24 • Evolution of photosynthesis I: basic leaf morphological traits and diffusion and photosynthetic structures

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24.1. INTRODUCTION

The photochemical and biochemical processes that utilise solar energy for the synthesis of complex organic molecules have been on Earth for more than 3.5 billion years (Blankenship, 1992). The original photosynthetic mechanisms are thought to have been similar to those of contemporary cyanobacteria, with an oxygen-evolving photosystem that was responsible for the oxygenation of our early atmosphere, although physical processes have also been suggested (Kump, 2008). Evidence of the ancient origins of photosynthesis is seen in the fossil records of stromatolites of Western Australia (Awramik, 1992). Molecular studies show that it is increasingly more likely that photosynthesis evolved after chemolithotrophy (Xiong and Bauer, 2002). The evolutionary path of type-I and type-II reaction-centre systems remains unresolved and there is some debate on whether the earliest O_2-producing cyanobacteria used water or bicarbonate as the terminal reductant that led to an aerobic atmosphere (Dismukes *et al.*, 2001). There is evidence that the reaction giving rise to molecular oxygen, and responsible for our contemporary aerobic atmosphere, arose only once and the structural characteristics of its catalytic centre and its mechanism have been conserved ever since (Barber, 2008a,b). The origin of this oxygenic photosynthesis is under debate, with some authors suggesting an origin as early as 3.8 GA (Buick, 2008).

The Calvin-Benson or C_3 cycle of carbon reduction evolved early in the history of life and this is reflected in its universal presence in photosynthetic plants. Evolution of the carbon-reducing steps of the Calvin cycle is thought to have occurred when the Earth's atmosphere was hypoxic and rich in CO_2, which may account for the extreme sensitivity of Rubisco, the enzyme responsible for CO_2 entry into the Calvin cycle, to contemporary oxygen levels (Ogren, 1984). Presumably, a long period of evolution in a hypoxic atmosphere resulted in a complex pathway of tightly coupled reactions that have not been amenable to evolutionary modification in a way that reduces O_2 inhibition of photosynthesis while retaining the original carbon-fixation function. Under current atmospheric conditions, the O_2 inhibition of photosynthesis occurs through oxygenation of RuBP and subsequent loss of CO_2 through the reactions of photorespiration.

Although the basic structures and mechanisms associated with oxygenic photosynthesis evolved in aquatic plants far before the appearance of land plants, the colonisation of terrestrial habitats would have required adaptations to new stressors. These included increased risk of desiccation owing to the severe water deficit of the atmosphere and increased risk of photoinhibition and photooxidation. All of these constraints are intrinsically linked to the ecophysiology of photosynthesis and are likely to have played a key role in the development and evolution of structures directly involved in photosynthesis (stomata, leaves), photosynthetic WUE (stomata, cuticles, leaf structure) and photo-protection (leaf structure, modifications of chloroplasts, pigments and antioxidants). The earliest plants on land appeared during the Silurian approximately 470 MA (Fig. 24.1), and consisted of filamentous terrestrial algae, lichens and thalloid liverworts (Marchantiopsida), which were followed by hornworts (Anthocerotopsida) and mosses (Bryopsida) (Kenrich and Crane, 1997; Yoshinaga and Kugita, 2004). The origins of vascular plants (Tracheophytes) date to around the mid-Silurian (approximately 428 MA, Fig. 24.1). The ancestral vascular plants are often classified within the Rhyniophytina or Rhyniopsids, and include the extinct genus *Cooksonia* (often considered the earliest vascular plant), *Rhynia* and *Stockmansella* (Kenrich and Crane, 1997; Edwards *et al.*, 1998; Niklas and Kutschera, 2009a,b). The first vascular plants with leaves, microphyll

Terrestrial Photosynthesis in a Changing Environment: A Molecular, Physiological and Ecological Approach, ed. J. Flexas, F. Loreto and H. Medrano. Published by Cambridge University Press. © Cambridge University Press 2012.

