers, where new cloned individuals are produced.

Natural vegetative structures

The **rhizome** is a modified underground stem serving as an organ of vegetative reproduction, e. g. Polypody, Iris, Couch Grass and Nettles.

Prostrate aerial stems, called **runners** or **stolons** are important vegetative reproduction organs in some species, such as the strawberry, numerous grasses, and some ferns.

Adventitious buds form on roots near the ground surface, on damaged stems (as on the stumps of cut trees), or on old roots. These develop into above-ground stems and leaves.

A form of budding called **suckering** is the reproduction or regeneration of a plant by shoots that arise from an existing root system. Species that characteristically produce suckers include Elm (*Ulmus*), Dandelion (*Taraxacum*), and members of the Rose Family (*Rosa*).

Another type of a vegetative reproduction is the production of bulbs. Plants like onion (*Allium cepa*), hyacinth (*Hyacinth*), narcissus (*Narcissus*) and tulips (*Tulipa*) reproduce by forming bulbs.

Other plants like potatoes (*Solanum tuberosum*) and dahlia (*Dahlia*) reproduce by a method similar to bulbs: they produce tubers.

Gladioli and crocuses (*Crocus*) reproduce by forming a bulb-like structure called a corm.

Human uses of asexual reproduction

The most common form of plant reproduction utilized by people is seeds, but a number of asexual methods are utilized which are usually enhancements of natural processes, including: cutting, grafting, budding, layering, division, sectioning of rhizomes or roots, stolons, tillers (suckers) and artificial propagation by laboratory tissue cloning. Asexual methods are most often used to propagate cultivars with individual desirable characteristics that do not come true from seed. Fruit tree propagation is frequently

performed by budding or grafting desirable cultivars (clones), onto rootstocks that are also clones, propagated by layering.

In horticulture, a "cutting" is a branch that has been cut off from a mother plant below an internode and then rooted, often with the help of a rooting liquid or powder containing hormones. When a full root has formed and leaves begin to sprout anew, the clone is a self-sufficient plant, genetically identical to the mother plant. Examples include cuttings from the stems of blackberries (*Rubus occidentalis*), African violets (*Saintpaulia*), verbenas (*Verbena*) to produce new plants. A related use of cuttings is grafting, where a stem or bud is joined onto a different stem. Nurseries offer for sale trees with grafted stems that can produce four or more varieties of related fruits, including apples. The most common usage of grafting is the propagation of cultivars onto already rooted plants, sometimes the rootstock is used to dwarf the plants or protect them from root damaging pathogens.

Since vegetatively propagated plants are clones, they are important tools in plant research. When a clone is grown in various conditions, differences in growth can be ascribes to environmental effects instead of genetic differences.

Sexual reproduction

Sexual reproduction involves two fundamental processes, meiosis which rearranges the genes and reduces the number of chromosomes, and fusion of gametes which restores the chromosome to a complete diploid number. In between these two processes, different types of plants vary. In plants and algae that undergo alternation of generations, a gametophyte is the multicellular structure, or phase, that is haploid, containing a single set of chromosomes:

The gametophyte produces male or female gametes (or both), by a process of cell division called mitosis. The fusion of male and female gametes produces a diploid zygote, which develops by repeated mitotic cell divisions into a multicellular sporophyte. Because the sporophyte is the product of the fusion of two haploid gametes, its cells are diploid, containing two sets of chromosomes. The mature sporophyte produces spores by a process called meiosis, sometimes referred to as "reduction division" because the chromosome pairs are separated once again to form single sets. The spores are therefore once again haploid and develop into a haploid gametophyte. In land plants such as ferns, mosses and liverworts the gametophyte is very small, as in ferns and their relatives. In flowering plants (angiosperms) It is reduced to only a few cells, where the female gametophyte (embryo sac) is known as a megagametophyte and the male gametophyte (pollen) is called a microgametophyte.

History of sexual reproduction

Unlike animals, plants are immobile, and cannot seek out sexual partners for reproduction. In the evolution of early plants, abiotic means, including water and wind,

transported sperm for reproduction. The first plants were aquatic and released sperm freely into the water to be carried with the currents. Primitive land plants like liverworts and mosses had motile sperm that swam in a thin film of water or were splashed in water droplets from the male reproduction organs onto the female organs. As taller and more complex plants evolved, modifications in the alternation of generations evolved; in the Paleozoic era progymnosperms reproduced by using spores dispersed on the wind. The seed plants including seed ferns, conifers and cordaites, which were all gymnosperms, evolved 350 million years ago; they had pollen grains that contained the male gametes for protection of the sperm during the process of transfer from the male to female parts. It is believed that insects fed on the pollen, and plants thus evolved to use insects to actively carry pollen from one plant to the next. Seed producing plants, which include the angiosperms and the gymnosperms, have heteromorphic alternation of generations with large sporophytes containing much reduced gametophytes. Angiosperms have distinctive reproductive organs called flowers, with carpels, and the female gametophyte is greatly reduced to a female embryo sac, with as few as eight cells. The male gametophyte consists of the pollen grains. The sperm of seed plants are non-motile, except for two older groups of plants, the Cycadophyta and the Ginkgophyta, which have flagellated sperm.

Flowering plants

Flowering plants are the dominant plant form on land and they reproduce by sexual and asexual means. Often their most distinguishing feature is their reproductive organs, commonly called flowers. Sexual reproduction in flowering plants involves the production of male and female gametes, the transfer of the male gametes to the female ovules in a process called pollination. After pollination occurs, fertilization happens and the ovules grow into seeds with in a fruit. After the seeds are ready for dispersal, the fruit ripens and by various means the seeds are freed from the fruit and after varying amounts of time and under specific conditions the seeds germinate and grow into the next generation.

The anther produces male gametophytes, the sperm is produced in pollen grains, which attach to the stigma on top of a carpel, in which the female gametophytes (inside ovules) are located. After the pollen tube grows through the carpel's style, the sex cell nuclei from the pollen grain migrate into the ovule to fertilize the egg cell and endosperm nuclei within the female gametophyte in a process termed double fertilization. The resulting zygote develops into an embryo, while the triploid endosperm (one sperm cell plus two female cells) and female tissues of the ovule give rise to the surrounding tissues in the developing seed. The ovary, which produced the female gametophyte(s), then grows into a fruit, which surrounds the seed(s). Plants may either self-pollinate or cross-pollinate. Nonflowering plants like ferns, moss and liverworts use other means of sexual reproduction.

Adaptations

An Orchid flower.

Flowers of wind pollinated plants tend to lack petals and or sepals. Typically large amounts of pollen are produced and pollination often occurs early in the growing season before leaves can interfere with the dispersal of the pollen. Many trees and all grasses and sedges are wind pollinated, as such they have no need for large fancy flowers. In plants that use insects or other animals to move pollen from one flower to the next, plants have developed greatly modified flower parts to attract pollinators and to facilitate the movement of pollen from one flower to the insect and from the insect back to the next flower. Plants have a number of different means to attract pollinators including color, scent, heat, nectar glands, eatable pollen and flower shape. Along with modifications involving the above structures two other conditions play a very important role in the sexual reproduction of flowering plants, the first is timing of flowering and the other is the size or number of flowers produced. Often plant species have a few large, very showy flower while others produce many small flowers, often flowers are collected together into large inflorescences to maximize their visual effect, becoming more noticeable to passing pollinators. Flowers are attraction strategies and sexual expressions are functional strategies used to produce the next generation of plants, with pollinators and plants having co-evolved, often to some extraordinary degrees, very often rendering mutual benefit.

Flower heads showing disk and ray florets.

The largest family of flowering plants is the orchids (Orchidaceae), estimated by some specialists to include up to 35,000 species, which often have highly specialized flowers used to attract insects and facilitate pollination. The stamens are modified to produce pollen in clusters called pollinium, which are attached to insects when crawling into the flower. The flower shapes are modified to force insects to pass by the pollen, which is "glued" to the insect. Some orchids are even more highly specialized, with flower shapes that mimic the shape of insects to attract them to 'mate' with the flowers, a few even have scents that mimic insect pheromones.

Another large group of flowering plants is the Asteraceae or sunflower family with close to 22,000 species, which also have highly modified inflorescences that are flowers collected together in heads composed of a composite of individual flowers called florets.

Heads with florets of one sex, when the flowers are pistillate or functionally staminate, or made up of all bisexual florets, are called homogamous and can include discoid and liguliflorous type heads. Some radiate heads may be homogamous too. Plants with heads that have florets of two or more sexual forms are called heterogamous and include radiate and disciform head forms, though some radiate heads may be heterogamous too.

Ferns

Ferns typically produce large diploid sporophytes with rhizomes, roots and leaves; and on fertile leaves called sporangium, spores are produced. The spores are released and germinate to produce short, thin gametophytes that are typically heart shaped, small and green in color. The gametophytes or thallus, produce both motile sperm in the antheridia and egg cells in separate archegonia. After rains or when dew deposits a film of water, the motile sperm are splashed away from the antheridia, which are normally produce on the top side of the thallus, and swim in the film of water to the antheridia where they fertilize the egg. To promote out crossing or cross fertilization the sperm are released before the eggs are receptive of the sperm, making it more likely that the sperm will fertilize the eggs of different thallus. A zygote is formed after fertilization, which grows into a new sporophytic plant. The condition of having separate sporephyte and gametophyte plants is call alternation of generations. Other plants with similar reproductive means include the *Psilotum*, *Lycopodium*, *Selaginella* and *Equisetum*.

Bryophytes

The bryophytes, which include liverworts, hornworts and mosses, reproduce both sexually and vegetatively. The gametophyte is the most commonly known phase of the plant. An early developmental stage in the gametophyte of mosses (immediately following germination of the meiospore) is called the protonema. All are small plants found growing in moist locations and like ferns, have motile sperm with flagella and need water to facilitate sexual reproduction. These plants start as a haploid spore that grows into the dominate form, which is a multicellular haploid body with leaf-like structures that photosynthesize. Haploid gametes are produced in antherida and archegonia by mitosis. The sperm released from the antherida respond to chemicals released by ripe archegonia and swim to them in a film of water and fertilize the egg cells thus producing a zygote. The zygote divides by mitotic division and grows into a sporophyte that is diploid. The multicellular diploid sporophyte produces structures called spore capsules, which are connected by seta to the archegonia. The spore capsules produce spores by meiosis, when ripe the capsules burst open and the spores are released. Bryophytes show considerable variation in their breeding structures and the above is a basic outline. Also in some species each plant is one sex while other species produce both sexes on the same plant.

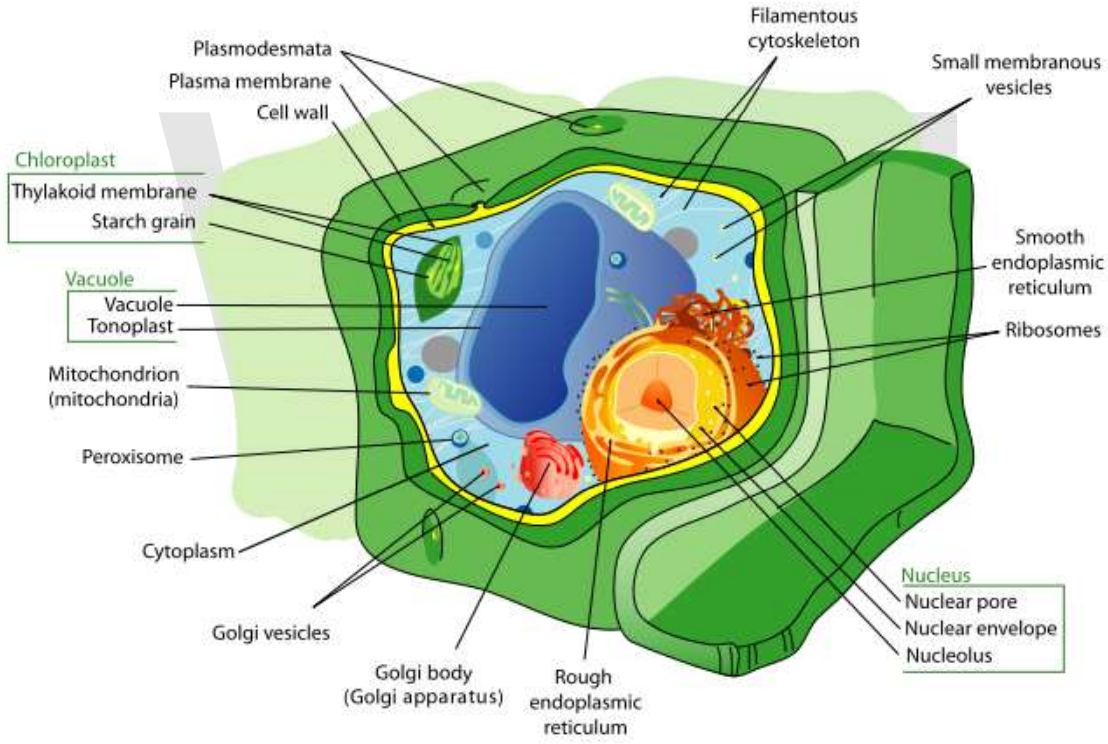
Sexual expression

Many plants have evolved a complex sexuality, which is expressed in different combinations of their reproductive organs. Some species have separate male and female

individuals, some have separate male and female flowers on the same plant, abut the majority of plants have both male and female parts in the same flower. Some plants change their gender expression depending on a number of factors like age, time of day, or because of environmental conditions. Plant sexuality also varies within different populations of some species.

Chapter 5

Plant Cell



Plant cell structure

Plant cells are eukaryotic cells that differ in several key respects from the cells of other eukaryotic organisms. Their distinctive features include:

- A large central vacuole, a water-filled volume enclosed by a membrane known as the *tonoplast* maintains the cell's turgor, controls movement of molecules between the cytosol and sap, stores useful material and digests waste proteins and organelles.

- A cell wall composed of cellulose and hemicellulose, pectin and in many cases lignin, are secreted by the protoplast on the outside of the cell membrane. This contrasts with the cell walls of fungi (which are made of chitin), and of bacteria, which are made of peptidoglycan.

- Specialised cell-cell communication pathways known as plasmodesmata, pores in the primary cell wall through which the plasmalemma and endoplasmic reticulum of adjacent cells are continuous.

- Plastids, the notables one being the chloroplasts, which contain chlorophyll and the biochemical systems for light harvesting and photosynthesis, but also amyloplasts specialized for starch storage, elaioplasts specialized for fat storage, and chromoplasts specialized for synthesis and storage of pigments. As in mitochondria, which have a genome encoding 37 genes, plastids have their own genomes of about 100-120 unique genes and, it is presumed, arose as prokaryotic endosymbionts living in the cells of an early eukaryotic ancestor of the land plants and algae.

- Unlike animal cells, plant cells are stationary.

- Cell division by construction of a phragmoplast as a template for building a cell plate late in cytokinesis is characteristic of land plants and a few groups of algae, the notable one being the Charophytes and the Order Trentepohliales

- The sperm of bryophytes have flagellae similar to those in animals, but higher plants, (including Gymnosperms and flowering plants) lack the flagellae and centrioles that are present in animal cells.

Cell types

- Parenchyma cells are living cells that have diverse functions ranging from storage and support to photosynthesis and phloem loading (transfer cells). Apart from the xylem and phloem in its vascular bundles, leaves are composed mainly of parenchyma cells. Some parenchyma cells, as in the epidermis, are specialized for light penetration and focusing or regulation of gas exchange, but others are among the least specialized cells in plant tissue, and may remain totipotent, capable of dividing to produce new populations of undifferentiated cells, throughout their lives. Parenchyma cells have thin, permeable primary walls enabling the transport of small molecules between them, and their cytoplasm is responsible for a wide range of biochemical functions such as nectar secretion, or the manufacture of secondary products that discourage herbivory. Parenchyma cells that contain many chloroplasts and are concerned primarily with photosynthesis are called chlorenchyma cells. Others, such as the majority of the parenchyma cells in potato tubers and the seed cotyledons of legumes, have a storage function.

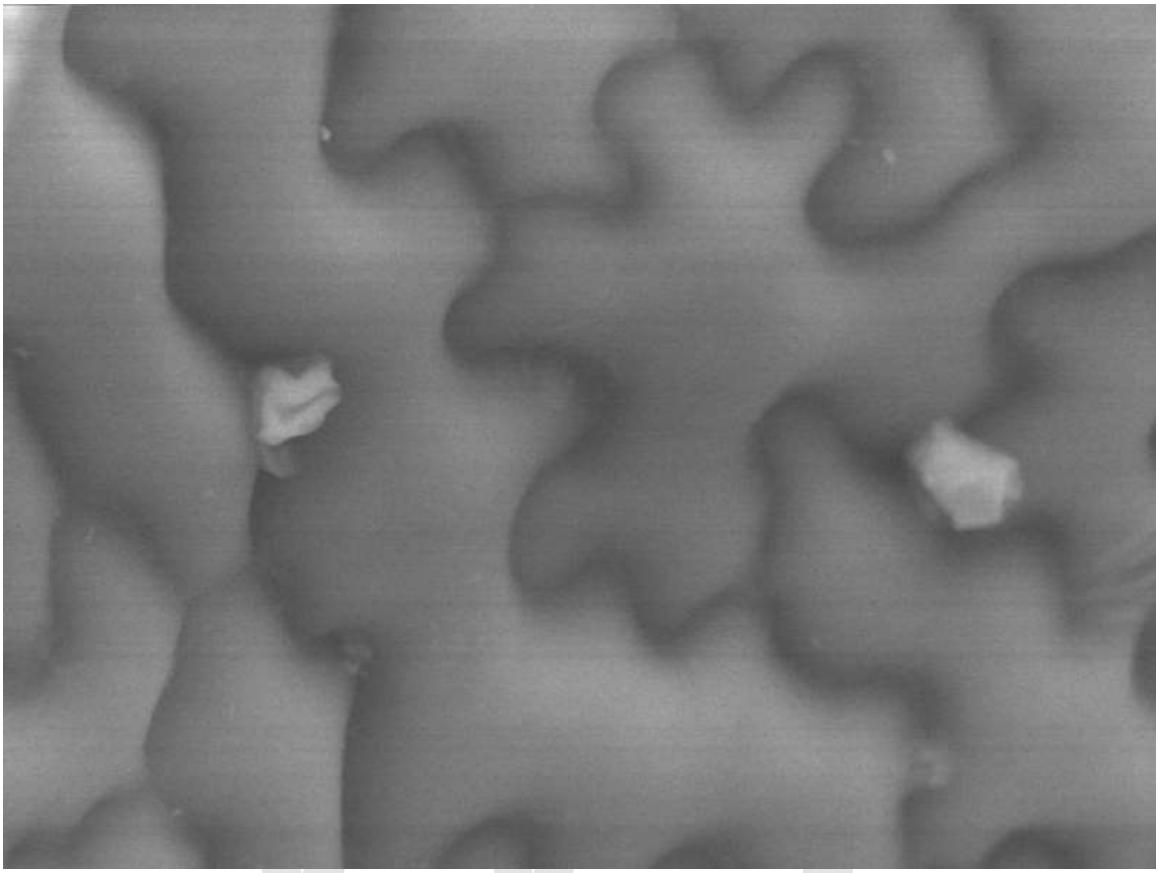
- Collenchyma cells - collenchyma cells are alive at maturity and have only a primary wall. These cells mature from meristem derivatives that initially resemble parenchyma, but differences quickly become apparent. Plastids do not develop, and the secretory apparatus (ER and Golgi) proliferates to secrete additional primary wall. The wall is most commonly thickest at the corners, where three or more cells come in contact, and thinnest where only two cells come in contact, though other arrangements of the wall thickening are possible.

Pectin and hemicellulose are the dominant constituents of collenchyma cell walls of dicotyledon angiosperms, which may contain as little as 20% of cellulose in *Petasites*. Collenchyma cells are typically quite elongated, and may divide transversely to give a septate appearance. The role of this cell type is to support the plant in axes still growing in length, and to confer flexibility and tensile strength on tissues. The primary wall lacks lignin that would make it tough and rigid, so this cell type provides what could be called plastic support - support that can hold a young stem or petiole into the air, but in cells that can be stretched as the cells around them elongate. Stretchable support (without elastic snap-back) is a good way to describe what collenchyma does. Parts of the strings in celery are collenchyma.

- Sclerenchyma cells - Sclerenchyma cells (from the Greek **skleros**, *hard*) are hard and tough cells with a function in mechanical support. They are of two broad types – sclereids or stone cells and fibres. The cells develop an extensive secondary cell wall that is laid down on the inside of the primary cell wall. The secondary wall is impregnated with lignin, making it hard and impermeable to water. Thus, these cells cannot survive for long' as they cannot exchange sufficient material to maintain active metabolism. Sclerenchyma cells are typically dead at functional maturity, and the cytoplasm is missing, leaving an empty central cavity.

Functions for sclereid cells (hard cells that give leaves or fruits a gritty texture) include discouraging herbivory, by damaging digestive passages in small insect larval stages, and physical protection (a solid tissue of hard sclereid cells form the pit wall in a peach and many other fruits). Functions of fibres include provision of load-bearing support and tensile strength to the leaves and stems of herbaceous plants. Sclerenchyma fibres are not involved in conduction, either of water and nutrients (as in the xylem) or of carbon compounds (as in the phloem), but it is likely that they may have evolved as modifications of xylem and phloem initials in early land plants.

Tissue types



cells of *Arabidopsis thaliana* epidermis

The major classes of cells differentiate from undifferentiated meristematic cells (analogous to the stem cells of animals) to form the tissue structures of roots, stems, leaves, flowers, and reproductive structures.

Xylem cells are elongated cells with lignified secondary thickening of the cell walls. Xylem cells are specialised for conduction of water, and first appeared in plants during their transition to land in the Silurian period more than 425 million years ago. The possession of xylem defines the vascular plants or Tracheophytes. Xylem tracheids are pointed, elongated xylem cells, the simplest of which have continuous primary cell walls and lignified secondary wall thickenings in the form of rings, hoops, or reticulate networks. More complex tracheids with valve-like perforations called bordered pits characterise the gymnosperms. The ferns and other pteridophytes and the gymnosperms have only xylem tracheids, while the angiosperms also have xylem vessels. Vessel members are hollow xylem cells aligned end-to-end, without end walls that are assembled into long continuous tubes. The bryophytes lack true xylem cells, but their sporophytes have a water-conducting tissue known as the hydrome that is composed of elongated cells of simpler construction.

Phloem is a specialised tissue for food conduction in higher plants. The conduction of food is a complex process that is carried in the plant with the help of special cell called phloem cells. These cells conduct inter- and intra-cellular fluid (food - proteins and other essential elements required by the plant for its metabolism) through the process of osmosis. This phenomenon is called ascent of sap in plants. Phloem consists of two cell types, the sieve tubes and the intimately-associated companion cells. The sieve tube elements lack nuclei and ribosomes, and their metabolism and functions are regulated by the adjacent nucleate companion cells. Sieve tubes are joined end-to-end with perforate end-plates between known as *sieve plates*, which allow transport of photosynthate between the sieve elements. The companion cells, connected to the sieve tubes via plasmodesmata, are responsible for loading the phloem with sugars. The bryophytes lack phloem, but moss sporophytes have a simpler tissue with analogous function known as the leptome.

Plant epidermal cells are specialised parenchyma cells covering the external surfaces of leaves, stems and roots. The epidermal cells of aerial organs arise from the superficial layer of cells known as the *tunica* (L1 and L2 layers) that covers the plant shoot apex, whereas the cortex and vascular tissues arise from innermost layer of the shoot apex known as the *corpus* (L3 layer). The epidermis of roots originates from the layer of cells immediately beneath the root cap.

The epidermis of all aerial organs, but not roots, is covered with a cuticle made of waxes and the polyester cutin. Several cell types may be present in the epidermis. Notable among these are the stomatal guard cells, glandular and clothing hairs or trichomes, and the root hairs of primary roots. In the shoot epidermis of most plants, only the guard cells have chloroplasts. The epidermal cells of the primary shoot are thought to be the only plant cells with the biochemical capacity to synthesize cutin.