Era	Period	Epoch	Began (MA)	Event
Cenozoic		Holocene	0.01	
	Quaternary	Pleistocene	1.8	
		Pliocene	5.3	Expansion of C_4 plants (8-5)
		Miocene	23	
		Oligocene	34	Origin of C_4 plants (30)
		Eocene	54	Cryptostomata (55)
	Tertiary	Paleocene	65	Poaceae and 'dumb-bell-shaped 'stomata (70)
Mesozoic	Cretaceous		145	Probable origin of terrestrial CAM plants
	Jurassic		200	First angiosperms (200)
	Triassic		251	
Paleozoic	Permian		299	First conifers (290)
	Carboniferous		359	Megaphylls (360)
	Devonian		416	Microphylls and 'kidney-shape'stomata (410)
	Silurian		443	Early vascular plants (420)
	Ordovician		488	Early land plants (470)
	Cambrian		542	

Fig. 24.1. Variations in atmospheric CO_2 (solid line) and O_2 (dashed line) over the past 400 million years predicted by the geochemical mass-balance model (from Beerling *et al.*, 1998 as reproduced in Keeley and Rundel, 2005).

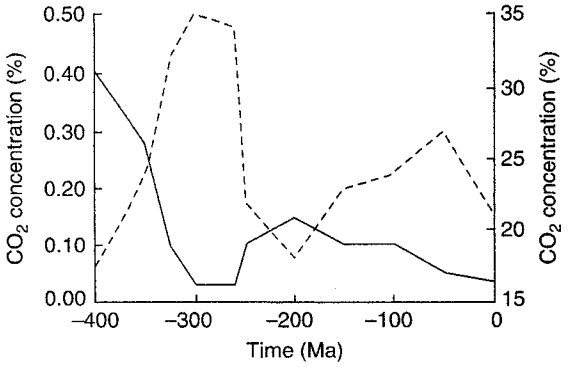

Fig. 24.2. Geological timescale based on the 2004 timescale endorsed by the International Commission on Stratigraphy. The approximate time of occurrence of the main evolutionary events related with photosynthesis is indicated.

plants or lycophytes date from the Devonian (approximately 410 MA, Fig. 24.1). Megaphyll plants or euphyllophytes did not appear until the Carboniferous (approximately 360 MA, Fig. 24.1). Euphyllophytes include Psilophyta (whisk ferns), Equisetophyta (horsetails), Ophioglossophyta (adder's tongues and grape ferns), Pteridophyta (true ferns) and seed plants, including gymnosperms (Gnetophyta, Cycadophyta, Ginkgophyta and Coniferophyta) and angiosperms (Pryer *et al.*, 2001). The earliest fossil records of horsetails, ferns and Cycadophyta date from the Carboniferous (approximately 360 MA, Fig. 24.1) and of Ginkgophyta and Coniferophyta from the Permian (approximately 290 MA, Fig. 24.1). The origin of Gnetophyta and its phylogenetic relations with other groups is under debate. They may have originated from Coniferophyta (Chaw *et al.*, 2000; Nickrent *et al.*, 2000) or be a sister group of them (Soltis *et al.*, 1999; Soltis and Soltis, 2003; Crane *et al.*, 2004) and sometimes are considered the closest to angiosperms among gymnosperms (Soltis *et al.*, 2002). Angiosperms did not appear until the boundary between Triassic and Jurassic (approximately 200 MA, Fig. 24.1), and their phylogenetic origin still remains debated (Friis *et al.*, 2005). *Amborella trichopoda* (Amborellaceae), a shrub with unisexual flowers and vesselless xylem endemic to New Caledonia is considered to be the closest extant relative to the phylogenetic root of angiosperms, closely followed by water lilies or Nymphaeales (Matthews and Donoghue, 1999).