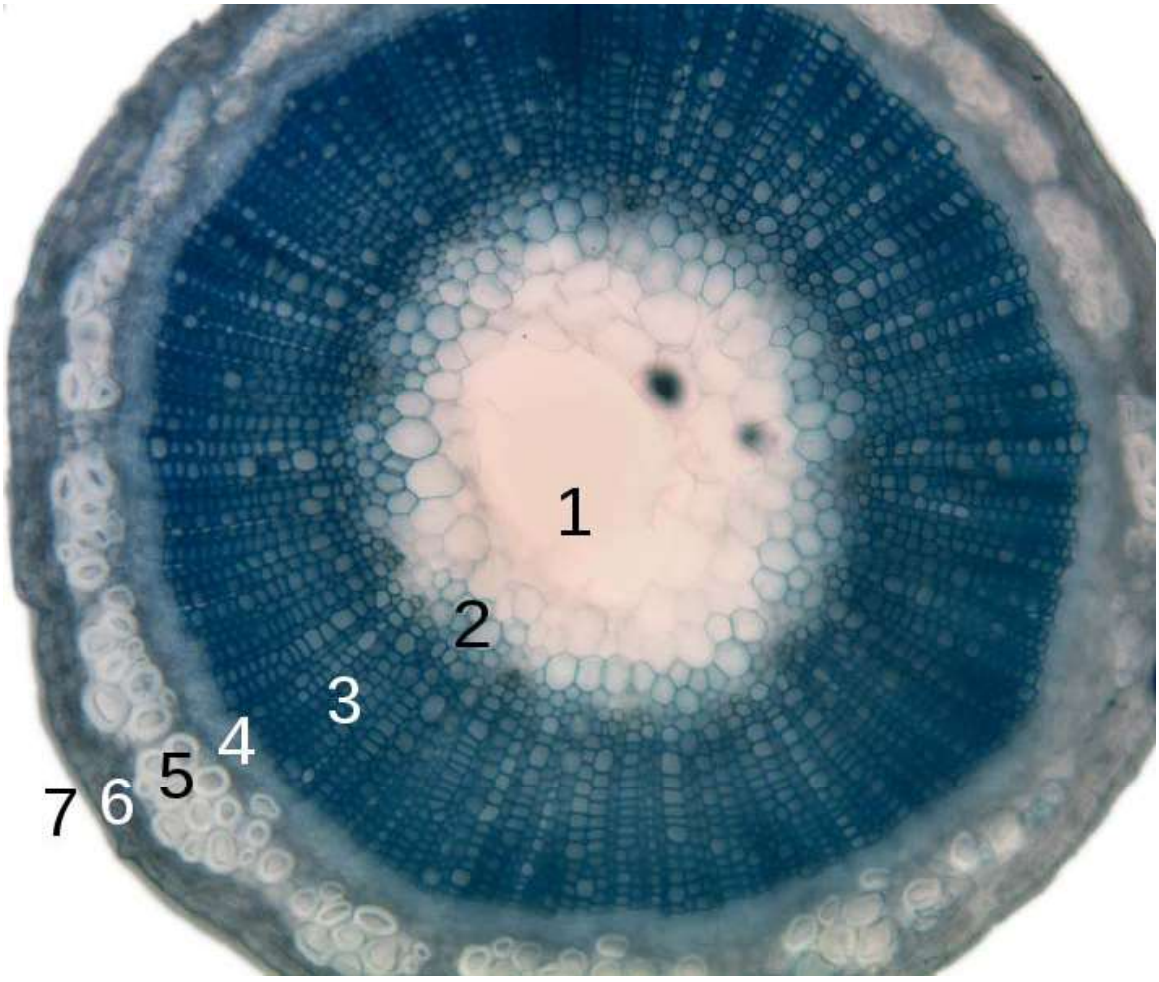
Organelles

- Cell membrane
- Cell wall
- Nuclear membrane
- Plasmodesma
- Vacuole
- Plastids
- Chloroplast
- Leucoplast
- Chromoplast
- Golgi Bodies
- Ribosome
- Endoplasmic reticulum
- Mitochondrion
- Lysosome
- Cytoplasm
- Nucleus

- DNA
- Chromatin
- RNA
- Cytoskeleton
- Nucleolus

Chapter 6

Ground Tissue



Cross-section of a flax plant stem:
1. Pith,
2. Protoxylem,
3. Xylem I,
4. Phloem I,
5. Sclerenchyma (bast fibre),

6. Cortex,
7. Epidermis

The types of **ground tissue** found in plants develop from *ground tissue* meristem and consists of three simple tissues:

- Parenchyma (cells with thin primary walls that retain their protoplasm)
- Collenchyma (cells with thick primary walls that retain their protoplasm)
- Sclerenchyma (cells with lignified secondary walls that have lost their protoplasm at maturity, i.e. are 'dead')

Parenchyma

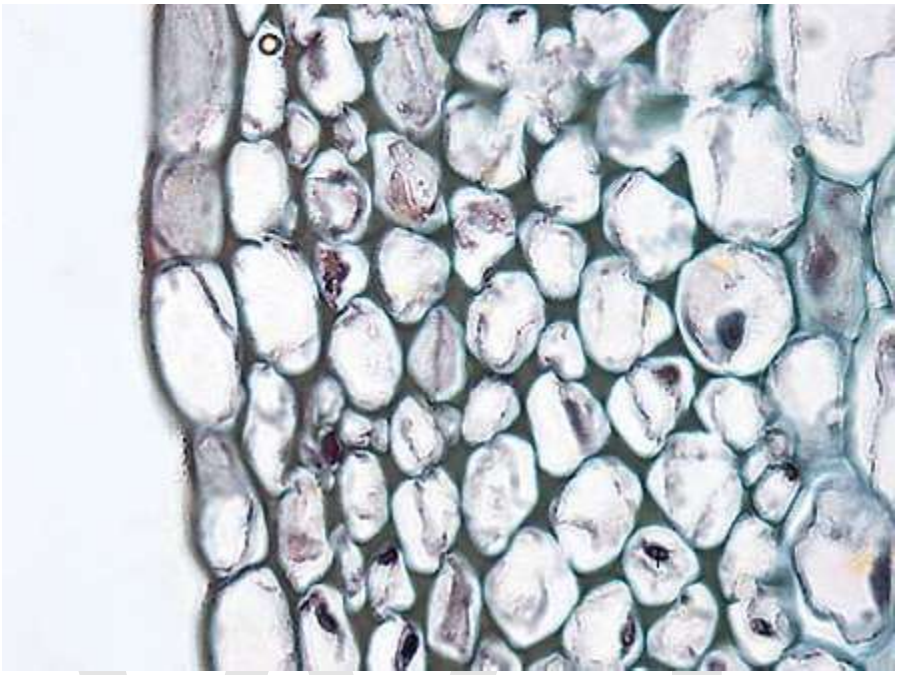
Parenchyma is the most common and versatile ground tissue. It forms, for example, the cortex and pith of stems, the cortex of roots, the mesophyll of leaves, the pulp of fruits, and the endosperm of seeds. Parenchyma cells are living cells and may remain meristematic at maturity, meaning that they are capable of cell division. They have thin but flexible cellulose cell walls, and are generally polygonal when close-packed, but approximately spherical when isolated from their neighbours. They have large central vacuoles, which allows the cells to store and regulate ions, waste products and water.

Parenchyma cells have a variety of functions:

- In leaves, they form the mesophyll and are responsible for photosynthesis and the exchange of gases, parenchyma cells in the mesophyll of leaves are a specialized parenchymatous tissue known as chlorenchyma (parenchyma with chloroplasts).
- Storage of starch, protein, fats and oils and water in roots, tubers (e.g. potato), seed endosperm (e.g. cereals) and cotyledons (e.g. pulses and groundnut)
- Secretion (e.g. hydathodes, nectaries and cells lining the inside of resin ducts)
- Wound repair and the potential for renewed meristematic activity
- Other specialized functions such as aeration (aerenchyma) and support

The form of parenchyma cells varies with their function. The epidermal parenchyma cells of a leaf are barrel shaped in cross section, but have a variety of outline shapes ranging from simple polygons to strongly branched and interlocked shapes resembling the pieces of a jigsaw puzzle, as in the leaves of *Arabidopsis thaliana*. In the epidermis of higher plants, only the guard cells have chloroplasts. This tissue serves as a barrier wall and protects the internal tissues from injury. In the spongy mesophyll of a leaf, parenchyma cells range from near-spherical and loosely arranged with large intercellular spaces to branched or stellate, mutually interconnected with their neighbours at the ends of the arms to form a three-dimensional network, as in the red kidney bean *Phaseolus vulgaris* and other mesophytes. These cells, with the epidermal guard cells of the stoma, form a system of air spaces and chambers that regulate the exchange of gases. They usually contain plastids.

Collenchyma



Cross section of collenchyma cells

The name 'collenchyma' derives from the Greek word κολλα ("kól-la"), meaning "glue", which refers to the thick, glistening appearance of the walls in fresh tissues. Collenchyma tissue is composed of elongated cells with unevenly thickened walls. They provide structural support, particularly in growing shoots and leaves. Collenchyma tissue composes, for example, the resilient strands in stalks of celery. Its growth is strongly affected by mechanical stress upon the plant. The walls of collenchyma in shaken plants (to mimic the effects of wind etc.), may be 40%-100% thicker than those not shaken. The wall is made up of cellulose and pectin.

There are three principal types of collenchyma:

- Angular collenchyma (thickened at intercellular contact points)
- Tangential collenchyma (cells arranged into ordered rows and thickened at the tangential face of the cell wall)
- Lacunar collenchyma (have intercellular space and thickening proximal to the intercellular space)

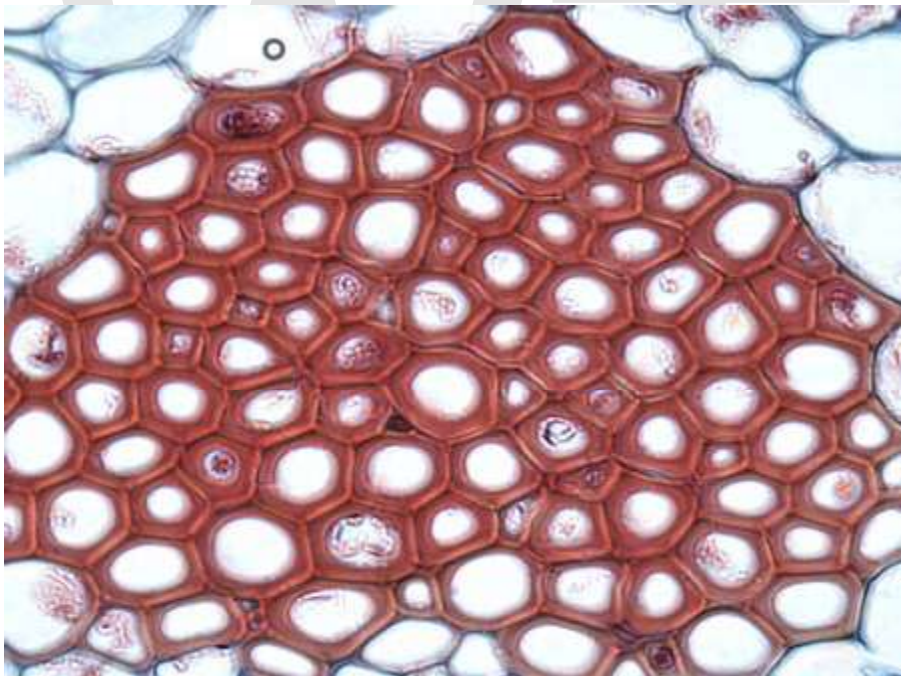
Collenchyma cells are most often found adjacent to outer growing tissues, the vascular cambium and are known for increasing structural support and integrity.

Sclerenchyma

Sclerenchyma is a supporting tissue in plants. Two groups of sclerenchyma cells exist: fibres and sclereids. Their walls consist of cellulose, hemicellulose and lignin. Sclerenchyma cells are the principal supporting cells in plant tissues that have ceased elongation. Sclerenchyma fibres are of great economical importance, since they constitute the source material for many fabrics (flax, hemp, jute, ramie).

Unlike the collenchyma, mature sclerenchyma is composed of dead cells with extremely thick cell walls (secondary walls) that make up to 90% of the whole cell volume. The term "sclerenchyma" is derived from the Greek σκληρός ("sklē-rós"), meaning "hard". It is the hard, thick walls that make sclerenchyma cells important strengthening and supporting elements in plant parts that have ceased elongation. The difference between fibres and sclereids is not always clear. Transitions do exist, sometimes even within one and the same plant.

Fibres



Cross section of sclerenchyma fibers

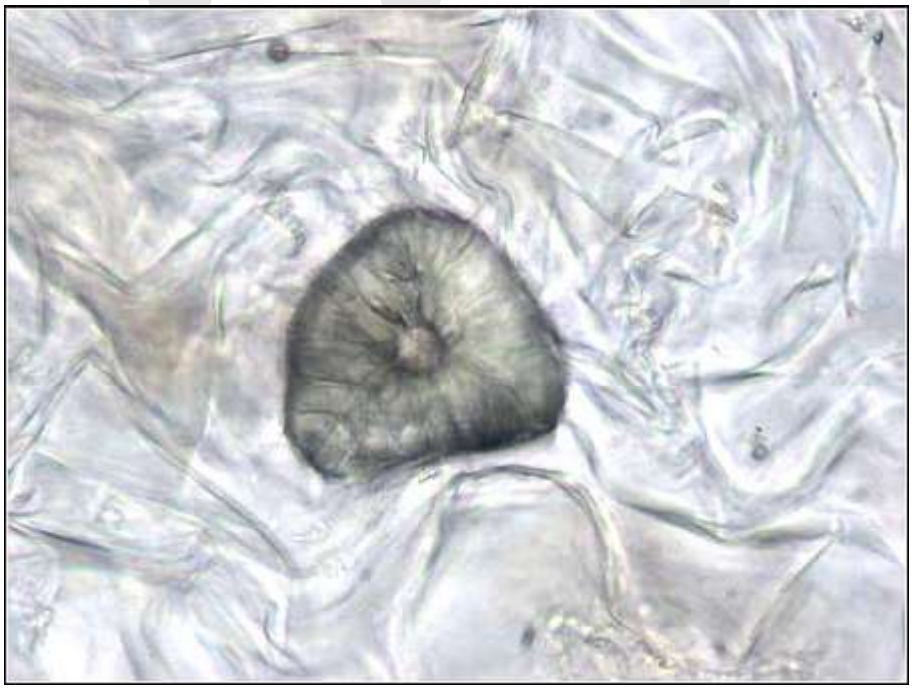
Fibres or bast are generally long, slender, so-called prosenchymatous cells, usually occurring in strands or bundles. Such bundles or the totality of a stem's bundles are colloquially called fibres. Their high load-bearing capacity and the ease with which they can be processed has since antiquity made them the source material for a number of things, like ropes, fabrics or mattresses. The fibres of flax (*Linum usitatissimum*) have been known in Europe and Egypt for more than 3000 years, those of hemp (*Cannabis sativa*) in China for just as long. These fibres, and those of jute (*Corchorus capsularis*)

and ramie (*Boehmeria nivea*, a nettle), are extremely soft and elastic and are especially well suited for the processing to textiles. Their principal cell wall material is cellulose.

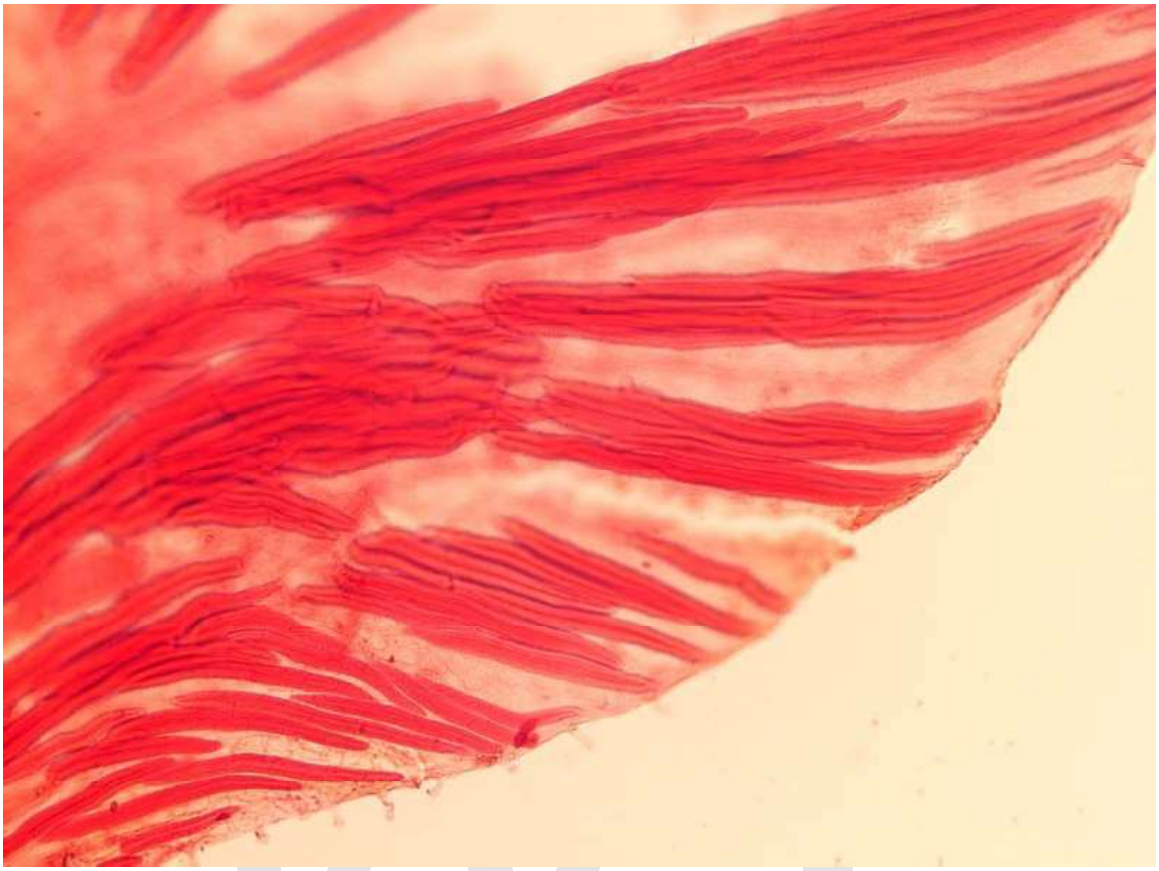
Contrasting are hard fibres that are mostly found in monocots. Typical examples are the fibres of many Gramineae, Agaves (sisal: *Agave sisalana*), lilies (*Yucca* or *Phormium tenax*), *Musa textilis* and others. Their cell walls contain, besides cellulose, a high proportion of lignin. The load-bearing capacity of *Phormium tenax* is as high as 20–25 kg/mm^2, the same as that of good steel wire (25 kg/ mm^2), but the fibre tears as soon as too great a strain is placed upon it, while the wire distorts and does not tear before a strain of 80 kg/mm^2. The thickening of a cell wall has been studied in *Linum*. Starting at the centre of the fibre are the thickening layers of the secondary wall deposited one after the other. Growth at both tips of the cell leads to simultaneous elongation. During development the layers of secondary material seem like tubes, of which the outer one is always longer and older than the next. After completion of growth the missing parts are supplemented, so that the wall is evenly thickened up to the tips of the fibres.

Fibres usually originate from meristematic tissues. Cambium and procambium are their main centers of production. They are usually associated with the xylem and phloem of the vascular bundles. The fibres of the xylem are always lignified, while those of the phloem are cellulosic. Reliable evidence for the fibre cells' evolutionary origin from tracheids exists. During evolution the strength of the tracheid cell walls was enhanced, the ability to conduct water was lost and the size of the pits reduced. Fibres that do not belong to the xylem are bast (outside the ring of cambium) and such fibres that are arranged in characteristic patterns at different sites of the shoot.

Sclereids



Fresh mount of a sclereid.



Long tapered sclereids supporting a leaf edge in *Dionysia kossinskyi*.

Sclereids are small bundles of sclerenchyma tissue in plants that form durable layers, such as the cores of apples and the gritty texture of pears. Sclereids are variable in shape. The cells can be isodiametric, prosenchymatic, forked or elaborately branched. They can be grouped into bundles, can form complete tubes located at the periphery or can occur as single cells or small groups of cells within parenchyma tissues. But compared with most fibres, sclereids are relatively short. Characteristic examples are brachysclereids or the stone cells (called stone cells because of their hardness) of pears (*Pyrus communis*) and quinces (*Cydonia oblonga*) and those of the shoot of the wax-plant (*Hoya carnosa*). The cell walls fill nearly all the cell's volume. A layering of the walls and the existence of branched pits is clearly visible. Branched pits such as these are called ramiform pits. The shell of many seeds like those of nuts as well as the stones of drupes like cherries or plums are made up from sclereids.

Chapter 7

Pectin

Polymer of D-Galacturonic Acid, Pectin. Shown here in powder form.

Pectin is a structural heteropolysaccharide contained in the primary cell walls of terrestrial plants. It was first isolated and described in 1825 by Henri Braconnot. It is produced commercially as a white to light brown powder, mainly extracted from citrus fruits, and is used in food as a gelling agent particularly in jams and jellies. It is also used in fillings, sweets, as a stabilizer in fruit juices and milk drinks and as a source of dietary fiber.

Biology

In plant cells, pectin consists of a complex set of polysaccharides (see below) that are present in most primary cell walls and particularly abundant in the non-woody parts of terrestrial plants. Pectin is present not only throughout primary cell walls but also in the middle lamella between plant cells where it helps to bind cells together.

The amount, structure and chemical composition of pectin differs between plants, within a plant over time and in different parts of a plant. During ripening, pectin is broken down by the enzymes pectinase and pectinesterase; in this process the fruit becomes softer as the middle lamella breaks down and cells become separated from each other. A similar process of cell separation caused by pectin breakdown occurs in the abscission zone of the petioles of deciduous plants at leaf fall.

Pectin is a natural part of human diet, but does not contribute significantly to nutrition. The daily intake of pectin from fruits and vegetables can be estimated to be around 5 g (assuming consumption of approximately 500 g fruits and vegetables per day).

In human digestion, pectin goes through the small intestine more or less intact. Pectin is thus a soluble dietary fiber.

Consumption of pectin has been shown to reduce blood cholesterol levels. The mechanism appears to be an increase of viscosity in the intestinal tract, leading to a reduced absorption of cholesterol from bile or food. In the large intestine and colon, microorganisms degrade pectin and liberate short-chain fatty acids that have positive influence on health (prebiotic effect).

Chemistry

Pectins are a family of complex polysaccharides that contain 1,4-linked α-D-galactosyluronic acid residues. Three pectic polysaccharides have been isolated from plant primary cell walls and structurally characterized. These are:

- Homogalacturonans
- Substituted galacturonans
- Rhamnogalacturonans

Homogalacturonans are linear chains of α-(1-4)-linked D-galacturonic acid. Substituted galacturonans are characterized by the presence of saccharide appendant residues (such as D-xylose or D-apiose in the respective cases of xylogalacturonan and apiogalacturonan) branching from a backbone of D-galacturonic acid residues. Rhamnogalacturonan I pectins (RG-I) contain a backbone of the repeating disaccharide: 4)-α-D-galacturonic acid-(1,2)-α-L-rhamnose-(1. From many of the rhamnose residues, sidechains of various neutral sugars branch off. The neutral sugars are mainly D-galactose, L-arabinose and D-xylose, the types and proportions of neutral sugars varying with the origin of pectin.

Another structural type of pectin is rhamnogalacturonan II (RG-II), which is a less frequent complex, highly branched polysaccharide. Rhamnogalacturonan II is classified by some authors within the group of substituted galacturonans since the rhamnogalacturonan II backbone is made exclusively of D-galacturonic acid units.

Isolated pectin has a molecular weight of typically 60–130,000 g/mol, varying with origin and extraction conditions.

In nature, around 80% of carboxyl groups of galacturonic acid are esterified with methanol. This proportion is decreased more or less during pectin extraction. The ratio of esterified to non-esterified galacturonic acid determines the behavior of pectin in food applications. This is why pectins are classified as high- vs. low-ester pectins – or in short HM vs. LM-pectins, with more or less than half of all the galacturonic acid esterified.

The non-esterified galacturonic acid units can be either free acids (carboxyl groups) or salts with sodium, potassium or calcium. The salts of partially esterified pectins are called pectinates, if the degree of esterification is below 5% the salts are called pectates, the insoluble acid form, pectic acid.

Some plants like sugar beet, potatoes and pears contain pectins with acetylated galacturonic acid in addition to methyl esters. Acetylation prevents gel-formation but increases the stabilising and emulsifying effects of pectin.

Amidated pectin is a modified form of pectin. Here, some of the galacturonic acid is converted with ammonia to carboxylic acid amide. These pectins are more tolerant of varying calcium concentrations that occur in use.

To prepare a pectin-gel, the ingredients are heated, dissolving the pectin. Upon cooling below gelling temperature, a gel starts to form. If gel formation is too strong, syneresis or a granular texture are the result, whilst weak gelling leads to excessively soft gels. In high-ester pectins at soluble solids content above 60% and a pH-value between 2.8 and 3.6, hydrogen bonds and hydrophobic interactions bind the individual pectin chains together. These bonds form as water is bound by sugar and forces pectin strands to stick together. These form a 3-dimensional molecular net that creates the macromolecular gel. The gelling-mechanism is called a low-water-activity gel or sugar-acid-pectin gel.

In low-ester pectins, ionic bridges are formed between calcium ions and the ionised carboxyl groups of the galacturonic acid. This is idealised in the so-called “egg box-model”. Low-ester pectins need calcium to form a gel, but can do so at lower soluble solids and higher pH-values than high-ester pectins.

Amidated pectins behave like low-ester pectins but need less calcium and are more tolerant of excess calcium. Also, gels from amidated pectin are thermo-reversible – they can be heated and after cooling solidify again, whereas conventional pectin-gels will afterwards remain liquid.

High-ester pectins set at higher temperatures than low-ester pectins. However, gelling reactions with calcium increase as the degree of esterification falls. Similarly, lower pH-values or higher soluble solids (normally sugars) increase gelling speed. Suitable pectins can therefore be selected for jams and for jellies, or for higher sugar confectionery jellies.

Sources and production

Apples, guavas, quince, plums, gooseberries, oranges and other citrus fruits, contain large amounts of pectin, while soft fruits like cherries, grapes and strawberries contain small amounts of pectin.

Typical levels of pectin in plants are (fresh weight):

- apples, 1–1.5%
- apricot, 1%
- cherries, 0.4%
- oranges 0.5–3.5%
- carrots approx. 1.4%
- citrus peels, 30%

The main raw-materials for pectin production are dried citrus peel or apple pomace, both by-products of juice production. Pomace from sugar-beet is also used to a small extent.

From these materials, pectin is extracted by adding hot dilute acid at pH-values from 1.5 – 3.5. During several hours of extraction, the protopectin loses some of its branching and chain-length and goes into solution. After filtering, the extract is concentrated in vacuum and the pectin then precipitated by adding ethanol or isopropanol. An old technique of precipitating pectin with aluminium salts is no longer used (apart from alcohols and polyvalent cations; pectin also precipitates with proteins and detergents).

Alcohol-precipitated pectin is then separated, washed and dried. Treating the initial pectin with dilute acid leads to low-esterified pectins. When this process includes ammonium hydroxide, amidated pectins are obtained. After drying and milling pectin is usually standardised with sugar and sometimes calcium-salts or organic acids to have optimum performance in a particular application.

Worldwide, approximately 40,000 metric tons of pectin are produced every year.

Uses

The main use for pectin is as a gelling agent, thickening agent and stabilizer in food. The classical application is giving the jelly-like consistency to jams or marmalades, which would otherwise be sweet juices. For household use, pectin is an ingredient in gelling sugar (also known as "Jam Sugar") where it is diluted to the right concentration with sugar and some citric acid to adjust pH. In some countries, pectin is also available as a

solution or an extract, or as a blended powder, for home jam making. For conventional jams and marmalades that contain above 60% sugar and soluble fruit solids, high-ester pectins are used. With low-ester pectins and amidated pectins less sugar is needed, so that diet products can be made. Pectin can also be used to stabilize acidic protein drinks, such as drinking yogurt, and as a fat substitute in baked goods. Typical levels of pectin used as a food additive are between 0.5 – 1.0% - this is about the same amount of pectin as in fresh fruit.

In medicine, pectin increases viscosity and volume of stool so that it is used against constipation and diarrhea. Until 2002, it was one of the main ingredients used in Kaopectate, along with kaolinite. Pectin is also used in throat lozenges as a demulcent. In cosmetic products, pectin acts as stabilizer. Pectin is also used in wound healing preparations and specialty medical adhesives, such as colostomy devices. Also, it is considered a natural remedy for nausea. Pectin rich foods are proven to help nausea.

In ruminant nutrition, depending on the extent of lignification of the cell wall, pectin is up to 90% digestible by bacterial enzymes. Ruminant nutritionists recommend that the digestibility and energy concentration in forages can be improved by increasing pectin concentration in the forage.

In the cigar industry, pectin is considered an excellent substitute for vegetable glue and many cigar smokers and collectors will use pectin for repairing damaged tobacco wrapper leaves on their cigars.

Pectin is also used in jellybeans.

Legal status

At the FAO/WHO joint Expert Committee on Food Additives and in the EU, no numerical acceptable daily intake (ADI) has been set, as pectin is considered safe.

In the US, pectin is GRAS – Generally recognized as safe. In most foods it can be used according to good manufacturing practices in the levels needed for its application, “quantum satis”.

In the International Numbering System (INS) pectin has the number 440. In Europe pectins are differentiated into the E numbers E440(i) for non-amidated pectins and E440 (ii) for amidated pectins. There are specifications in all national and international legislation defining its quality and regulating its use.

History

Pectin was first isolated and described in 1825 by Henri Braconnot, though the action of pectin to make jams and marmalades was known long before. To obtain well set jams

from fruits that had little or only poor quality pectin, pectin-rich fruits or their extracts were mixed into the recipe.

During industrialization, the makers of fruit preserves soon turned to producers of apple juice to obtain dried apple pomace that was cooked to extract pectin.

Later, in the 1920s and 1930s, factories were built that commercially extracted pectin from dried apple pomace and later citrus-peel in regions that produced apple juice in both the USA and in Europe.

At first pectin was sold as a liquid extract, but nowadays pectin is often used as dried powder that is easier to store and handle than a liquid.

Chapter 8

Aerenchyma and Amyloplast

Aerenchyma

Aerenchyma is an air channel in the roots of some plants, which allows exchange of gases between the shoot and the root. The channel of large air-filled cavities provides a low-resistance internal pathway for the exchange of gases such as oxygen and ethylene between the plant above the water and the submerged tissues.

Aerenchyma form in roots subject to anoxia such as what occurs during flooding of plants and soil . For example, Blom et al. (1994) investigated the adaptive responses of plants to flooding along the banks of the Rhine River, which included such morphological changes such as aerenchyma formation.

Aerenchyma formation

Aerenchyma of Schoenoplectus validus

In maize, an aerenchyma is formed from highly selective cell death and dissolution in the root cortex during anoxia in the roots . When plant roots are submerged or the surrounding soil flooded, hypoxia develops, as soil microorganisms consume oxygen faster than diffusion occurs. Nitrification is inhibited as low oxygen occurs and toxic compounds are formed, as anaerobic bacteria use nitrate, manganese, and sulfate as alternative electron acceptors . The reduction-oxidation potential of the rhizhosphere decreases and metal ions such as iron and manganese precipitate.

In general, low oxygen stimulates trees and plants to produce ethylene . Yet Visser *et al.*., in 1997, found that ethylene slows down primary and adventitious root elongation and formation. Thus, in addition to supplying root tissues with oxygen, aerenchymas assist in diffusing the accumulation of ethylene in order to prevent elongation inhibition (Visser et al. 1997).

Formation of Aerenchyma

Aerenchymas are formed by cell differentiation and collapse (lysigenous aerenchyma) or by cell separation without collapse (schizogenous aerenchyma). The differentiation or separation forms large continuous air spaces that allow diffusion of oxygen from shoot to root . Different experiments defined how cell collapse occurs. Cell death was blocked by antagonists of phospholipid metabolism, of cytolsolic Ca^{2+} or Ca-calmodulin, and of protein kinases. By contrast, reagents that activate G-proteins raise cytolsolic Ca^{2+} or inhibit phosphatases-promoted cell death (two references He *et al.*. 1996). An enzyme that was linked to this process is cellulase, which assists in cell wall breakage. In maize, a protein that is homologous to the enzyme XET (a protein that breaks the β-1,4 links between glucans and xyulosyl, the cross-linking molecule in plant cell walls) was found..

Advantages of aerenchyma

The large air-filled cavities provide a low-resistance internal pathway for the exchange of gases between the plant organs above the water and the submerged tissues. Some of the oxygen transported through the aerenchyma leaks through root pores into the surrounding soil. The resulting small rhizosphere of oxygenated soil around individual roots support microorganisms that prevent the influx of potentially toxic soil components such as sulfide, iron, and manganese. Nitrifying bacteria provide the roots with a favourable nitrogen source .

During drought, aerenchymas allow plant roots to grow deeper for water, even through compacted layers; thick and tough roots are formed. As the roots dieback and decay, the resulting voids are paths in which new roots can grow and elongate when resources are available.

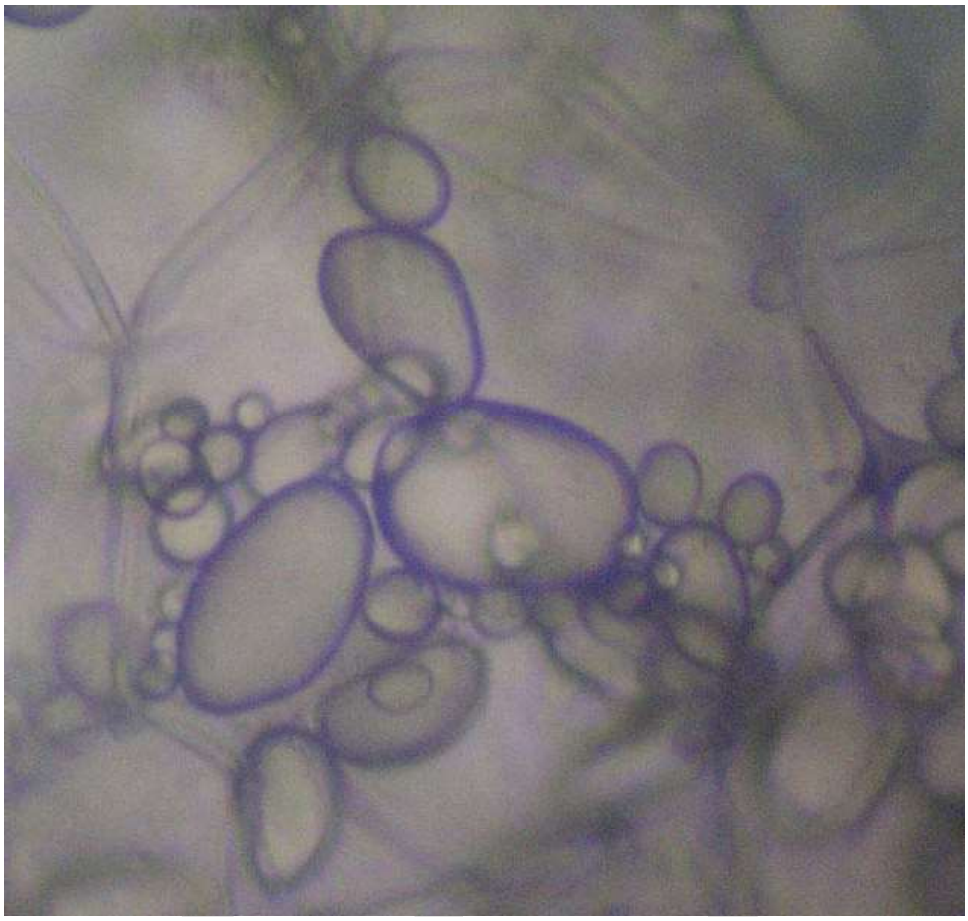
Disadvantages of Aerenchyma

Not all plants are able to develop aerenchymas.

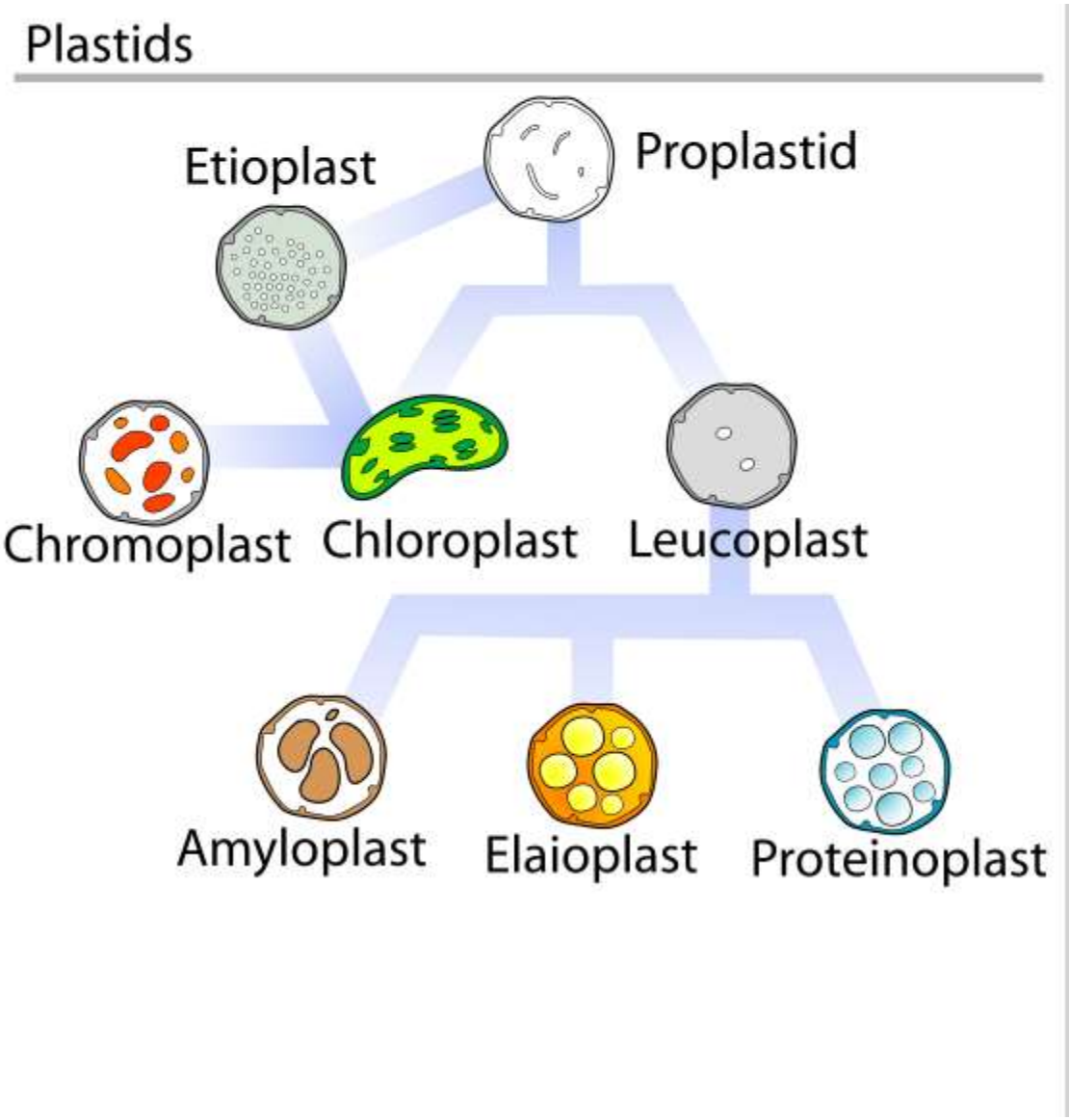
Aerenchymous roots may experience the following problems

- Water and nutrient uptake may be less efficient; large intercellular spaces decrease the tissue available to transport water and nutrients from the root surface to the root xylem (Visser *et al.*. 1996, 2000a).
- Large root diameters reduce biomass-to-surface ratio, resulting in less uptake of water and nutrients and the reduced opportunity to explore all microzones for nutrients.
- Some roots with aerenchymas are less likely to resist the physical strain of compacted soils. Those roots that penetrate and survive dense and compact drained soils have a higher bulk density and a strongly lignified layer of cells surrounding the aerenchyma, which strengthens the root. This dense, lignified layer prevents radial leakage of oxygen from the aerenchyma and may block some water and nutrient uptake (Colmer *et al.*. 1998; Visser *et al.*. 2000).
- During drought, roots with aerenchyma may be less tolerant to water stress as the open structure of the cortex is probably a low-resistance pathway for water vapor, as well as for air, thereby increasing the susceptibility of the root to water loss.

Amyloplast



Amyloplasts in a potato cell



Types of plastid

Amyloplasts are non-pigmented organelles found in some plant cells. They are responsible for the synthesis and storage of starch granules, through the polymerization of glucose. Amyloplasts also convert this starch back into sugar when the plant needs energy. Large numbers of amyloplasts can be found in fruit and in underground storage tissues of some plants, such as in potato tubers.

Amyloplasts are plastids, specifically leucoplasts. Plastids are a specialized class of cellular organelles that carry their own genome and are believed to be descendants of cyanobacteria (blue-green algae) which formed a symbiotic relationship with the eukaryotic cell.

Starch synthesis and storage also takes place in chloroplasts, a type of pigmented plastid involved in photosynthesis. Amyloplasts and chloroplasts are closely related, and amyloplasts can turn into chloroplasts; this is for instance observed when potato tubers are exposed to light and turn green.

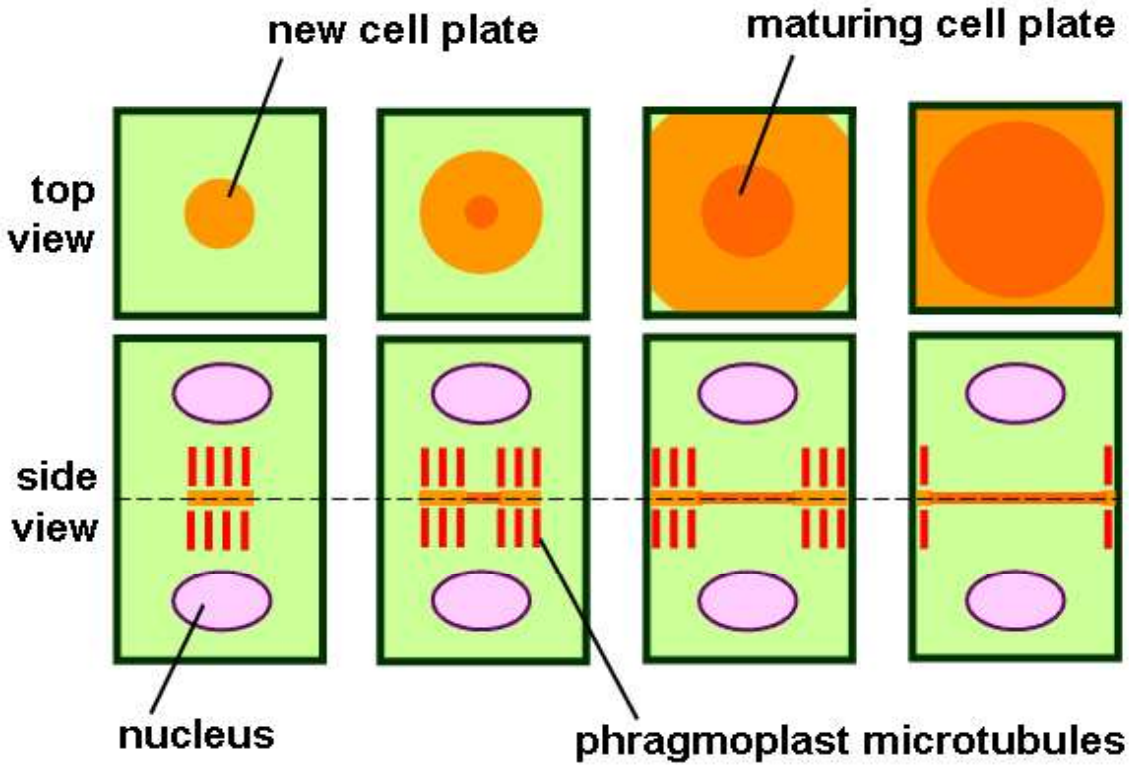
Sensing gravity

In the root cap (a tissue at the tip of the root) some specialized amyloplasts are involved in the perception of air by the plant (gravitropism). These specialized amyloplasts are denser than the cytoplasm and can sediment according to the gravity vector. They are found in a special subset of cells of the root cap (a tissue at the tip of the root) called statocytes. Statoliths are enmeshed in a web of actin and it is thought that their sedimentation transmits the gravitropic signal by activating mechanosensing channels. The gravitropic signal then leads to reorientation of auxin efflux carriers and subsequent redistribution of auxin streams in root cap and root as a whole. The changed relations in concentration of auxin leads to differential growth of the root tissues. Taken together, the root is then turning, following the gravity stimuli. They are also found in the endodermic layer of the inflorescence stem. The redistribution of auxin causes the shoot to turn in a direction opposite that of the gravity stimuli.

Chapter 9

Cell Plate, Leucoplast and Oleosin

Cell plate



Phragmoplast and cell plate formation in a plant cell during cytokinesis. Left side: Phragmoplast forms and cell plate starts to assemble in the center of the cell. Toawards the right: Phragmoplast enlarges in a donut-shape towards the outside of the cell, leaving behind mature cell plate in the center. The cell plate will transform into the new cell wall once cytokinesis is complete.

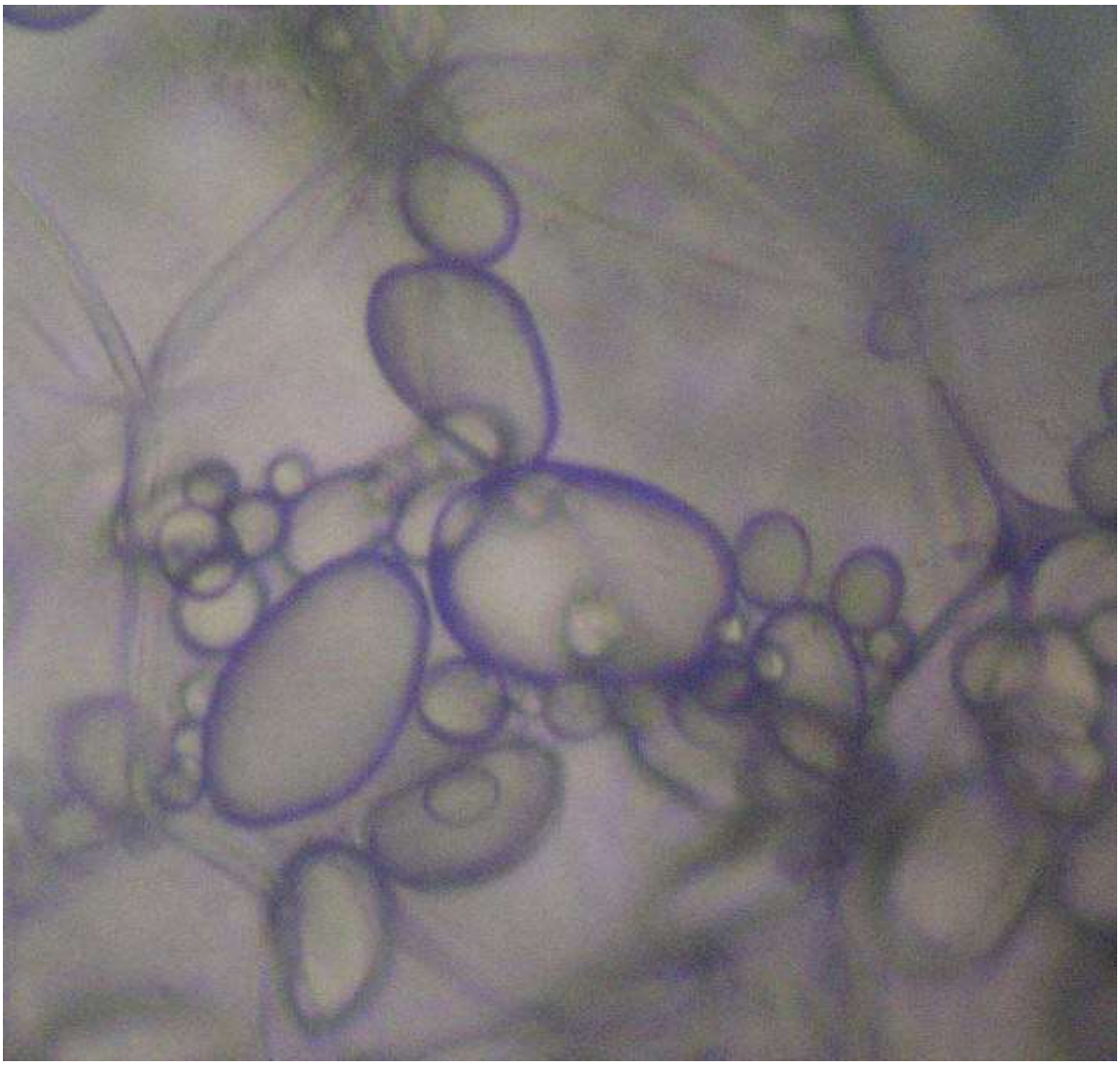
Cytokinesis in terrestrial plants occurs by **cell plate** formation. This process entails the delivery of Golgi-derived and endosomal vesicles carrying cell wall and cell membrane components to the plane of cell division and the subsequent fusion of these vesicles within this plane.