All of these early land plants were undoubtedly C_3 plants. Changes in the composition of the atmosphere may have triggered the evolution of megaphylls first (Beerling, 2005) and later on CO_2-concentrating mechanisms. Indeed, a significant part of photosynthetic evolution in vascular plants has been the development of mechanisms for reducing photorespiration by concentrating CO_2 around Rubisco, thus returning this enzyme to an atmospheric condition that resembles the primitive earth. These CO_2-concentrating mechanisms are known as C_4 photosynthesis and CAM (Chapters 5 and 6) and their evolution is covered in the next chapter. Other CO_2-concentrating mechanisms exist in some algae and cyanobacteria (Kaplan and Reinhold, 1999; Raven *et al.*, 2008), but these are mostly important for aquatic habitats and hence are out of the scope of this book. An exception would be the presence of pyrenoids in hornworts, which are a unique feature of early land plants (Vaughn *et al.*, 1992).

The present chapter focuses on the evolution of land-plant traits directly or indirectly related to photosynthesis, based on paleontological and ecophysiological evidence. The latter is based on the study of extant plants belonging to the different evolutionary groups of land plants (with the exception of Rhyniopsids, which are all extinct). Therefore, ecophysiological aspects must be viewed with some care as current plants within a given group may have suffered evolutionary changes as compared with their ancestors. Even within a given species, examples of ecotypic variation have shown that both morphological and ecological evolution can occur rapidly in decades or even less. Therefore, it is possible, although not demonstrable, that the ecophysiological responses of the ancestors of present plants were different from contemporary representatives. The leaf traits to be

covered here are: (1) chloroplast biochemistry, including Rubisco, photosystems and photoprotective mechanisms; (2) plant cuticles and stomata; and (3) mesophyll structure and leaf form.

24.2. CHLOROPLAST BIOCHEMISTRY: RUBISCO, PHOTOSYSTEMS AND PHOTOPROTECTIVE MECHANISMS

Despite some debate as to the evolutionary path of type-I and type-II reaction-centre systems (Blankenship, 1992) and as to the age of Rubisco (Nisbet *et al.*, 2007), it is clear that the basic molecular components of chloroplasts evolved early in the history of life, probably approximately 3 billion years ago. This was long before chloroplasts originated from a cyanobacterium through endosymbiosis (Raven and Allen, 2003) and therefore before plants colonised the land. Following the emergence of land plants, continued evolution of these components is evident.

24.2.1. Rubisco

Concerning Rubisco, although the basic structure of form I in higher plants is similar to that of cyanobacteria (Nisbet *et al.*, 2007), substantial divergence has occurred including variations in the organelle location of the genes and their arrangement, mechanism of Rubisco synthesis, polypeptide sequence and efficacy of the substrates' binding and inhibitor action (Newman and Cattolico, 1990). For instance, in eucaryotes, contrary to ancestral cyanobacteria, the large subunit of Rubisco is encoded by multiple identical copies of *rbc*L in the chloroplast genome (Eilenberg *et al.*, 1998), whereas the small subunit *rbc*S gene family, which has between 2 and 12 nuclear genes, encodes small subunit peptides that are synthesised as precursor polypeptides in the cytosolic ribosomes and imported into the chloroplast in an ATP-dependent reaction (Spreitzer and Salvucci, 2002). Moreover, RuBP has no effect on the in-vitro activation of Rubisco by CO_2 and Mg^{2+} in cyanobacteria, but it induces a large decrease in the rate of activation in land plants (Newman and Cattolico, 1990).

Most relevant to the ecophysiology of photosynthesis is the evolutionary divergence in the functional properties of Rubisco, i.e., its specificity factor for CO_2 over O_2 ($S_{c/o}$) and catalytic constants (Raven, 2000). Nature has been unable to avoid the lack of specificity of Rubisco for CO_2. The reason is probably a result of the low oxygen concentration present in the atmosphere during the early evolution of Rubisco.

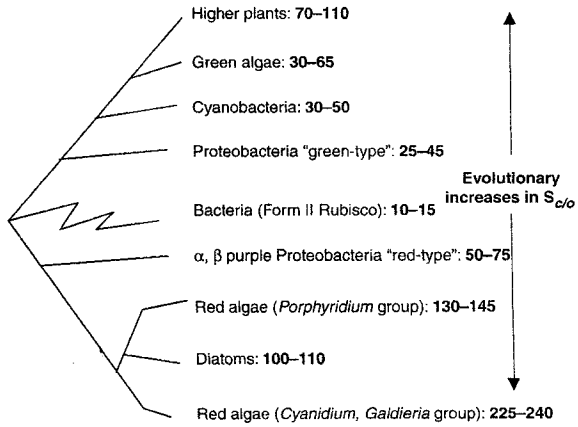

Fig. 24.3. Evolutionary trends of Rubisco $S_{c/o}$ variations. $S_{c/o}$ ranges obtained from various sources.

Comparison of $S_{c/o}$ values (Fig. 24.3), which vary over a 20-fold range from divergent photosynthetic organisms, shows that Rubisco specificity increases from the lower photosynthetic forms to higher plant forms (Jordan and Ogren, 1981a,b, 1983, 1984; Tortell, 2000), although the highest known specificities are for the red algae *Galdieria partita* (Uemura *et al.*, 1997).

Despite the strong phylogeny dependence of Rubisco specificity, it has been hypothesised that the enzyme could have evolved according to the organisms' specific needs for CO_2 assimilation. These would have depended on the environmental conditions in which the organism evolved, which would explain the large specificity in *Galdieria*, an organism inhabiting acidic hot springs rich in sulfur dioxide and low in CO_2 (Horken and Tabita, 1999). This view is also supported by comparing $S_{c/o}$ values along the green-plant lineage. Within this evolutionary line, chlorophyte algae containing CO_2-concentrating mechanisms (CCMs) present significantly lower $S_{c/o}$ than algae lacking CCMs (Raven, 2000). The latter, in turn, present high specificities similar to those of terrestrial C_3 plants including horsetails, gymnosperms and angiosperms (Kent and Tomany, 1995; Raven, 2000). Similarly among higher plants, those having evolved new CCMs in more recent times, such as C_4 metabolism, present lower $S_{c/o}$ values than C_3 species (Kent and Tomany, 1995; Raven, 2000; see Chapters 5, 6 and 25). Even within C_3 plants, Delgado *et al.* (1995) and Kent and Tomany (1995) hypothesised that hot environments together with water stress may impose an increased selection pressure on Rubisco for improved specificity. This was recently supported by Galmés *et al.* (2005) in a survey

of mediterranean C_3 species. It was shown that the diversity found in $S_{c/o}$ (from the lowest reported values in C_3 plants around 75 in several herbaceous species to the highest of 110 in the evergreen sclerophyll semi-shrub *Limonium gibertii*) was not related to the phylogeny of the studied plants, but strongly and positively correlated to the aridity of each species natural habitat and to leaf sclerophylly. Both aridity (see Chapters 20 and 29) and increased leaf thickness (see Chapter 12) result in low CO_2 availability in chloroplasts, which in the long term may exert a selection pressure towards more efficient Rubisco, just as it may have occurred when atmospheric CO_2 concentration dropped after land colonisation by plants. Further support for environmentally driven evolution of Rubisco comes from phylogenetic and maximum likelihood analyses of codon substitution models in the *rbc*L gene, which suggest that this gene has evolved under positive Darwinian selection (Kapralov *et al.*, 2006, 2007; Iida *et al.*, 2009). Still, *rbc*L is a slowly evolving gene, allowing it to be used to construct phylogenies and to assess the evolutionary potential of floras in biodiversity hotspots (Forest *et al.*, 2007; Nickrent *et al.*, 2000).