After formation of an early tubulo-vesicular network at the center of the cell, the initially labile cell plate consolidates into a tubular network and eventually a fenestrated sheet. The cell plate grows outward from the center of the cell to the parental plasma membrane with which it will fuse, thus completing cell division. Formation and growth of the cell plate is dependent upon the phragmoplast, which is required for proper targeting of Golgi-derived vesicles to the cell plate.

As the cell plate matures in the central part of the cell, the phragmoplast disassembles in this region and new elements are added on its outside. This process leads to a steady expansion of the phragmoplast, and concomitantly, to a continuous retargeting of Golgi-derived vesicles to the growing edge of the cell plate. Once the cell plate reaches and fuses with the plasma membrane the phragmoplast disappears. This event not only marks the separation of the two daughter cells, but also initiates a range of biochemical modifications that transform the callose-rich, flexible cell plate into a cellulose-rich, stiff primary cell wall.

The heavy dependence of cell plate formation on active Golgi stacks explains why plant cells, unlike mammalian cells, do not disassemble their secretion machinery during cell division.

Leucoplast



Leucoplasts, specifically, amyloplasts

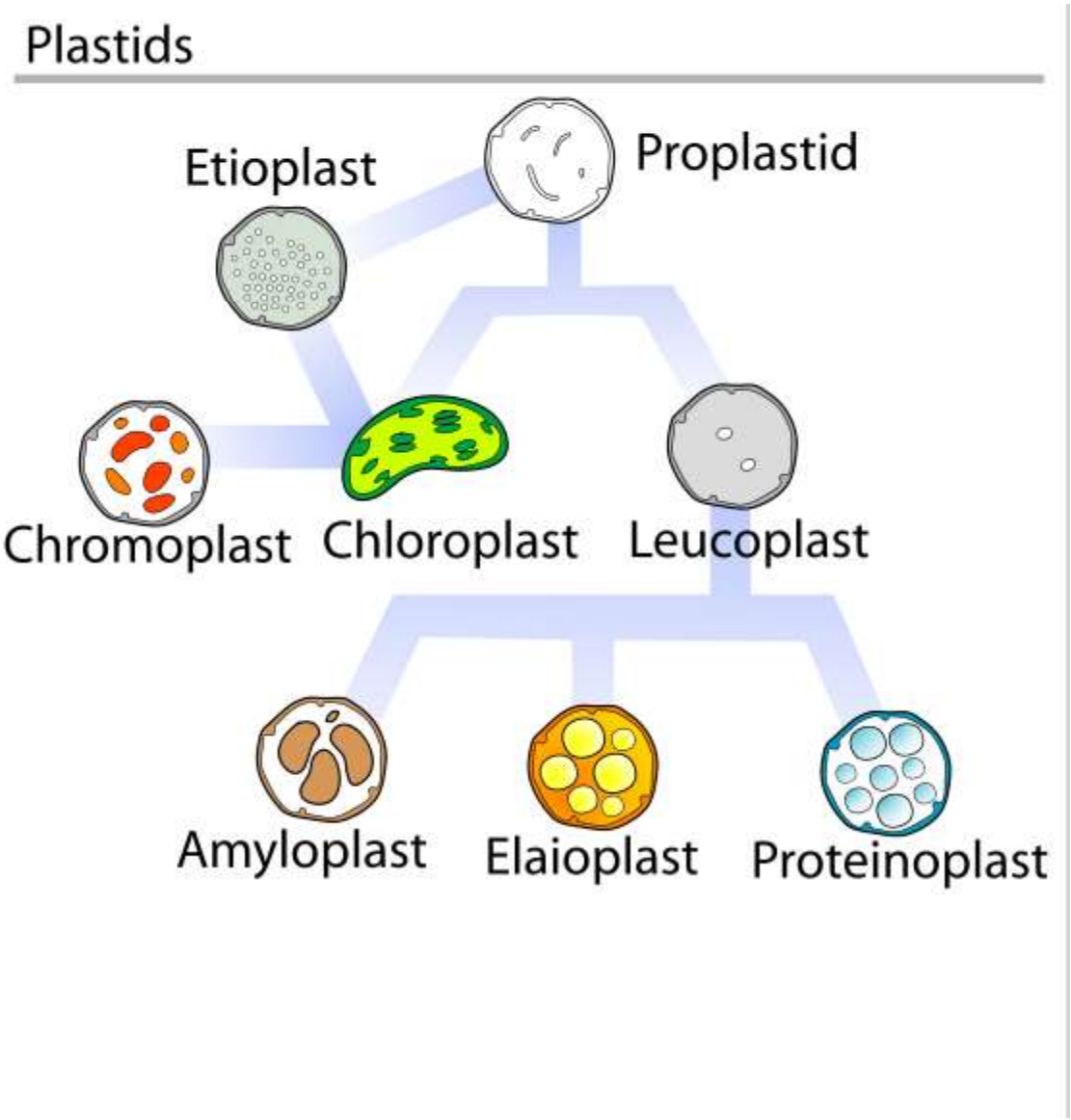
Leucoplasts are a category of plastid and as such are organelles found in plant cells. They are non-pigmented, in contrast to other plastids such as the chloroplast.

Lacking pigments, leucoplasts are not green, so they are predictably located in roots and non-photosynthetic tissues of plants. They may become specialized for bulk storage of starch, lipid or protein and are then known as amyloplasts, elaioplasts, or proteinoplasts respectively. However, in many cell types, leucoplasts do not have a major storage function and are present to provide a wide range of essential biosynthetic functions, including the synthesis of fatty acids, many amino acids, and tetrapyrrole compounds such as haem. In general, leucoplasts are much smaller than chloroplasts and have a variable morphology, often described as amoeboid. Extensive networks of stromules

interconnecting leucoplasts have been observed in epidermal cells of roots, hypocotyls and petals, and in callus and suspension culture cells of tobacco. In some cell types at certain stages of development, leucoplasts are clustered around the nucleus with stromules extending to the cell periphery, as observed for proplastids in the root meristem.

Etioplasts, which are pre-granal, immature chloroplasts but can also be chloroplasts which have been deprived of light, lack active pigment and can technically be considered leucoplasts. After several minutes exposure to light, etioplasts begin to transform into functioning chloroplasts and cease being leucoplasts.

Compare



- Plastid
  - Chloroplast and etioplast
  - Chromoplast
  - Leucoplast
    - Amyloplast
    - Elaioplast
    - Proteinoplast

Oleosin

Oleosins are structural proteins found in oil bodies and found in plant cells. Oil bodies are not considered organelles because they have a single layer membrane and lack the pre-requisite double layer membrane in order to be considered an organelle. They are found in plant parts with high oil content that undergo extreme desiccation as part of their maturation process, and help stabilize the bodies.

Oleosins comprise of three parts. The N- and C-terminal domains are amphipathic, whereas the middle part is strongly hydrophobic. Models show oleosins having a hairpin-like hydrophobic shape that is inserted inside the triacylglyceride (TAG), while the hydrophilic parts are left outside oil bodies.

Oleosins have been found on oil bodies of seeds, tapetum cells, and pollen but not fruits. Instead of a stabilizer of oil bodies, oleosins are believe to be involved in water-uptaking of pollen on stigma.

Use in Purification of Recombinant Protein

Oleosins provide an easy way of purifying proteins which have been produced recombinantly in plants. If the protein is made as a fusion protein with oleosin and a protease recognition site is incorporated between them, the fusion protein will sit in the membrane of the oil body, which can be easily isolated by centrifugation. The oil droplets can then be mixed with aqueous medium again, and oleosin cleaved from the protein of interest. Centrifugation will cause two phases to separate again, and the aqueous medium now contains the purified protein.

Chapter 10

Guard Cell

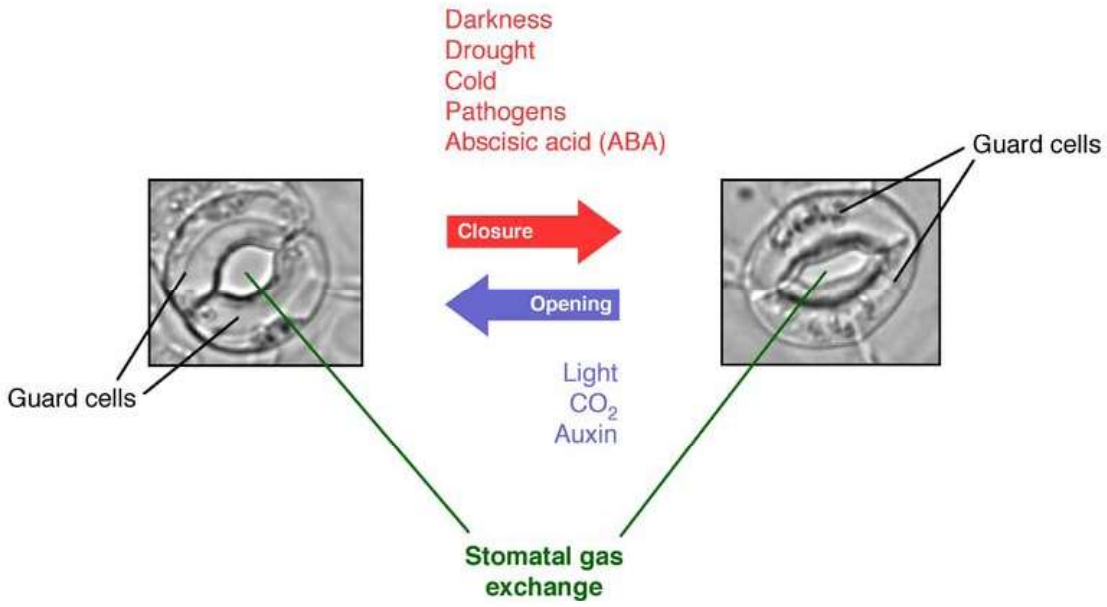


Figure 1. A stomatal pore in the surface (epidermis) of a leaf as viewed through a microscope. The central stomatal pore is formed by a pair of guard cells. The stomatal pore can either open (left) or close (right) depending on the environmental conditions.

Guard cells are specialized cells located in the leaf epidermis of plants. Pairs of guard cells surround tiny stomatal airway pores (Figure 1). These tiny holes in the surface of leaves are necessary for gas exchange into and out of the plant; carbon dioxide (CO_2) enters the plant allowing the carbon fixation reactions of photosynthesis to occur. Oxygen (O_2) exits the plant as a byproduct of photosynthesis. The opening and closing of the stomatal gas exchange holes is regulated by swelling and shrinking of the two surrounding guard cells (Figure 1). Due to the presence of the stomatal pores on plant leaf surfaces, water evaporates through the stomatal openings causing plants to lose water. Over 95% of water loss from plants can occur by evaporation (transpiration) through the stomatal pores. Therefore, it is important for plants to be able to balance the amount of CO_2 being brought into the plant with the amount of water escaping as a result of the open stomatal pores. Hence, the guard cells are the gate keepers of the plants ability to take in CO_2 from the atmosphere for photosynthesis – while regulating how much water

plants lose to the atmosphere. Opening and closure of the stomatal pore (Figure 1) is mediated by changes in the turgor pressure of the two guard cells. The turgor pressure of guard cells is controlled by movements of large quantities of ions and sugars into and out of the guard cells. When guard cells take up these solutes, the water potential (Ψ) inside the cells decreases, causing osmotic water flow into the guard cells. This leads to a turgor pressure increase causing swelling of the guard cells and the stomatal pores open (Figure 2). The ions that are taken up by guard cells are mainly potassium (K^+) ions and chloride (Cl^-) ions. In addition guard cells take up sugars that also contribute to opening of the stomatal pores.

Water loss and water use efficiency

Water stress (drought and salt stress) is one of the major environmental problems causing severe losses in agriculture and in nature. Drought tolerance of plants is mediated by several mechanisms that work together, including stabilizing and protecting the plant from damage caused by desiccation and also controlling how much water plants loose through the stomatal pores during drought. A plant hormone, abscisic acid (ABA), is produced in response to drought. A major type of ABA receptor has been identified. Future research is needed to test if these receptors can be used to engineer drought tolerance in plants. The plant hormone ABA causes the stomatal pores to close in response to drought, which reduces plant water loss via transpiration to the atmosphere and allows plants to avoid or slow down water loss during droughts. The use of drought tolerant crop plants would lead to a reduction in crop losses during droughts. Since guard cells control water loss of plants, the investigation on how stomatal opening and closure are regulated could lead to the development of plants with improved avoidance or slowing of desiccation and better water use efficiency.

Ion uptake and release

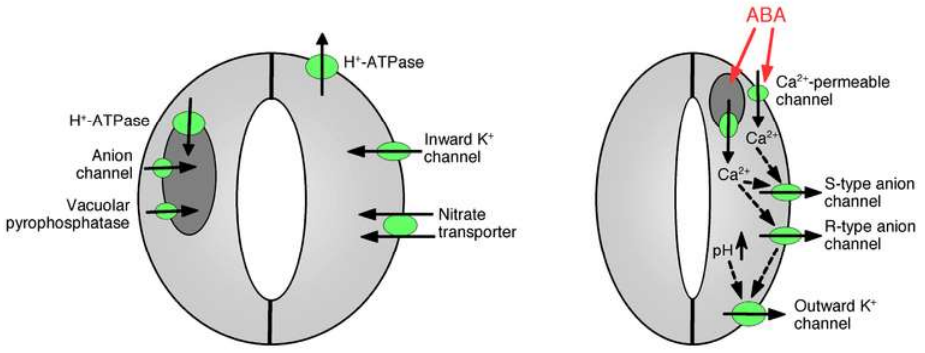


Figure 2. Ion channels and pumps regulating stomatal opening and closure.

Ion uptake into guard cells causes stomatal opening: The opening of gas exchange pores requires the uptake of potassium ions into guard cells. Potassium channels and pumps have been identified and shown to function in the uptake of ions and opening of stomatal

apertures (Figure 2). Ion release from guard cells causes stomatal pore closing: Other ion channels have been identified that mediate release of ions from guard cells, which results in osmotic water efflux from guard cells due to osmosis, shrinking of the guard cells, and closing of stomatal pores (Figures 1 and 2). Specialized potassium efflux channels participate in mediating release of potassium from guard cells. Anion channels were identified as important controllers of stomatal closing. Anion channels have several major functions in controlling stomatal closing: (a) They allow release of anions, such as chloride and malate from guard cells, which is needed for stomatal closing. (b) Anion channels are activated by signals that cause stomatal closing, for example by intracellular calcium and ABA. The resulting release of negatively charged anions from guard cells results in an electrical shift of the membrane to more positive voltages (depolarization) at the intracellular surface of the guard cell plasma membrane. This electrical depolarization of guard cells leads to activation of the outward potassium channels and the release of potassium through these channels (Figure 2). At least two major types of anion channels have been characterized in the plasma membrane: S-type anion channels and R-type anion channels.

Vacuolar ion transport

Vacuoles are large intracellular storage organelles in plants cells. In addition to the ion channels in the plasma membrane, vacuolar ion channels have important functions in regulation of stomatal opening and closure because vacuoles can occupy up to 90% of guard cell's volume. Therefore, a majority of ions are released from vacuoles when stomata are closed. Vacuolar K^+ (VK) channels and fast vacuolar channels can mediate K^+ release from vacuoles. Vacuolar K^+ (VK) channels are activated by elevation in the intracellular calcium concentration. Another type of calcium-activated channel, is the slow vacuolar (SV) channel. SV channels have been shown to function as cation channels that are permeable to Ca^{2+} ions, but their exact functions are not yet known in plants.

Signal transduction

Guard cells perceive and process environmental and endogenous stimuli such as light, humidity, CO_2, temperature, drought, and plant hormones to trigger cellular responses resulting in stomatal opening or closure. These signal transduction pathways determine for example how quickly a plant will loose water during a drought period. Guard cells have become a model for single cell signaling. Using Arabidopsis thaliana, the investigation of signal processing in single guard cells has become open to the power of genetics. Cytosolic and nuclear proteins and chemical messengers that function in stomatal movements have been identified that mediate the transduction of environmental signals thus controlling CO_2 intake into plants and plant water loss. Research on guard cell signal transduction mechanisms is producing an understanding of how plants can improve their response to drought stress by reducing plant water loss. Guard cells also provide an excellent model for basic studies on how a cell integrates numerous kinds of input signals to produce a response (stomatal opening or closing). These responses require coordination of numerous cell biological processes in guard cells, including signal

reception, ion channel and pump regulation, membrane trafficking, transcription, cytoskeletal rearrangements and more. A challenge for future research is to assign the functions of some of the identified proteins to these diverse cell biological processes.

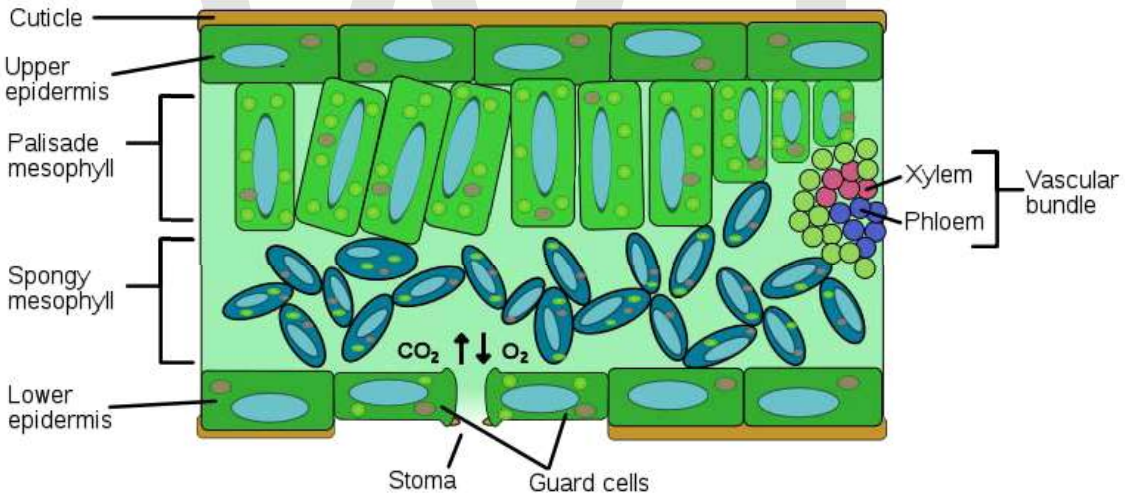
Development

During the development of plant leaves, the specialized guard cells differentiate from "guard mother cells". The density of the stomatal pores in leaves is regulated by environmental signals, including the continuingly increasing atmospheric CO_2 concentration, which reduces the density of stomatal pores in the surface of leaves in many plant species by presently unknown mechanisms. The genetics of stomatal development can be directly studied by imaging of the leaf epidermis using a microscope (Figure 1). Several major control proteins that function in a pathway mediating the development of guard cells and the stomatal pores have been identified.

Chapter 11

Palisade Cell, Phragmoplast and Phragmosome

Palisade cell

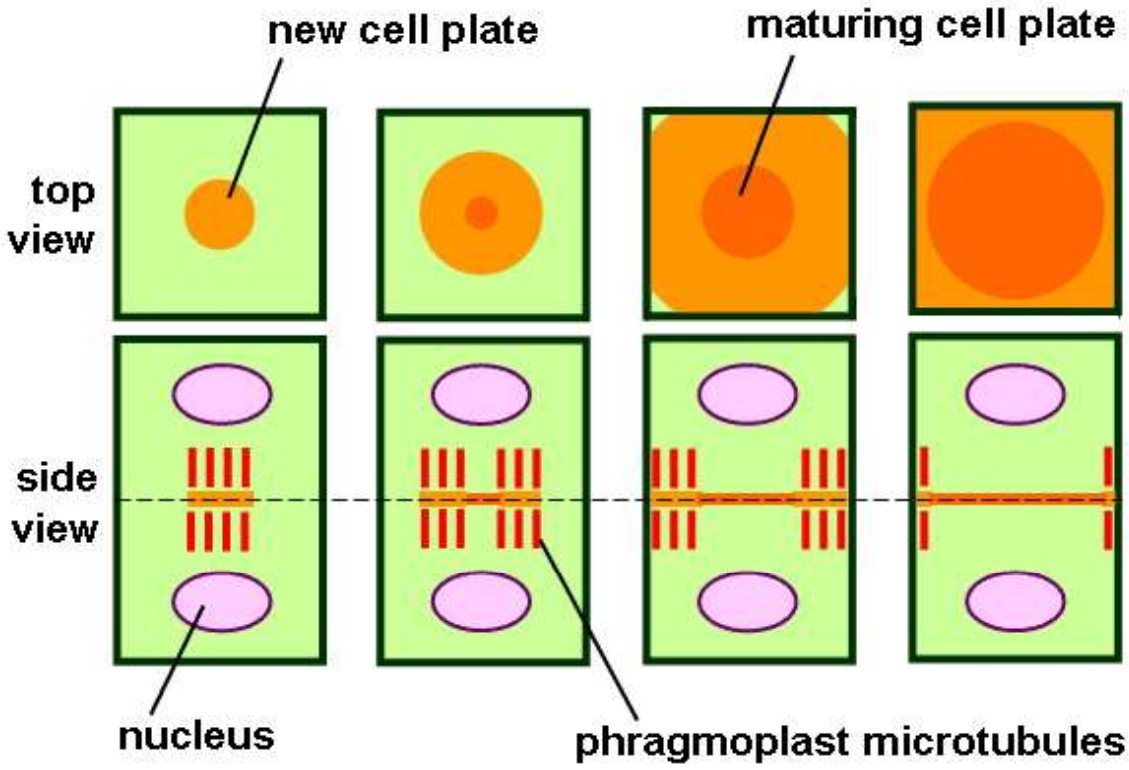


Cross-section of a leaf

Palisade cells are cells found within the mesophyll in leaves of dicotyledonous plants. They contain chloroplasts, which convert the energy stored in photons to chemical energy through photosynthesis, which is made up of two main stages; the light-dependent reactions and light-independent reactions. The cylindrical shape of palisade cells allows a large amount of light to be absorbed by the chloroplasts. Beneath the palisade mesophyll are the spongy mesophyll cells, irregularly-shaped cells that having many intercellular spaces to allow the passage of gases, such as the intake of carbon dioxide for photosynthesis to take place. The stomata is the way in which these gases are exchanged, as well as the transpiration of water from the xylem, either by the apoplast or symplast

pathway. Palisade cells are positioned towards the upper surface of the leaf and contain the largest number of chloroplasts per cell in plants. This makes them the primary site of photosynthesis in a plant's leaves. They have a very large surface area in order for them to absorb more light during photosynthesis.

Phragmoplast



Phragmoplast and cell plate formation in a plant cell during cytokinesis. Left side: Phragmoplast forms and cell plate starts to assemble in the center of the cell. Towards the right: Phragmoplast enlarges in a donut-shape towards the outside of the cell, leaving behind mature cell plate in the center. The cell plate will transform into the new cell wall once cytokinesis is complete.

The **phragmoplast** is a plant cell specific structure that forms during late cytokinesis. It serves as a scaffold for cell plate assembly and subsequent formation of a new cell wall separating the two daughter cells.

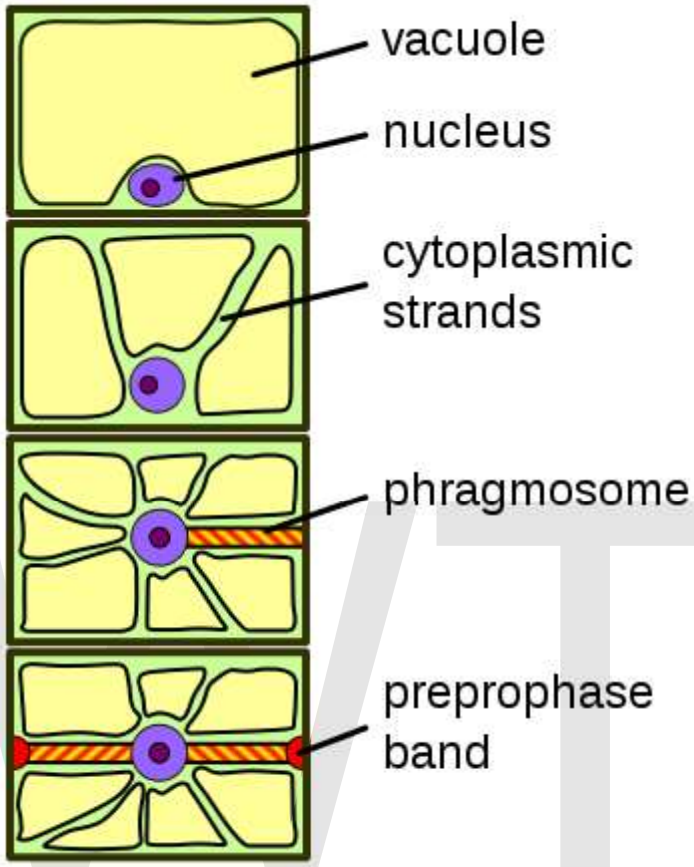
The phragmoplast is a complex assembly of microtubules (MTs), microfilaments (MFs), and endoplasmic reticulum (ER) elements, that assemble in two opposing sets perpendicular to the plane of the future cell plate during anaphase and telophase. It is initially barrel-shaped and forms from the mitotic spindle between the two daughter nuclei while nuclear envelopes reassemble around them. The cell plate initially forms as a disc between the two halves of the phragmoplast structure. While new cell plate material

is added to the edges of the growing plate, the phragmoplast microtubules disappear in the center and regenerate at the edges of the growing cell plate. The two structures grow outwards until they reach the outer wall of the dividing cell. If a phragmosome was present in the cell, the phragmoplast and cell plate will grow through the space occupied by the phragmosome. They will reach the parent cell wall exactly at the position formerly occupied by the preprophase band.

The microtubules and actin filaments within the phragmoplast serve to guide vesicles with cell wall material to the growing cell plate. Actin filaments are also possibly involved in guiding the phragmoplast to the site of the former preprophase band location at the parent cell wall. While the cell plate is growing, segments of smooth endoplasmic reticulum are trapped within it, later forming the plasmodesmata connecting the two daughter cells.

The phragmoplast can only be observed in bryophytes and vascular plants and a few algae. Some algae use another type of microtubule array, a phycoplast, during cytokinesis.

Phragmosome



Phragmosome formation in a highly vacuolated plant cell. From top to bottom: 1) Interphase cell with large central vacuole. 2) Cytoplasmic strands starting to penetrate vacuole. 3) Nucleus migration into center and formation of the phragmosome. 4) Phragmosome formation completed and formation of preprophase band marking future cell division plane.

The **phragmosome** is a sheet of cytoplasm forming in highly vacuolated plant cells in preparation for mitosis. In contrast to animal cells, plant cells often contain large central vacuoles occupying up to 90% of the total cell volume and pushing the nucleus against the cell wall. In order for mitosis to occur, the nucleus has to move into the center of the cell. This happens during G2 phase of the cell cycle just after DNA replication.

Initially, cytoplasmic strands form that penetrate the central vacuole and provide pathways for nuclear migration. Actin filaments along these cytoplasmic strands pull the nucleus into the center of the cell. These cytoplasmic strands fuse into a transverse sheet of cytoplasm along the plane of future cell division, forming the phragmosome.

Phragmosome formation is only clearly visible in dividing plant cells that are highly vacuolated.

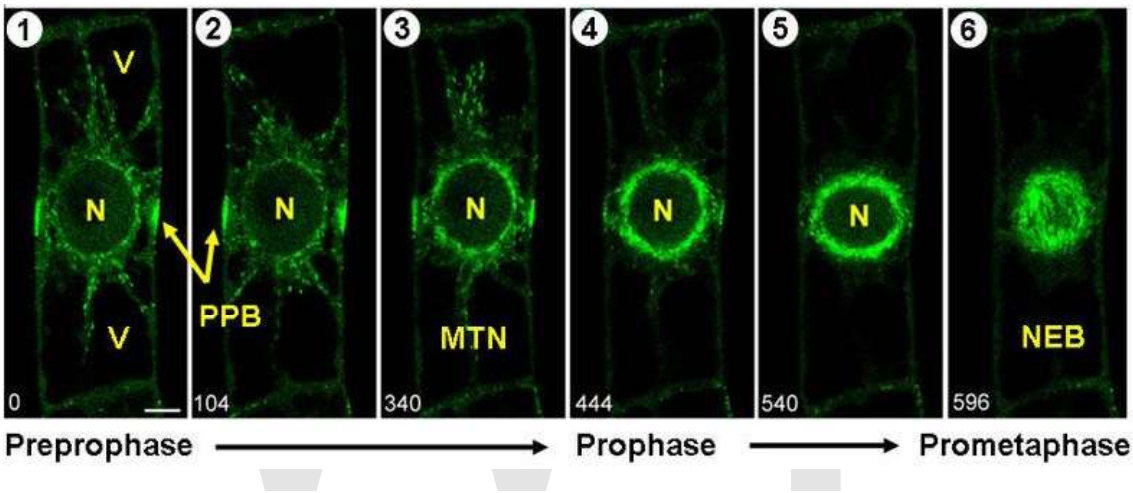
Just before mitosis, a dense band of microtubules appears around the phragmosome and the future division plane just below the plasma membrane. This preprophase band marks the equatorial plane of the future mitotic spindle as well as the future fusion sites for the new cell plate with the existing cell wall. It disappears as soon as the nuclear envelope breaks down and the mitotic spindle forms.

When mitosis is completed, the cell plate and new cell wall form starting from the center along the plane occupied by the phragmosome. The cell plate grows outwards until it fuses with the cell wall of the dividing cell at exactly the spots predicted by the preprophase band.

Chapter 12

Preprophase and Preprophase Band

Preprophase



Microtubule dynamics during preprophase and prophase in plant cell mitosis, modified from Donukshe et al. The images follow a tobacco BY-2 cell through the first stages of mitosis (c. 12 minutes). The growing ends of microtubules are shown in green (labeled with green fluorescent protein fused to the microtubule plus end binding protein EB1 of *Arabidopsis thaliana*). N = Nucleus, V = Vacuole, PPB = Preprophase band, MTN = Microtubule nucleation starts at the nuclear envelope, NEB = Nuclear envelope breakdown at the onset of prometaphase.

Preprophase is an additional phase during mitosis in plant cells that does not occur in other eukaryotes such as animals or fungi. It precedes prophase and is characterized by two distinct events:

- The formation of the preprophase band, a dense microtubule ring underneath the plasma membrane.
- The initiation of microtubule nucleation at the nuclear envelope.

Function of preprophase in the cell cycle

Plant cells are fixed with regards to their neighbor cells within the tissues they are growing in. In contrast to animals where certain cells can migrate within the embryo to form new tissues, the seedlings of higher plants grow entirely based on the orientation of cell division and subsequent elongation and differentiation of cells within their cell walls. Therefore, the accurate control of cell division planes and placement of the future cell wall in plant cells is crucial for the correct architecture of plant tissues and organs.

The preprophase stage of somatic plant cell mitosis serves to establish the precise location of the division plane and future cell wall before the cell enters prophase. This is achieved through the formation of a transient microtubule structure, the preprophase band, and a so far unknown mechanism by which the cell is able to "memorize" the position of the preprophase band to guide the new cell wall growing during cytokinesis to the correct location. In gametophyte tissues during the reproductive phase of the plant life cycle, cell division planes may be established without the use of a preprophase band.

In highly vacuolated plant cells, preprophase may be preceded by the formation of a phragmosome. The function of the phragmosome is to suspend the cell nucleus in the center of the cell in preparation for mitosis. If a phragmosome is visible, the preprophase band will appear at its outer edge.

Preprophase band formation

At the beginning of preprophase, the cortical microtubules of a plant cell disappear and aggregate into a dense ring underneath the plasma membrane. This preprophase band runs around the equatorial plane of the future mitotic spindle and marks the plane of cell division and future fusion site for the cell plate. It consists of microtubules and microfilaments (actin) and persists into prophase. Spindle formation occurs during prophase with the axis perpendicular to the plane surrounded by the preprophase band.

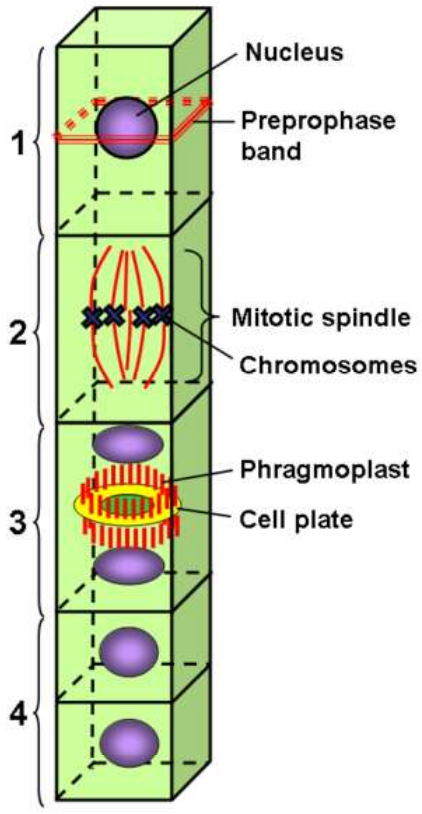
Microtubule nucleation

In contrast to animal cells, plant cells do not possess centrosomes to organize their mitotic spindles. Instead, the nuclear envelope acts as a microtubule organizing center (MTOC) for spindle formation during preprophase. The first sign is a clear, actin-free zone appearing around the nuclear envelope. This zone fills with microtubules nucleating on the surface of the nucleus. The preprophase spindle forms by self-assembly of these microtubules in the cytoplasm surrounding the nuclear envelope. It is reinforced through chromosome (kinetochore)-mediated spindle assembly after the nuclear envelope breaks down at the end of prophase.

Transition into prophase

During progression from preprophase into prophase, the randomly oriented microtubules align parallel along the nuclear surface according to the spindle axis. This structure is called the *prophase spindle*. Triggered by nuclear envelope breakdown at the end of prophase, the preprophase band disappears and the prophase spindle matures into the metaphase spindle occupying the space of the former nucleus. Experiments with drugs destroying microfilaments indicate that actin may play a role in keeping the cellular "memory" of the position of the division plane after the preprophase band breaks down to direct cytokinesis in telophase.

Preprophase band



The preprophase band predicts the cell division plane: 1) Preprophase band formation during preprophase. 2) Metaphase spindle orients with the equator along the plane marked by preprophase band. 3) Phragmoplast and cell plate form along the plane marked by preprophase band. 4) The new cell wall of the daughter cells connects with the parent cell wall along the line of the former preprophase band location.

The **preprophase band** is a microtubule array found in plant cells that are about to undergo cell division and enter the preprophase stage of the plant cell cycle. Besides the phragmosome, it is the first microscopically visible sign that a plant cell is about to enter mitosis. The preprophase band was first observed and described by Jeremy Pickett-Heaps and Donald Northcote at Cambridge University in 1966.

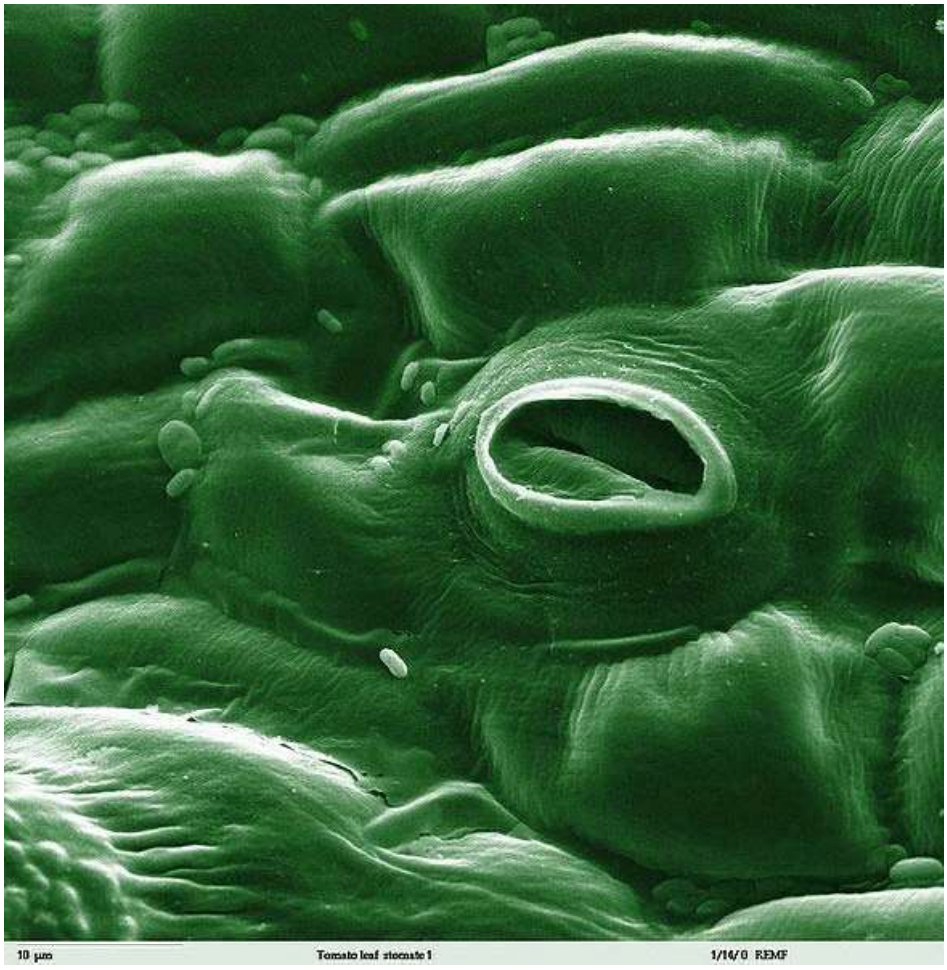
Just before mitosis starts, the preprophase band forms as a dense band of microtubules around the phragmosome and the future division plane just below the plasma membrane. It encircles the nucleus at the equatorial plane of the future mitotic spindle when dividing cells enter the G2 phase of the cell cycle after DNA replication is complete. The preprophase band consists mainly of microtubules and microfilaments (actin) and is generally 2-3 μm wide. When stained with fluorescent markers, it can be seen as two bright spots close to the cell wall on either side of the nucleus.

Plant cells lack centrosomes as microtubule organizing centers. Instead, the microtubules of the mitotic spindle aggregate on the nuclear surface and are reoriented to form the spindle at the end of prophase. The preprophase band also functions in properly orienting the mitotic spindle, and contributes to efficient spindle formation during prometaphase

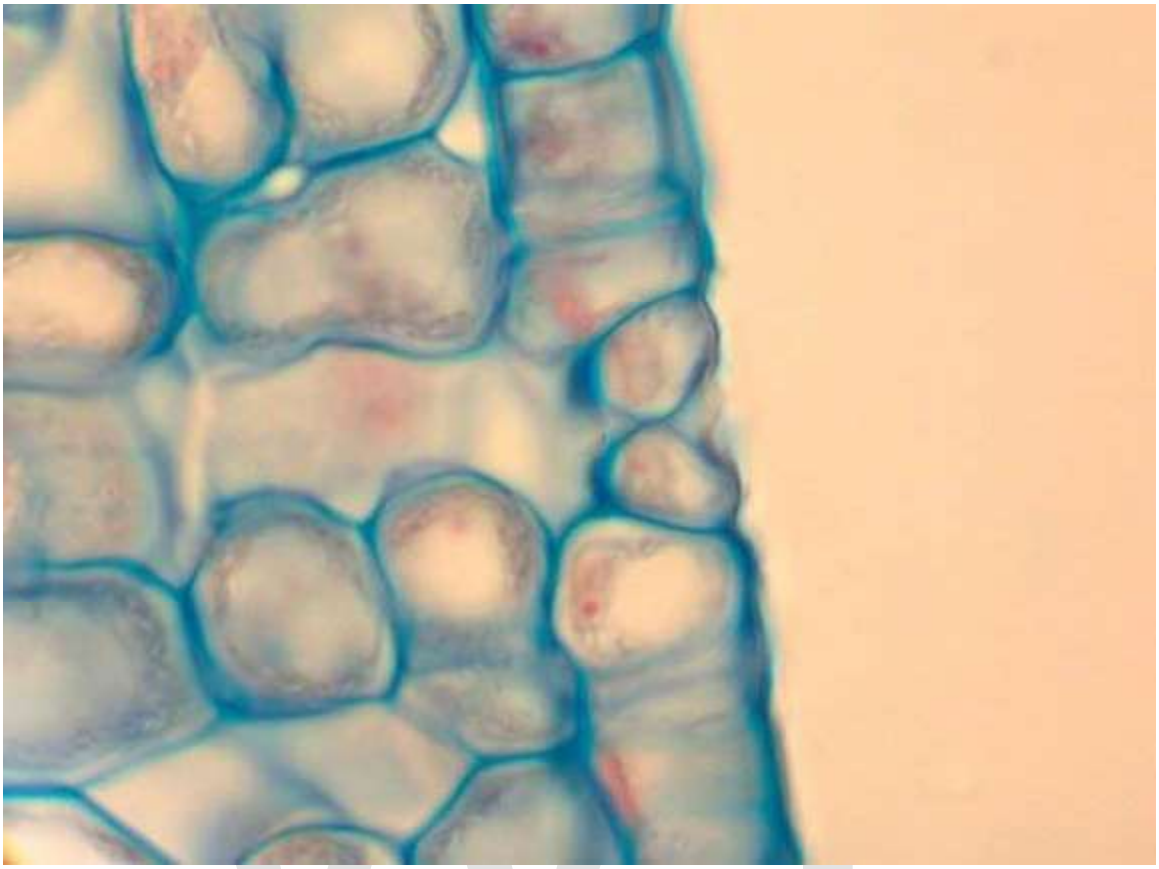
The preprophase band disappears as soon as the nuclear envelope breaks down and the mitotic spindle forms, leaving behind an actin-depleted zone. However, its position marks the future fusion sites for the new cell plate with the existing cell wall during telophase. When mitosis is completed, the cell plate and new cell wall form starting from the center along the plane occupied by the phragmosome. The cell plate grows outwards until it fuses with the cell wall of the dividing cell at exactly the spots predicted by the position of the preprophase band.

Chapter 13

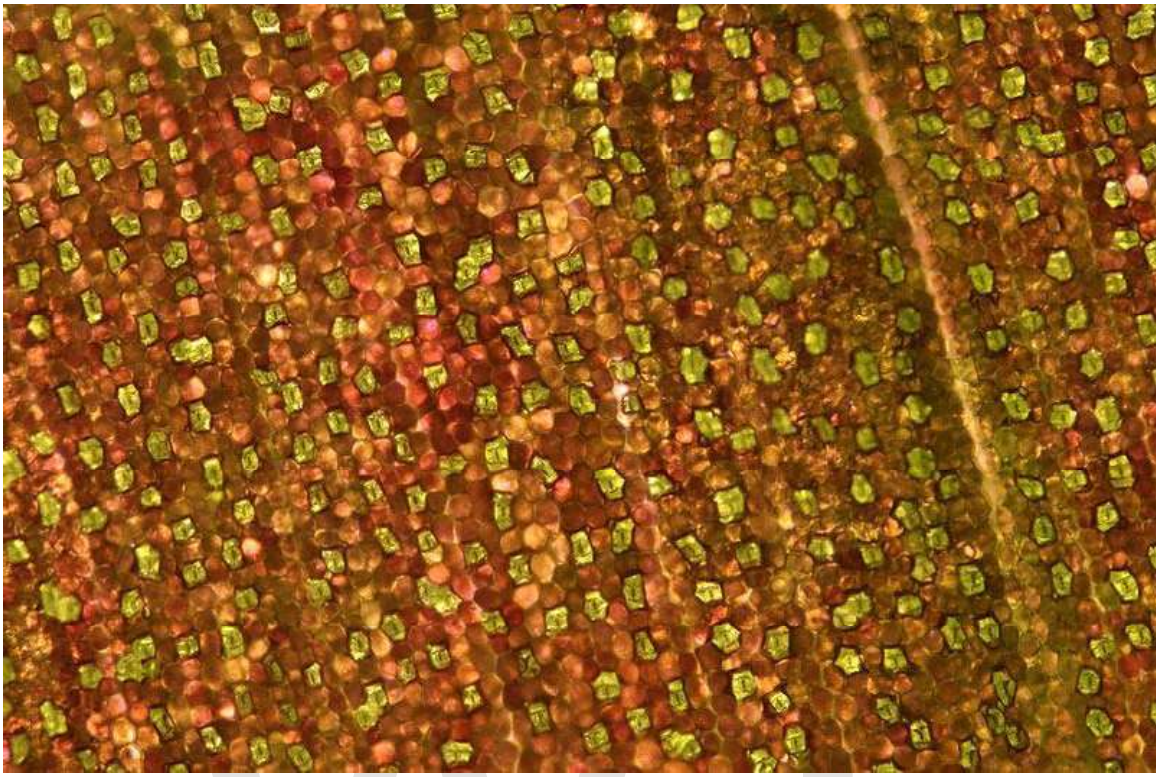
Stoma



Stoma in a tomato leaf shown via colorized scanning electron microscope image



A stoma in cross section



The underside of a leaf. In this species stomata appear green (due to chlorophyl) while the epidermal cells appear red due to additional pigmentation.

In botany, a **stoma** (also **stomate**; plural **stomata**) is a pore, found in the leaf and stem epidermis that is used for gas exchange. The pore is bordered by a pair of specialized parenchyma cells known as guard cells which are responsible for regulating the size of the opening. The term *stoma* is also used collectively to refer to an entire stomatal complex, both the pore itself and its accompanying guard cells. Air containing carbon dioxide and oxygen enters the plant through these openings where it is used in photosynthesis and respiration, respectively. Oxygen produced by photosynthesis in the **spongy layer** cells (parenchyma cells with pectin) of the leaf interior exits through these same openings. Also, water vapor is released into the atmosphere through these pores in a process called transpiration.

Stomata are present in the sporophyte generation of all land plant groups except liverworts. Dicotyledons usually have more stomata on the lower epidermis than the upper epidermis. Monocotyledons, on the other hand, usually have the same number of stomata on the two epidermes. In plants with floating leaves, stomata may be found only on the upper epidermis; submerged leaves may lack stomata entirely.

The word *stoma* derives from Greek στόμα, "mouth".

Function

Carbon gain and water loss

Carbon dioxide, a key reactant in photosynthesis, is present in the atmosphere at a concentration of about 384 ppm (as of March 2008). Most plants require the stomata to be open during daytime. The problem is that the air spaces in the leaf are saturated with water vapour, which exits the leaf through the stomata (this is known as transpiration). Therefore, plants cannot gain carbon dioxide without simultaneously losing water vapour.

Alternative approaches

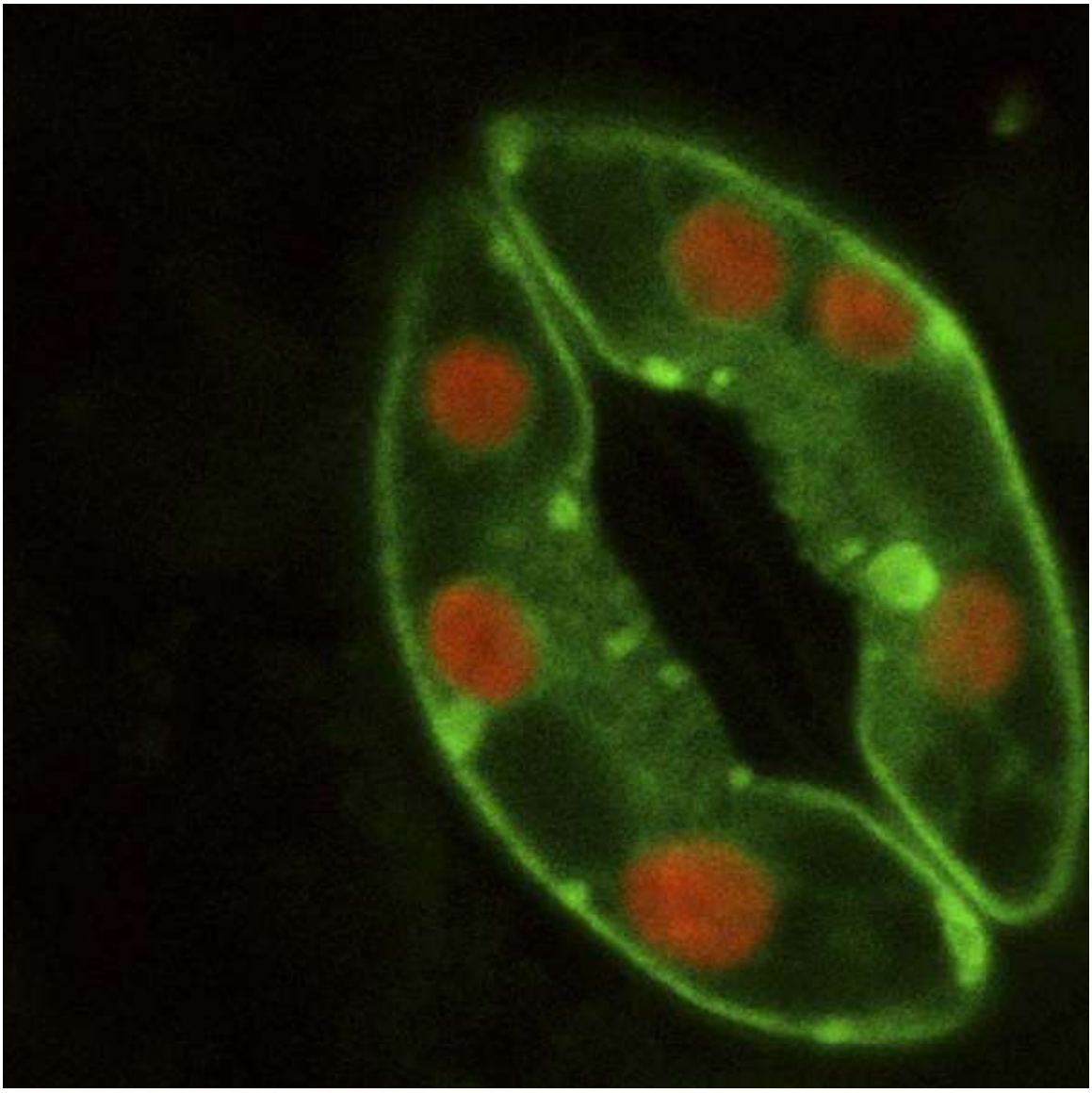
Ordinarily, carbon dioxide is fixed to ribulose-1,5-bisphosphate (BTAC) by the enzyme RuBisCO in mesophyll cells exposed directly to the air spaces inside the leaf. This exacerbates the carbon/water tradeoff for two reasons: first, Rubisco has a relatively low affinity for carbon dioxide and second, it fixes oxygen to RuBP, wasting energy and carbon in a process called photorespiration. For both of these reasons, Rubisco needs high carbon dioxide concentrations, which means high stomatal apertures and consequently high water loss.

However, plants possess another enzyme that can also fix carbon dioxide: PEP carboxylase or BTAC. This enzyme has high carbon dioxide affinity, so a given rate of carbon dioxide fixation can be achieved with less stomatal opening, and hence less water loss. However, the products of carbon fixation by PEPCase must be converted in an energy-intensive process to continue through the carbon reactions of photosynthesis. As a result, the PEPCase alternative is only preferable where water is more limiting but light — which provides the energy in this case — is plentiful, and/or where high temperatures increase the solubility of oxygen relative to that of carbon dioxide, magnifying Rubisco's oxygenation problem.

CAM plants

A group of mostly desert plants called "CAM" plants (Crassulacean acid metabolism, after the family Crassulaceae, which includes the species in which the CAM process was first discovered) open their stomata at night (when water evaporates more slowly from leaves for a given degree of stomatal opening), use PEPcarboxylase to fix carbon dioxide and store the products in large vacuoles. The following day, they close their stomata and release the carbon dioxide fixed the previous night into the presence of RuBisCO. This saturates RuBisCO with carbon dioxide, allowing minimal photorespiration. This approach, however, is severely limited by the capacity to store fixed carbon in the vacuoles, so it is preferable only when water is severely limiting.

Opening and closure



Confocal microscopy image of an *Arabidopsis thaliana* stoma showing two guard cells exhibiting fluorescence from green fluorescent protein and native chlorophyll (red)

However, most plants do not have the aforementioned facility and must therefore open and close their stomata during the daytime in response to changing conditions, such as light intensity, humidity, and carbon dioxide concentration. It is not entirely certain how these responses work. However, the basic mechanism involves regulation of osmotic pressure.

When conditions are conducive to stomatal opening (e.g., high light intensity and high humidity), a proton pump drives protons (H^+) from the guard cells. This means that the cells' electrical potential becomes increasingly negative. The negative potential opens

potassium voltage - gated channels and so an uptake of potassium ions (K^+) occurs. To maintain this internal negative voltage so that entry of potassium ions does not stop, negative ions balance the influx of potassium. In some cases chloride ions enter, while in other plants the organic ion malate is produced in guard cells. This increase in solute concentration lowers the water potential inside the cell, which results in water diffusing into the cell through osmosis. This increases the cell's volume and turgor pressure. Then, because of rings of cellulose microfibrils that prevent the width of the guard cells from swelling, and thus only allow the extra turgor pressure to elongate the guard cells, whose ends are held firmly in place by surrounding epidermal cells, the two guard cells lengthen by bowing apart from one another, creating an open pore through which gas can move.

When the roots begin to sense a water shortage in the soil, abscisic acid (ABA) is released. ABA binds to receptor proteins in the guard cells' plasma membrane and cytosol, which first raises the pH of the cytosol of the cells and cause the concentration of free Ca^{2+} to increase in the cytosol due to influx from outside the cell and release of Ca^{2+} from internal stores such as the endoplasmic reticulum and vacuoles. This causes the chloride (Cl^-) and inorganic ions to exit the cells. Secondly, this stops the uptake of any further K^+ into the cells and subsequently the loss of K^+. The loss of these solutes causes an increase in water potential, which results in water diffusing back out of the cell by osmosis. This makes the cell flaccid, which results in the closing of the stomatal pores.

However there is a another hypothesis--starch and sugar hypothesis. During the day, plant experience photosynthesis and cause partial pressure of Carbon Dioxide to drop and cause the pH value to rise. This cause the starch(almost insoluble) to convert to sugar and dissolve in the cytoplasm, causing the water potential in guard cell to decrease and draw in water from the neighboring cell. The guard cell become turgid and the stomata expend. When there is no light, photosynthesis stop and partial pressure of carbon dioxide increase, causing the pH value in guard cell to rise. Soluble sugar is converted to insoluble starch and the water potential in guard cell increase and water is drown out of the guard cell and the stomata close. Yet this hypothesis has some flaw. For example, the CAM plant undergo photosynthesis in day, but it's stomata open in the night for gases exchange and close in the day to avoid great lost of water.

Interestingly, guard cells have more chloroplasts than the other epidermal cells from which guard cells are derived. Their function is controversial.

Inferring stomatal behavior from gas exchange

The degree of stomatal resistance can be determined by measuring leaf gas exchange of a leaf. The transpiration rate is dependent on the diffusion resistance provided by the stomatal pores, and also on the humidity gradient between the leaf's internal air spaces and the outside air. Stomatal resistance (or its inverse, stomatal conductance) can therefore be calculated from the transpiration rate and humidity gradient. This allows scientists to investigate how stomata respond to changes in environmental conditions, such as light intensity and concentrations of gases such as water vapor, carbon dioxide, and ozone. Evaporation (E) can be calculated as;

$E = (e_i - e_a) / P\, r$

where *ei* and *ea* = partial pressures of water in the leaf and in the ambient air; P = atmospheric pressure; and r = stomatal resistance. The inverse of r is conductance to water vapor (g), so the equation can be rearranged to;

$E = (e_i - e_a)\, g / P$

and solved for g;

$g = EP / (e_i - e_a)$

The rate of evaporation from a leaf can be determined using a photosynthesis system. These scientific instruments measure the amount of water vapour leaving the leaf and the vapor pressure of the ambient air. Photosynthetic systems may calculate water use efficiency (A/E), stomatal conductance (gs), intrinsic water use efficiency (A/gs), and sub-stomatal CO_2 concentration (Ci). These scientific instruments are commonly used by plant physiologists to measure CO_2 uptake and thus measure photosynthetic rate.

Evolution

The fossil record has little to say about the evolution of stomata. They may have evolved by the modification of conceptacles from plants' alga-like ancestors. It is clear, however, that the evolution of stomata must have happened at the same time as the waxy cuticle was evolving - these two traits together constituted a major advantage for primitive terrestrial plants.

Development

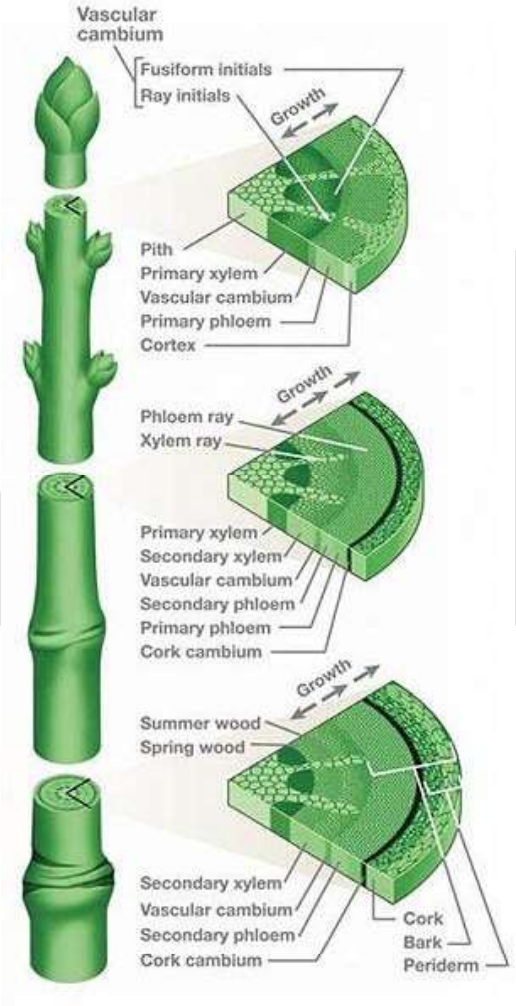
There are three major epidermal cell types which all ultimately derive from the L1 tissue layer of the shoot apical meristem, called protodermal cells: trichomes, pavement cells and guard cells, all of which are arranged in a nonrandom fashion. An asymmetrical cell division occurs in protodermal cells resulting in one large cell that is fated to become a pavement cell and a smaller cell called a meristemoid that will eventually differentiate into the guard cells that surround a stoma. This meristemoid then divides asymmetrically one to three times before differentiating into a guard mother cell. The guard mother cell then makes one symmetrical division, which forms a pair of guard cells.

Stomata as pathogenic pathways

Stomata are an obvious hole in the leaf by which, as was presumed for a while, pathogens can enter unchallenged. However, it has been recently shown that stomata do in fact sense the presence of some, if not all, pathogens. However, with the virulent bacteria applied to *Arabidopsis* plant leaves in the experiment, the bacteria released the chemical coronatine, which forced the stomata open again within a few hours.

Chapter 14

Xylem



Multiple cross sections of a flowering plant stem showing primary and secondary **xylem** and phloem

In vascular plants, **xylem** is one of the two types of transport tissue, phloem being the other. The word "xylem" is derived from classical Greek ξυλον (*xylon*), "wood", and indeed the best-known xylem tissue is wood, though it is found throughout the plant. Its basic function is to transport water but it also transports some nutrients through the plant.

Structure

The most distinctive xylem cells are the long tracheary elements that transport water. Tracheids and vessel elements are distinguished by the multitude of spots that occur on the vessel elements. In these spots both outer and inner walls are missing. The vessel elements are connected to each other in the ends, forming continuous tubes that are called *vessels*.

Xylem also contains other cell types, for example parenchyma.

Xylem can be found:

- in vascular bundles, present in non-woody plants and non-woody parts of plants with wood
- in secondary xylem, laid down by a meristem called the vascular cambium in woody plants
- as part of a stelar arrangement not divided into bundles, as in many ferns

In transitional stages of plants with secondary growth, the first two categories are not mutually exclusive, although usually a vascular bundle will contain *primary xylem* only.

The branching pattern exhibited by xylem follows Murray's law.

Primary and secondary xylem

Primary xylem is the xylem that is formed during primary growth from procambium. It includes protoxylem and metaxylem. Metaxylem develops after the protoxylem but before secondary xylem. It is distinguished by wider vessels and tracheids. Xylem development occurs in a number of patterns, which vary in the relative position of the protoxylem and metaxylem, e.g. endarch in which the protoxylem is towards the centre of the stem or root, or exarch in which the metaxylem is towards the centre.

Secondary xylem is the xylem that is formed during secondary growth from vascular cambium. Although secondary xylem is also found in members of the "gymnosperm" groups Gnetophyta and Ginkgophyta and to a lesser extent in members of the Cycadophyta, the two main groups in which secondary xylem can be found are:

1. conifers (*Coniferae*): there are some six hundred species of conifers. All species have secondary xylem, which is relatively uniform in structure throughout this group. Many conifers become tall trees: the secondary xylem of such trees is marketed as **softwood**.
2. angiosperms (*Angiospermae*): there are some quarter of a million to four hundred thousand species of angiosperms. Within this group secondary xylem has not been found in the monocots. In the remainder of the angiosperms, this secondary xylem may or may not be present; this may vary even within a species, depending on

growing circumstances. In view of the size of this group, it will be no surprise that no absolutes apply to the structure of secondary xylem within the angiosperms. Many non-monocot angiosperms become trees, and the secondary xylem of these is marketed as **hardwood**.

Main function - upwards water transport

The xylem transports water and soluble mineral nutrients from the roots throughout the plant. It is also used to replace water lost during transpiration and photosynthesis. Xylem sap consists mainly of water and inorganic ions, although it can contain a number of organic chemicals as well. This transport is not powered by energy spent by the tracheary elements themselves, which are dead by maturity and no longer have living contents. Two phenomena cause xylem sap to flow:

- **Transpirational pull**: the most important cause of xylem sap flow is the evaporation of water from the surfaces of mesophyll cells to the atmosphere. This transpiration causes millions of minute menisci to form in the mesophyll cell wall. The resulting surface tension causes a negative pressure or tension in the xylem that pulls the water from the roots and soil.
- **Root pressure**: If the water potential of the root cells is more negative than that of the soil, usually due to high concentrations of solute, water can move by osmosis into the root from the soil. This causes a positive pressure that forces sap up the xylem towards the leaves. In some circumstances, the sap will be forced from the leaf through a hydathode in a phenomenon known as guttation. Root pressure is highest in the morning before the stomata open and allow transpiration to begin. Different plant species can have different root pressures even in a similar environment; examples include up to 145 kPa in *Vitis riparia* but around zero in *Celastrus orbiculatus*.

The primary force that creates the capillary action movement of water upwards in plants is the adhesion between the water and the surface of the xylem conduits. Capillary action provides the force that establishes an equilibrium configuration, balancing gravity. When transpiration removes water at the top, the flow is needed to return to the equilibrium.

Transpirational pull results from the evaporation of water from the surfaces of cells in the leaves. This evaporation causes the surface of the water to recess into the pores of the cell wall. By capillary action, the water forms concave menisci inside the pores. The high surface tension of water pulls the concavity outwards, generating enough force to lift water as high as a hundred meters from ground level to a tree's highest branches.

Transpirational pull requires that the vessels transporting the water are very small in diameter, otherwise cavitation would break the water column. And as water evaporates from leaves, more is drawn up through the plant to replace it. When the water pressure within the xylem reaches extreme levels due to low water input from the roots (if, for example, the soil is dry), then the gases come out of solution and form a bubble - an embolism forms, which will spread quickly to other adjacent cells, unless *bordered pits*

are present (these have a plug-like structure called a torus, that seals off the opening between adjacent cells and stops the embolism from spreading).

Cohesion-tension theory

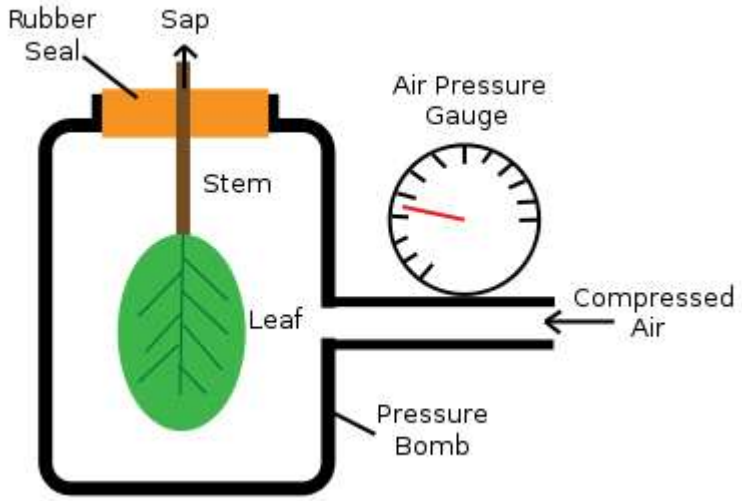
The *cohesion-tension theory* is a theory of intermolecular attraction commonly observed in the process of water traveling upwards (against the force of gravity) through the xylem of plants which was put forward by John Joly and Henry Horatio Dixon. Despite numerous objections, this is the most widely accepted theory for the transport of water through a plant's vascular system based on the classical research of Dixon-Joly (1894) , Askenasy (1895) , and Dixon (1914,1924) .

Water is a polar molecule due to the high electronegativity of the oxygen atom, which is an uncommon molecular configuration whereby the oxygen atom has two lone pairs of electrons. When two water molecules approach one another they form a hydrogen bond. The negatively charged oxygen atom of one water molecule forms a hydrogen bond with a positively charged hydrogen atom in another water molecule. This attractive force has several manifestations. Firstly, it causes water to be liquid at room temperature, while other lightweight molecules would be in a gaseous phase. Secondly, it (along with other intermolecular forces) is one of the principal factors responsible for the occurrence of surface tension in liquid water. This attractive force between molecules allows plants to draw water from the root (via osmosis) and then through the xylem to the leaf where photosynthesis converts water and carbon dioxide into glucose.

Water is constantly lost by transpiration in the leaf. When one water molecule is lost another is pulled along by the processes of cohesion and adhesion. Transpiration pull, utilizing capillary action and the inherent surface tension of water, is the primary mechanism of water movement in plants. However, it is not the only mechanism involved. Any use of water in leaves produces forces water to move into them.

Transpiration in leaves creates tension (negative pressure) in the mesophyll cells. Because of this tension, water is literally being pulled up from the roots into the leaves, helped by cohesion (the pull between individual water molecules, due to hydrogen bonds) and adhesion (the stickiness between water molecules and the hydrophilic cell walls of plants). This mechanism of water flow works because of water potential (water flows from high to low potential), and the rules of simple diffusion.

Measurement of pressure



A diagram showing the setup of a pressure bomb

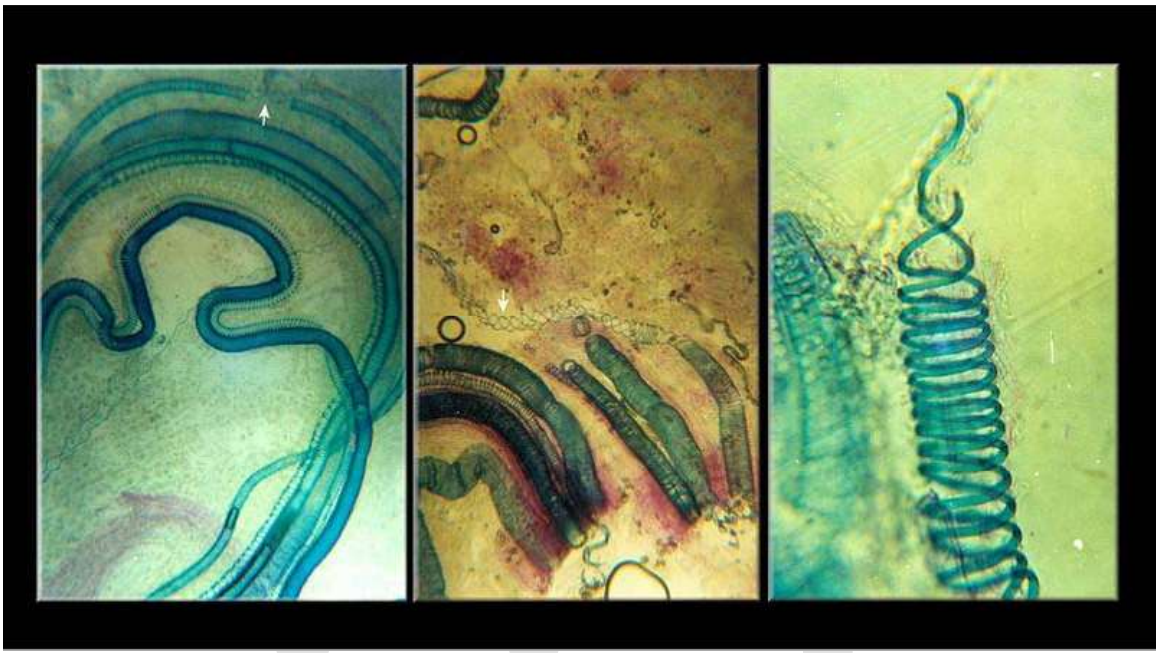
Until recently, the negative pressure (suction) of transpirational pull could only be measured indirectly, by applying external pressure with a pressure bomb to counteract it. When the technology to perform direct measurements with a pressure probe was developed, there was initially some controversy about whether the classic theory was correct, because some workers were unable to demonstrate negative pressures. More recent measurements do tend to validate the classic theory, for the most part. Xylem transport is driven by a combination of transpirational pull from above and root pressure from below, which makes the interpretation of measurements more complicated.

Evolution

Xylem appeared early in the history of terrestrial plant life. Fossil plants with anatomically preserved xylem are known from the Silurian (more than 400 million years ago), and trace fossils resembling individual xylem cells may be found in earlier Ordovician rocks. The earliest true and recognizable xylem consists of tracheids with a helical-annular reinforcing layer added to the cell wall. This is the only type of xylem found in the earliest vascular plants, and this type of cell continues to be found in the *protoxylem* (first-formed xylem) of all living groups of plants. Several groups of plants later developed pitted tracheid cells, it seems, through convergent evolution. In living plants, pitted tracheids do not appear in development until the maturation of the *metaxylem* (following the *protoxylem*).

In most plants, pitted tracheids function as the primary transport cells. The other type of tracheary element, besides the tracheid, is the vessel element. Vessel elements are joined by perforations into vessels. In vessels, water travels by *bulk flow*, as in a pipe, rather than by diffusion through cell membranes. The presence of vessels in xylem has been

considered to be one of the key innovations that led to the success of the angiosperms. However, the occurrence of vessel elements is not restricted to angiosperms, and they are absent in some archaic or "basal" lineages of the angiosperms: (e.g., Amborellaceae, Tetracentraceae, Trochodendraceae, and Winteraceae), and their secondary xylem is described by Arthur Cronquist as "primitively vesselless". Cronquist considered the vessels of *Gnetum* to be convergent with those of angiosperms. Whether the absence of vessels in basal angiosperms is a primitive condition is contested, the alternative hypothesis states that vessel elements originated in a precursor to the angiosperms and were subsequently lost.



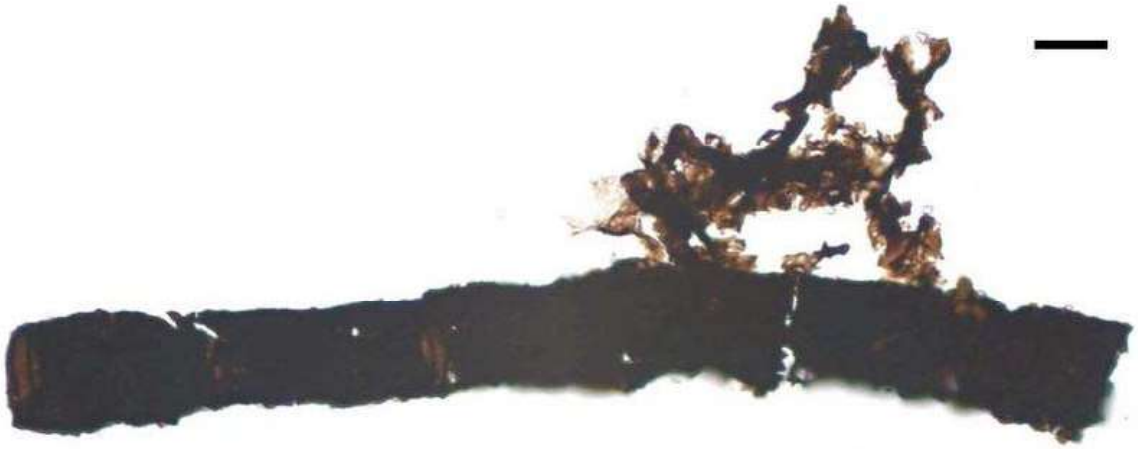
Photos showing xylem elements in the shoot of a fig tree (*Ficus alba*): crushed in hydrochloric acid, between slides and cover slips.

To photosynthesise, plants must absorb CO_2 from the atmosphere. However, this comes at a price: while stomata are open to allow CO_2 to enter, water can evaporate. Water is lost much faster than CO_2 is absorbed, so plants need to replace it, and have developed systems to transport water from the moist soil to the site of photosynthesis. Early plants sucked water between the walls of their cells, then evolved the ability to control water loss (and CO_2 acquisition) through the use of stomata. Specialised water transport tissues soon evolved in the form of hydroids, tracheids, then secondary xylem, followed by an endodermis and ultimately vessels.

The high CO_2 levels of Silurian-Devonian times, when plants were first colonising land, meant that the need for water was relatively low . As CO_2 was withdrawn from the atmosphere by plants, more water was lost in its capture, and more elegant transport mechanisms evolved. As water transport mechanisms, and waterproof cuticles, evolved, plants could survive without being continually covered by a film of water. This transition from poikilohydry to homoiohydry opened up new potential for colonisation. Plants then

needed a robust internal structure that held long narrow channels for transporting water from the soil to all the different parts of the above-soil plant, especially to the parts where photosynthesis occurred.

During the Silurian, CO_2 was readily available, so little water needed expending to acquire it. By the end of the Carboniferous, when CO_2 levels had lowered to something approaching today's, around 17 times more water was lost per unit of CO_2 uptake. However, even in these "easy" early days, water was at a premium, and had to be transported to parts of the plant from the wet soil to avoid desiccation. This early water transport took advantage of the **cohesion-tension** mechanism inherent in water. Water has a tendency to diffuse to areas that are drier, and this process is accelerated when water can be wicked along a fabric with small spaces. In small passages, such as that between the plant cell walls (or in tracheids), a column of water behaves like rubber – when molecules evaporate from one end, they literally pull the molecules behind them along the channels. Therefore transpiration alone provided the driving force for water transport in early plants. However, without dedicated transport vessels, the cohesion-tension mechanism cannot transport water more than about 2 cm, severely limiting the size of the earliest plants. This process demands a steady supply of water from one end, to maintain the chains; to avoid exhausing it, plants developed a waterproof cuticle. Early cuticle may not have had pores but did not cover the entire plant surface, so that gas exchange could continue. However, dehydration at times was inevitable; early plants cope with this by having a lot of water stored between their cell walls, and when it comes to it sticking out the tough times by putting life "on hold" until more water is supplied.



A banded tube from the late Silurian/early Devonian. The bands are difficult to see on this specimen, as an opaque carbonaceous coating conceals much of the tube. Bands are just visible in places on the left half of the image

To be free from the constraints of small size and constant moisture that the parenchymatic transport system inflicted, plants needed a more efficient water transport system. During the early Silurian, they developed specialized cells, which were lignified (or bore similar chemical compounds) to avoid implosion; this process coincided with cell death, allowing their innards to be emptied and water to be passed through them.

These wider, dead, empty cells were a million times more conductive than the inter-cell method, giving the potential for transport over longer distances, and higher CO_2 diffusion rates.

The first macrofossils to bear water-transport tubes *in situ* are the early Devonian pretracheophytes *Aglaophyton* and *Horneophyton*, which have structures very similar to the **hydroids** of modern mosses. Plants continued to innovate new ways of reducing the resistance to flow within their cells, thereby increasing the efficiency of their water transport. Bands on the walls of tubes, in fact apparent from the early Silurian onwards, are an early improvisation to aid the easy flow of water. Banded tubes, as well as tubes with pitted ornamentation on their walls, were lignified and, when they form single celled conduits, are considered to be **tracheids**. These, the "next generation" of transport cell design, have a more rigid structure than hydroids, allowing them to cope with higher levels of water pressure. Tracheids may have a single evolutionary origin, possibly within the hornworts, uniting all tracheophytes (but they may have evolved more than once).

Water transport requires regulation, and dynamic control is provided by stomata. By adjusting the amount of gas exchange, they can restrict the amount of water lost through transpiration. This is an important role where water supply is not constant, and indeed stomata appear to have evolved before tracheids, being present in the non-vascular hornworts.

An endodermis probably evolved during the Silu-Devonian, but the first fossil evidence for such a structure is Carboniferous. This structure in the roots covers the water transport tissue and regulates ion exchange (and prevents unwanted pathogens etc. from entering the water transport system). The endodermis can also provide an upwards pressure, forcing water out of the roots when transpiration is not enough of a driver.

Once plants had evolved this level of controlled water transport, they were truly homoiohydric, able to extract water from their environment through root-like organs rather than relying on a film of surface moisture, enabling them to grow to much greater size. As a result of their independence from their surroundings, they lost their ability to survive desiccation – a costly trait to retain.

During the Devonian, maximum xylem diameter increased with time, with the minimum diameter remaining pretty constant. By the middle Devonian, the tracheid diameter of some plant lineages had plateaued. Wider tracheids allow water to be transported faster, but the overall transport rate depends also on the overall cross-sectional area of the xylem bundle itself. The increase in vascular bundle thickness further seems to correlate with the width of plant axes, and plant height; it is also closely related to the appearance of leaves and increased stomatal density, both of which would increase the demand for water.

While wider tracheids with robust walls make it possible to achieve higher water transport pressures, this increases the problem of cavitation. Cavitation occurs when a bubble of air forms within a vessel, breaking the bonds between chains of water

molecules and preventing them from pulling more water up with their cohesive tension. A tracheid, once cavitated, cannot have its embolism removed and return to service (except in a few advanced angiosperms which have developed a mechanism of doing so). Therefore it is well worth plants' while to avoid cavitation occurring. For this reason, pits in tracheid walls have very small diameters, to prevent air entering and allowing bubbles to nucleate. Freeze-thaw cycles are a major cause of cavitation. Damage to a tracheid's wall almost inevitably leads to air leaking in and cavitation, hence the importance of many tracheids working in parallel.

Cavitation is hard to avoid, but once it has occurred plants have a range of mechanisms to contain the damage. Small pits link adjacent conduits to allow fluid to flow between them, but not air – although ironically these pits, which prevent the spread of embolisms, are also a major cause of them. These pitted surfaces further reduce the flow of water through the xylem by as much as 30%. Conifers, by the Jurassic, developed an ingenious improvement, using valve-like structures to isolate cavitated elements. These torus-margo structures have a blob floating in the middle of a donut; when one side depressurises the blob is sucked into the torus and blocks further flow. Other plants simply accept cavitation; for instance, oaks grow a ring of wide vessels at the start of each spring, none of which survive the winter frosts. Maples use root pressure each spring to force sap upwards from the roots, squeezing out any air bubbles.

Growing to height also employed another trait of tracheids – the support offered by their lignified walls. Defunct tracheids were retained to form a strong, woody stem, produced in most instances by a secondary xylem. However, in early plants, tracheids were too mechanically vulnerable, and retained a central position, with a layer of tough sclerenchyma on the outer rim of the stems. Even when tracheids do take a structural role, they are supported by sclerenchymatic tissue.

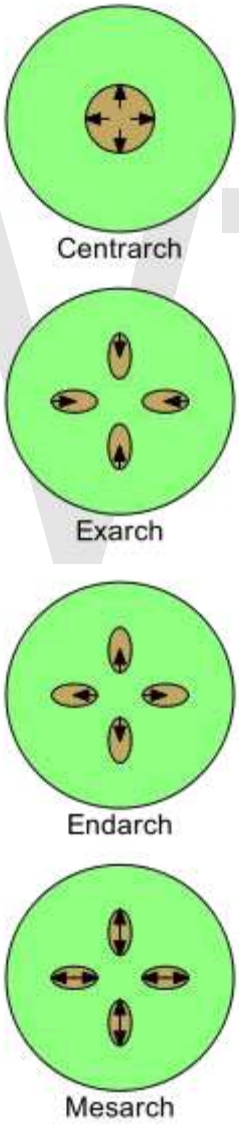
Tracheids end with walls, which impose a great deal of resistance on flow; vessel members have perforated end walls, and are arranged in series to operate as if they were one continuous vessel. The function of end walls, which were the default state in the Devonian, was probably to avoid embolisms. An embolism is where an air bubble is created in a tracheid. This may happen as a result of freezing, or by gases dissolving out of solution. Once an embolism is formed, it usually cannot be removed; the affected cell cannot pull water up, and is rendered useless.

End walls excluded, the tracheids of prevascular plants were able to operate under the same hydraulic conductivity as those of the first vascular plant, *Cooksonia*.

The size of tracheids is limited as they comprise a single cell; this limits their length, which in turn limits their maximum useful diameter to 80 μm. Conductivity grows with the fourth power of diameter, so increased diameter has huge rewards; **vessel elements**, consisting of a number of cells, joined at their ends, overcame this limit and allowed larger tubes to form, reaching diameters of up to 500 μm, and lengths of up to 10 m.

Vessels first evolved during the dry, low CO_2 periods of the late Permian, in the horsetails, ferns and Selaginellales independently, and later appeared in the mid Cretaceous in angiosperms and gnetophytes. Vessels allow the same cross-sectional area of wood to transport around a hundred times more water than tracheids! This allowed plants to fill more of their stems with structural fibres, and also opened a new niche to vines, which could transport water without being as thick as the tree they grew on. Despite these advantages, tracheid-based wood is a lot lighter, thus cheaper to make, as vessels need to be much more reinforced to avoid cavitation.

Development



Patterns of xylem development: xylem in brown; arrows show direction of development from protoxylem to metaxylem

Xylem development can be described by four terms: **centrarch**, **exarch**, **endarch** and **mesarch**. As it develops in young plants, its nature changes from *protoxylem* to *metaxylem* (i.e. from *first xylem* to *after xylem*). The patterns in which protoxylem and metaxylem are arranged is important in the study of plant morphology.

Protoxylem and metaxylem

As a young vascular plant grows, one or more strands of primary xylem form in its stems and roots. The first xylem to develop is called 'protoxylem'. In appearance protoxylem is usually distinguished by narrower vessels formed of smaller cells. Some of these cells have walls which contain thickenings in the form of rings or helices. Functionally, protoxylem can extend: the cells are able to grow in size and develop while a stem or root is elongating. Later, 'metaxylem' develops in the strands of xylem. Metaxylem vessels and cells are usually larger; the cells have thickenings which are typically either in the form of ladderlike transverse bars (scalariform) or continuous sheets except for holes or pits (pitted). Functionally, metaxylem completes its development after elongation ceases when the cells no longer need to be able grow in size.

Patterns of protoxylem and metaxylem

There are four main patterns to the arrangement of protoxylem and metaxylem in stems and roots.

Centrarch refers to the case in which the primary xylem forms a single cylinder in the centre of the stem and develops from the centre outwards. The protoxylem is thus found in the central core and the metaxylem in a cylinder around it. This pattern was common in early land plants, such as "rhyniophytes".

The other three terms are used where there is more than one strand of primary xylem.

Exarch is used when there is more than one strand of primary xylem in a stem or root, and the xylem develops from the outside inwards towards the centre, i.e. centripetally. The metaxylem is thus closest to the centre of the stem or root and the protoxylem closest to the periphery. The roots of vascular plants are normally considered to have exarch development.

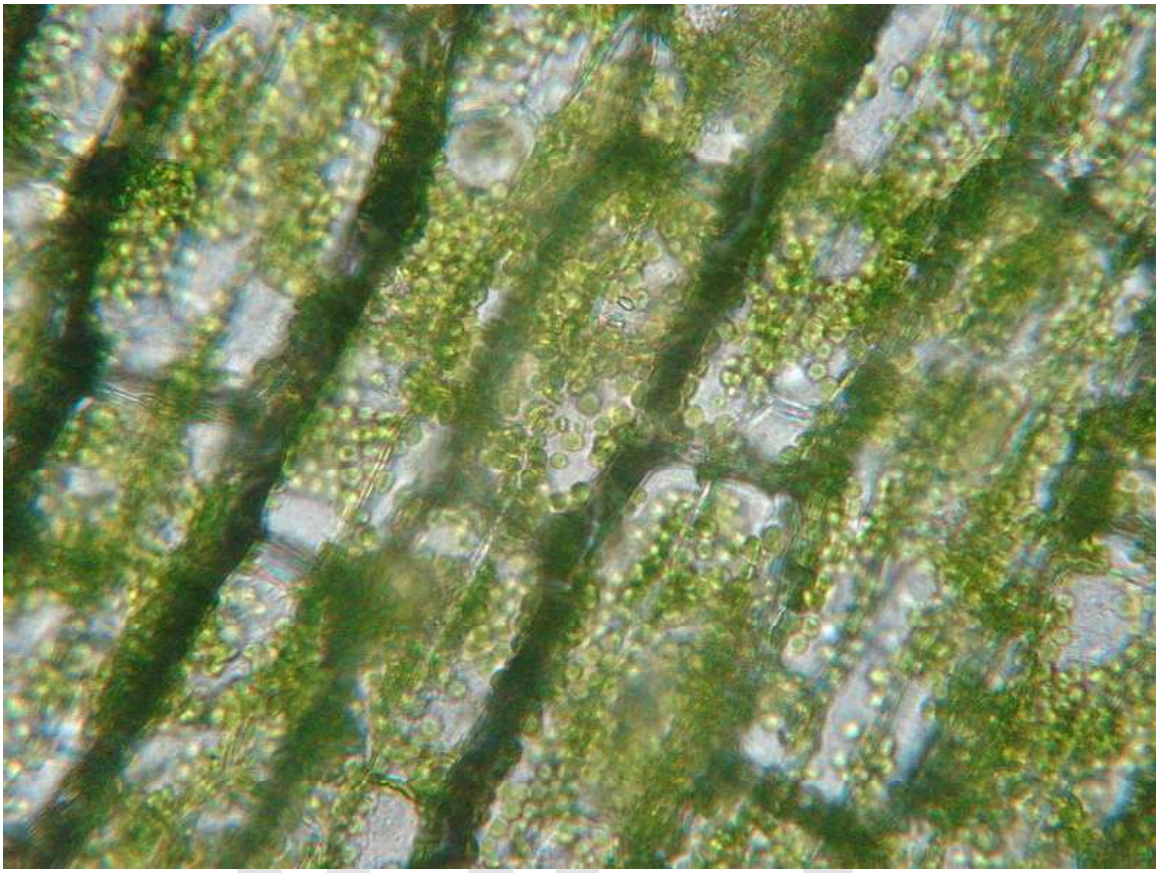
Endarch is used when there is more than one strand of primary xylem in a stem or root, and the xylem develops from the inside outwards towards the periphery, i.e. centrifugally. The protoxylem is thus closest to the centre of the stem or root and the metaxylem closest to the periphery. The stems of seed plants typically have endarch development.

Mesarch is used when there is more than one strand of primary xylem in a stem or root, and the xylem develops from the middle of a strand in both directions. The metaxylem is thus on both the peripheral and central sides of the strand with the protoxylem between the metaxylem (possibly surrounded by it). The leaves and stems of many ferns have mesarch development.

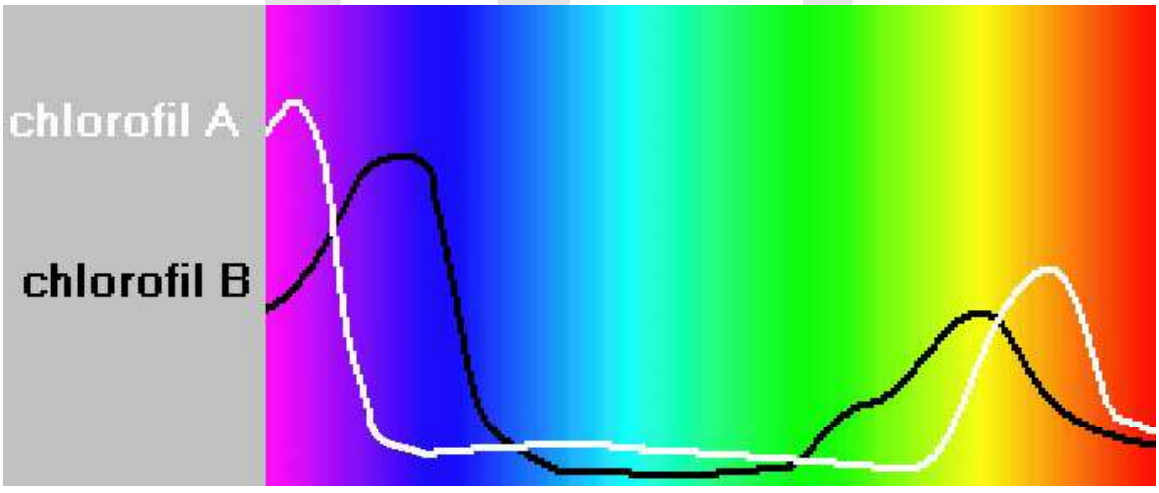
Chapter 15

Chlorophyll

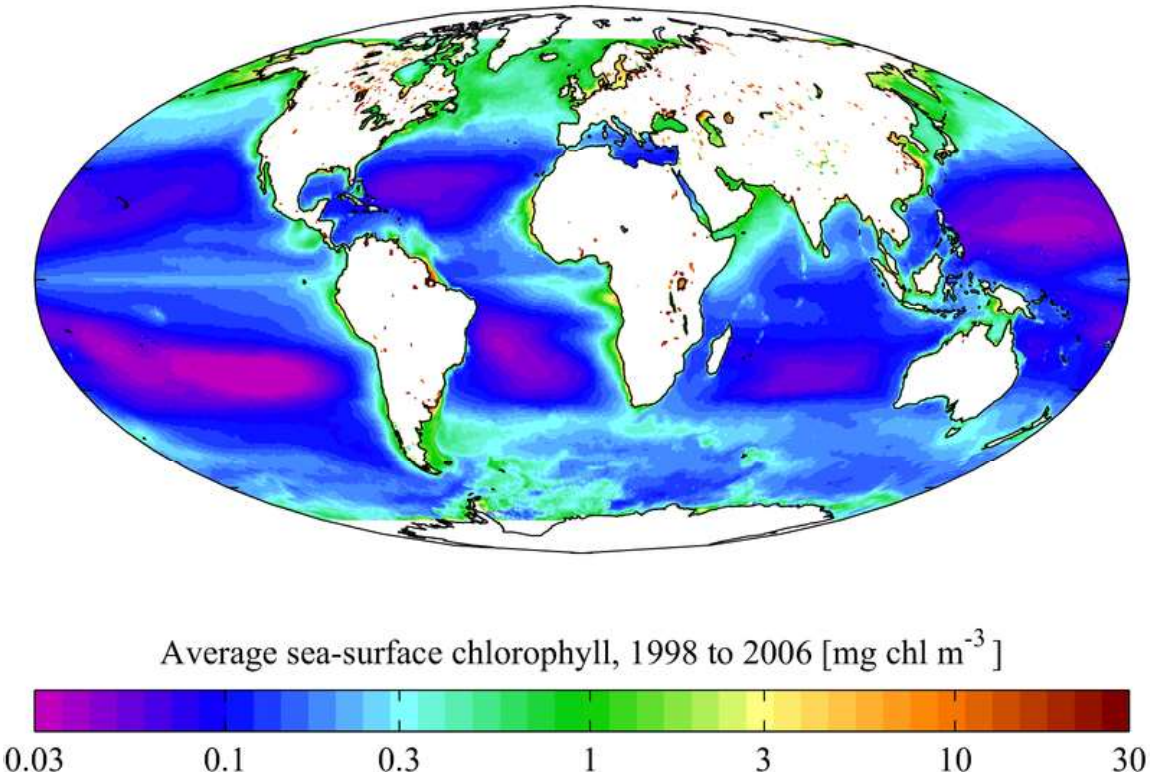
Chlorophyll gives leaves their green color and absorbs light that is used in photosynthesis.



Chlorophyll is found in high concentrations in chloroplasts of plant cells.



Absorption maxima of chlorophylls against the spectrum of white light.



SeaWiFS-derived average sea surface **chlorophyll** for the period 1998 to 2006.

Chlorophyll (also **chlorophyl**) is a green pigment found in almost all plants, algae, and cyanobacteria. Its name is derived from the Greek words *chloros* ("green") and *phyllon* ("leaf"). Chlorophyll is an extremely important biomolecule, critical in photosynthesis, which allows plants to obtain energy from light. Chlorophyll absorbs light most strongly in the blue portion of the electromagnetic spectrum, followed by the red portion. However, it is a poor absorber of green and near-green portions of the spectrum; hence the green color of chlorophyll-containing tissues. Chlorophyll was first isolated by Joseph Bienaimé Caventou and Pierre Joseph Pelletier in 1817.

Chlorophyll and photosynthesis

Chlorophyll is vital for photosynthesis, which allows plants to obtain energy from light.

Chlorophyll molecules are specifically arranged in and around photosystems that are embedded in the thylakoid membranes of chloroplasts. In these complexes, chlorophyll serves two primary functions. The function of the vast majority of chlorophyll (up to several hundred molecules per photosystem) is to absorb light and transfer that light energy by resonance energy transfer to a specific chlorophyll pair in the reaction center of the photosystems.

The two currently accepted photosystem units are Photosystem II and Photosystem I, which have their own distinct reaction center chlorophylls, named P680 and P700,

respectively. These pigments are named after the wavelength (in nanometers) of their red-peak absorption maximum. The identity, function and spectral properties of the types of chlorophyll in each photosystem are distinct and determined by each other and the protein structure surrounding them. Once extracted from the protein into a solvent (such as acetone or methanol), these chlorophyll pigments can be separated in a simple paper chromatography experiment and, based on the number of polar groups between chlorophyll a and chlorophyll b, will chemically separate out on the paper.

The function of the reaction center chlorophyll is to use the energy absorbed by and transferred to it from the other chlorophyll pigments in the photosystems to undergo a charge separation, a specific redox reaction in which the chlorophyll donates an electron into a series of molecular intermediates called an electron transport chain. The charged reaction center chlorophyll ($P680^+$) is then reduced back to its ground state by accepting an electron. In Photosystem II, the electron that reduces $P680^+$ ultimately comes from the oxidation of water into O_2 and H^+ through several intermediates. This reaction is how photosynthetic organisms such as plants produce O_2 gas, and is the source for practically all the O_2 in Earth's atmosphere. Photosystem I typically works in series with Photosystem II; thus the $P700^+$ of Photosystem I is usually reduced, via many intermediates in the thylakoid membrane, by electrons ultimately from Photosystem II. Electron transfer reactions in the thylakoid membranes are complex, however, and the source of electrons used to reduce $P700^+$ can vary.

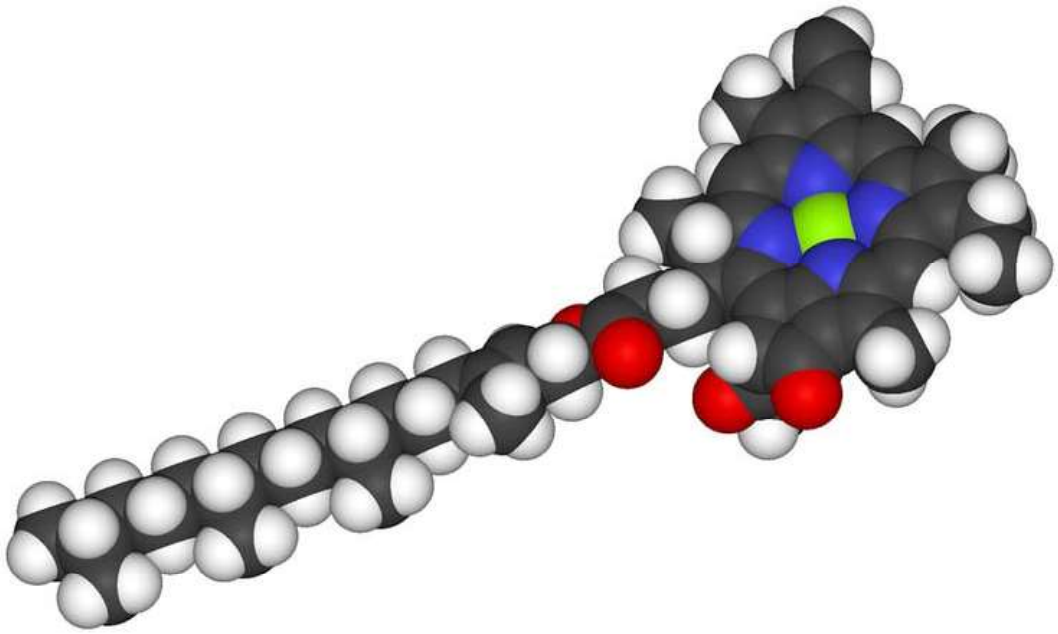
The electron flow produced by the reaction center chlorophyll pigments is used to shuttle H^+ ions across the thylakoid membrane, setting up a chemiosmotic potential used mainly to produce ATP chemical energy; and those electrons ultimately reduce $NADP^+$ to NADPH, a universal reductant used to reduce CO_2 into sugars as well as for other biosynthetic reductions.

Reaction center chlorophyll–protein complexes are capable of directly absorbing light and performing charge separation events without other chlorophyll pigments, but the absorption cross section (the likelihood of absorbing a photon under a given light intensity) is small. Thus, the remaining chlorophylls in the photosystem and antenna pigment protein complexes associated with the photosystems all cooperatively absorb and funnel light energy to the reaction center. Besides chlorophyll *a*, there are other pigments, called accessory pigments, which occur in these pigment–protein antenna complexes.

There is as yet no satisfactory scientific explanation as to why chlorophyll has evolved to "ignore" green and near-green light, which are a major part of the visible spectrum.

A green sea slug, *Elysia chlorotica*, has been found to use the chlorophyll it has eaten to perform photosynthesis for itself. This process is known as kleptoplasty, and no other animal has been found to have this ability.

Chemical structure

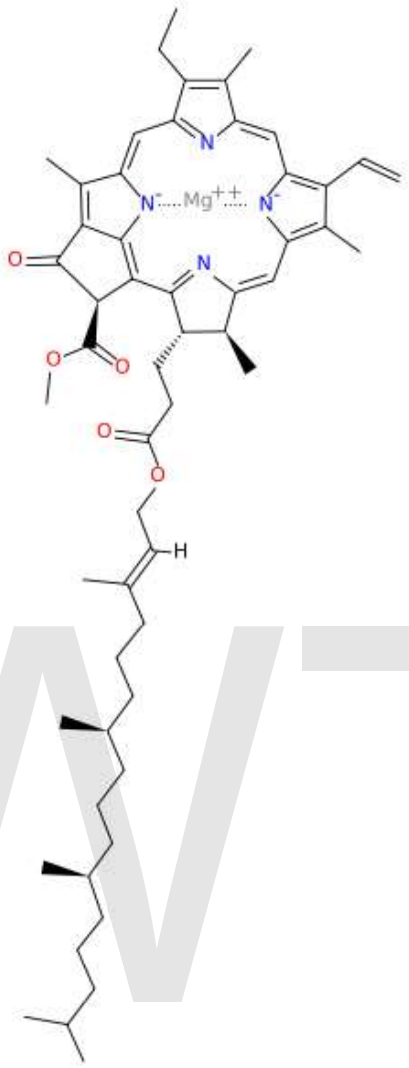


Space-filling model of the chlorophyll a molecule

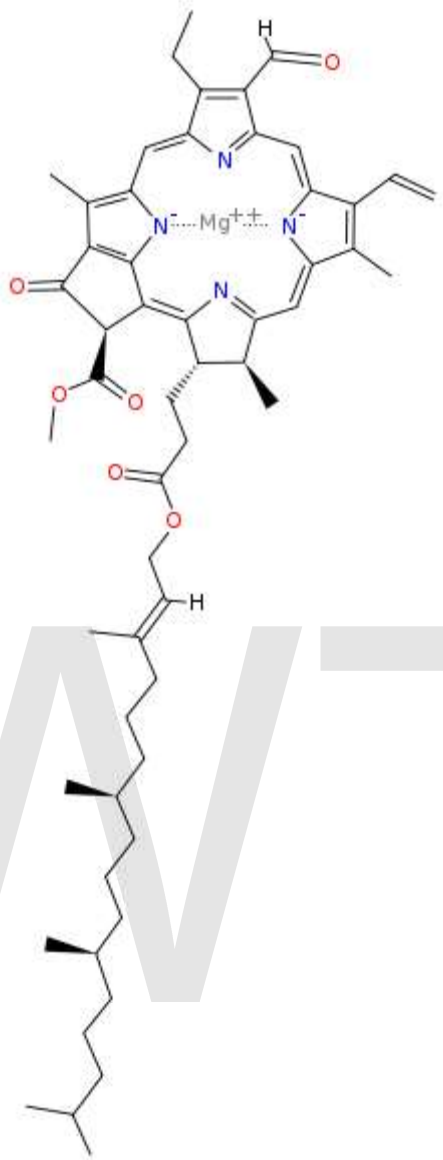
Chlorophyll is a chlorin pigment, which is structurally similar to and produced through the same metabolic pathway as other porphyrin pigments such as heme. At the center of the chlorin ring is a magnesium ion. For the structures depicted here, some of the ligands attached to the Mg^{2+} center are omitted for clarity. The chlorin ring can have several different side chains, usually including a long phytol chain. There are a few different forms that occur naturally, but the most widely distributed form in terrestrial plants is chlorophyll *a*. The general structure of chlorophyll *a* was elucidated by Hans Fischer in 1940, and by 1960, when most of the stereochemistry of chlorophyll *a* was known, Robert Burns Woodward published a total synthesis of the molecule as then known. In 1967, the last remaining stereochemical elucidation was completed by Ian Fleming, and in 1990 Woodward and co-authors published an updated synthesis. In 2010, a near-infrared light photosynthetic pigment called Chlorophyll f may have been discovered in cyanobacteria and other oxygenic microorganisms that form stromatolites. Based on NMR data, optical and mass spectra, it is thought to have a structure of $C_{55}H_{70}O_6N_4Mg$ or [2-formyl]-chlorophyll a.

The different structures of chlorophyll are summarized below:

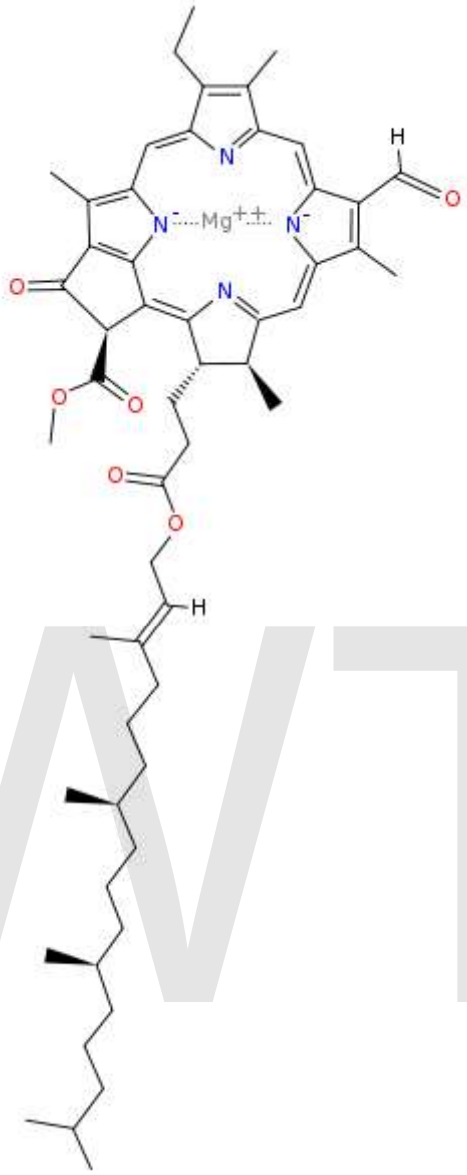
	Chlorophyll a	**Chlorophyll b**	**Chlorophyll c1**	**Chlorophyll c2**	**Chlorophyll d**	**Chlorophyll f**
Molecular formula	$C_{55}H_{72}O_5N_4Mg$	$C_{55}H_{70}O_6N_4Mg$	$C_{35}H_{30}O_5N_4Mg$	$C_{35}H_{28}O_5N_4Mg$	$C_{54}H_{70}O_6N_4Mg$	$C_{55}H_{70}O_6N_4Mg$
C2 group	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$	-CHO
C3 group	$-CH=CH_2$	$-CH=CH_2$	$-CH=CH_2$	$-CH=CH_2$	-CHO	$-CH=CH_2$
C7 group	$-CH_3$	-CHO	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$
C8 group	$-CH_2CH_3$	$-CH_2CH_3$	$-CH_2CH_3$	$-CH=CH_2$	$-CH_2CH_3$	$-CH_2CH_3$
C17 group	$-CH_2CH_2COO$-Phytyl	$-CH_2CH_2COO$-Phytyl	$-CH=CHCOOH$	$-CH=CHCOOH$	$-CH_2CH_2COO$-Phytyl	$-CH_2CH_2COO$-Phytyl
C17-C18 bond	Single (chlorin)	Single (chlorin)	Double (porphyrin)	Double (porphyrin)	Single (chlorin)	Single (chlorin)
Occurrence	Universal	Mostly plants	Various algae	Various algae	Cyanobacteria	Cyanobacteria



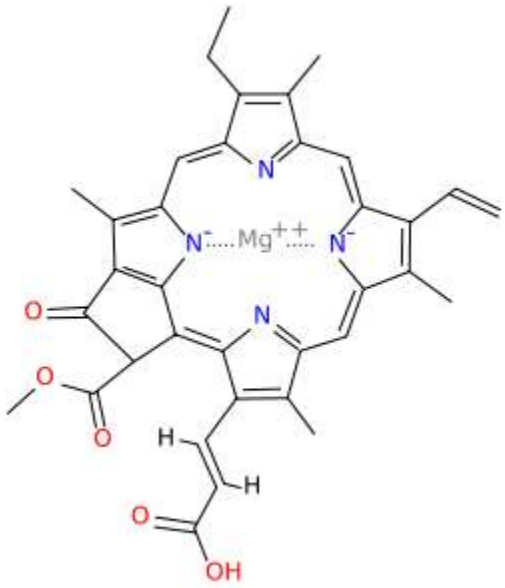
Structure of chlorophyll *a*



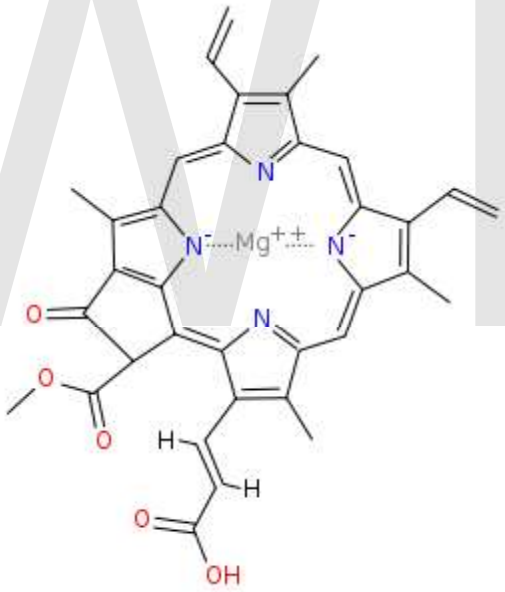
Structure of chlorophyll *b*



Structure of chlorophyll *d*

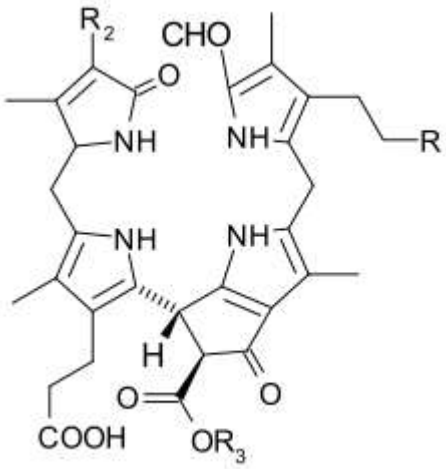


Structure of chlorophyll *c1*



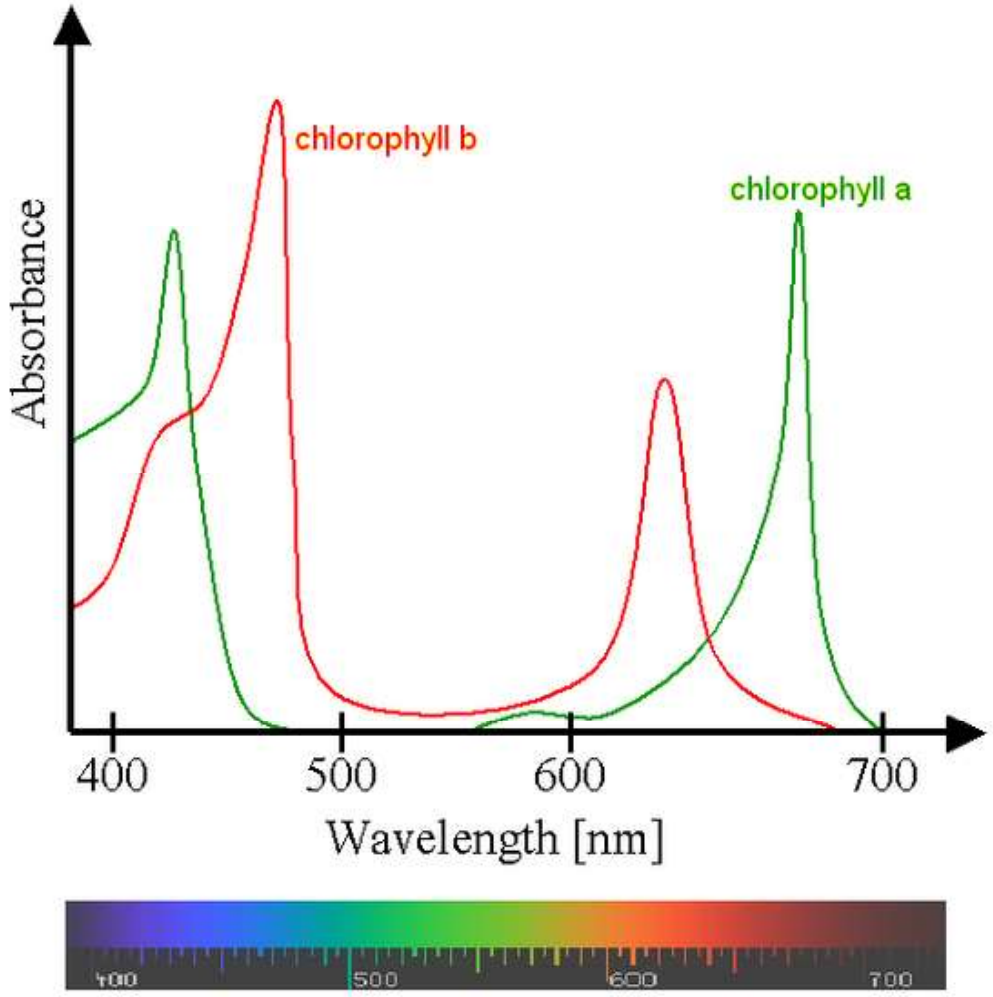
Structure of chlorophyll *c2*

When leaves degreen in the process of plant senescence, chlorophyll is converted to a group of colourless tetrapyrroles known as **nonfluorescent chlorophyll catabolites** (NCC's) with the general structure:



These compounds have also been identified in several ripening fruits.

Spectrophotometry



Absorbance spectra of free chlorophyll *a* (green) and *b* (red) in a solvent. The spectra of chlorophyll molecules are slightly modified *in vivo* depending on specific pigment-protein interactions.

Measurement of the absorption of light is complicated by the solvent used to extract it from plant material, which affects the values obtained,

- In diethyl ether, chlorophyll *a* has approximate absorbance maxima of 430 nm and 662 nm, while chlorophyll *b* has approximate maxima of 453 nm and 642 nm.
- The absorption peaks of chlorophyll *a* are at 665 nm and 465 nm. Chlorophyll *a* fluoresces at 673 nm (maximum) and 726 nm. The peak molar absorption coefficient of chlorophyll *a* exceeds 10^5 M^{-1} cm^{-1}, which is among the highest for small-molecule organic compounds.

By measuring chlorophyll fluorescence, plant ecophysiology can be investigated. Chlorophyll fluorometers are used by plant researchers to assess plant stress.

Biosynthesis

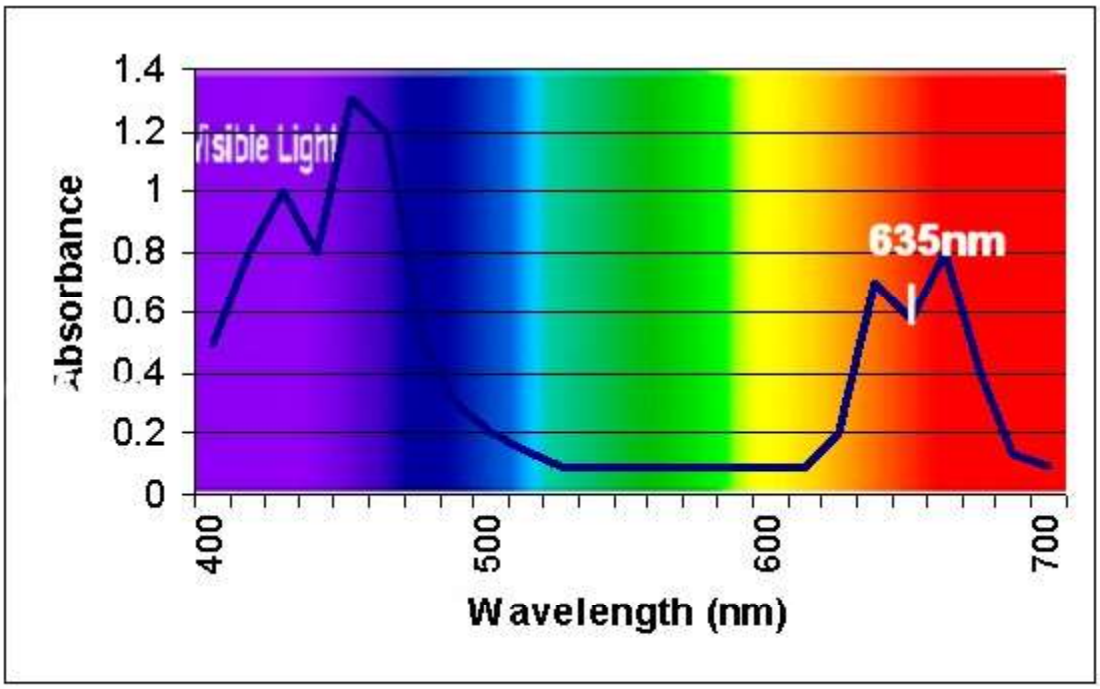
In plants, chlorophyll may be synthesized from succinyl-CoA and glycine, although the immediate precursor to chlorophyll *a* and *b* is protochlorophyllide. In Angiosperm plants, the last step, conversion of protochlorophyllide to chlorophyll, is light-dependent and such plants are pale (etiolated) if grown in the darkness. Non-vascular plants and green algae have an additional light-independent enzyme and grow green in the darkness as well.

Chlorophyll itself is bound to proteins and can transfer the absorbed energy in the required direction. Protochlorophyllide occurs mostly in the free form and, under light conditions, acts as a photosensitizer, forming highly toxic free radicals. Hence, plants need an efficient mechanism of regulating the amount of chlorophyll precursor. In angiosperms, this is done at the step of aminolevulinic acid (ALA), one of the intermediate compounds in the biosynthesis pathway. Plants that are fed by ALA accumulate high and toxic levels of protochlorophyllide; so do the mutants with the damaged regulatory system.

Chlorosis is a condition in which leaves produce insufficient chlorophyll, turning them yellow. Chlorosis can be caused by a nutrient deficiency of iron--called iron chlorosis—or by a shortage of magnesium or nitrogen. Soil pH sometimes plays a role in nutrient-caused chlorosis; many plants are adapted to grow in soils with specific pH levels and their ability to absorb nutrients from the soil can be dependent on this. Chlorosis can also be caused by pathogens including viruses, bacteria and fungal infections, or sap-sucking insects.

Measuring chlorophyll

The chlorophyll content of leaves can be non-destructively measured using hand-held, battery-powered meters.



The absorption spectrum of chlorophyll, showing the transmittance band measured by a CCM200 Chlorophyll Meter to calculate the relative chlorophyll content

Chlorophyll Content meters measure the optical absorption of a leaf to estimate it's chlorophyll content. Chlorophyll molecules absorb in the blue and red bands, but not the green and infra-red bands. Chlorophyll content meters measure the amount of absorption at the red band to estimate the amount of chlorophyll present in the leaf. To compensate for varying leaf thickness, Chlorophyll Meters also measure absorption at the infrared band which is not significantly affected by chlorophyll. For instance, the CCM200plus Chlorophyll Meter measures the transmittance at 653 nm (in the red band) and transmittance at 931 nm (in the infrared band). The percentage of transmittance at 931 nm, relative to the percentage of transmittance at 653 nm, estimates the relative chlorophyll content of the leaf.

The measurements made by these devices are simple, quick and relatively inexpensive. They now, typically, have large data storage capacity, averaging and graphical displays.

Culinary use

Chlorophyll is registered as a food additive (colorant), and its E number is **E140**. Chefs use chlorophyll to color a variety of foods and beverages green, such as pasta and absinthe. Chlorophyll is not soluble in water and is first mixed with a small quantity of oil to obtain the desired result. Extracted Liquid Chlorophyll was considered unstable and always denatured, until 1997 when Frank S. & Lisa Sagliano used freeze-drying of liquid chlorophyll at the University of Florida and stabilized it as a powder, preserving it for future use.