In general, a negative relationship has been described between $S_{c/o}$ and the catalytic rate of carboxylation (Raven, 2000; Zhu *et al.*, 2004; Tcherkez *et al.*, 2006), so that species with a higher affinity for CO_2 present much lower rates of photosynthesis. However, it has been shown recently that both *Galdieria* and *Limonium* simultaneously maintain both high $S_{c/o}$ and catalytic rates, which may open the possibility for genetically engineering plants with improved Rubisco carboxylation (Parry *et al.*, 2007). The variability found in Rubisco functional attributes deserve more attention in future studies as current models of leaf photosynthesis assume constant $S_{c/o}$ values among C_3 plants (Long and Bernacchi, 2003, see Chapter 8).

24.2.2. Photochemistry and photoprotection

Similar to Rubisco, the structure of PSI and II has not significantly changed from cyanobacteria to higher plants (Blankenship, 1992). Similarly, most of the known photoprotective mechanisms of higher plants (see Chapter 16), such as the xanthophyll cycle (VAZ), several tocopherols and all forms of SODs, are already present with little variability in all green algae and many diatoms (Asada, 2000; Baroli and Niyogi, 2000). As an example of evolutionary variations in the operation of VAZ-related energy dissipation processes, higher plants require protein PsbS for its operation (Li *et al.*, 2000). However, green algae, such as *Chlamydomonas*, lack PsbS and require a different protein, LHCRS, for VAZ-related energy dissipation. The moss *Physcomitrella patens* presents LHCRS and PsbS, both being functional and necessary for energy dissipation, suggesting that upon land colonisation photosynthetic organisms evolved a unique mechanism for excess energy dissipation before losing the ancestral one found in algae (Alboresi *et al.*, 2010). Other photoprotective cycles, such as the lutein epoxide cycle, may have evolved more recently as they are exclusive to some families of higher plants (Garcia-Plazaola *et al.*, 2007). Interestingly, lutein epoxide concentrations are highest in basal angiosperms with lower concentrations in gymnosperms and angiosperms, although no clear trend among subgroups of the latter has been found. This irregular distribution of the lutein epoxide cycle suggests that it is not determined phylogenetically but rather by ecological constraints (Esteban *et al.*, 2009). In addition, Esteban *et al.* (2009) have recently shown that although α-tocopherol and the VAZ pigments are present in all green plants, from green algae to angiosperms, there is an evolutionary trend to increase the content of α-tocopherol while decreasing VAZ pigments, which suggests a complementary role of these two photoprotective mechanisms. Hence, VAZ pigments are more abundant in green algae, liverworts and mosses, whereas α-tocopherol is more abundant in ferns, gymnosperms and angiosperms, the highest concentrations corresponding to dicots.

In addition to the evolution of molecular photoprotective mechanisms, it is likely that morphological traits conferring photoprotection (e.g., leaf hairiness, leaf angles, leaf veination) may have suffered different selection pressures depending on the habitat conditions of plants (e.g., Morales *et al.*, 2002; Esteban *et al.*, 2008), but any evolutionary evidence concerning these traits is lacking.

24.3. CUTICLES AND STOMATA

Ever since vascular plants colonised land approximately 400 million years ago, they have been protected by a cuticle, whereas all forms of non-terrestrial plants lack a cuticle (Edwards *et al.*, 1982). On the other hand the earliest well-authenticated stomata in fossil plants date from 410 million years ago, although stomata may have probably appeared earlier (Edwards *et al.*, 1998). Therefore, both cuticles and stomata evolved early after the colonisation of terrestrial environments and far before the appearance of leaves, which has led to the suggestion that the primary function of both structures was to protect plants from desiccation (Kerstiens,

1996, 2006; Raven, 2002a). Indeed, desiccation tolerance is a character apparently being lost during the course of evolution. Although it is common in lichens, bryophytes and lycophytes, less than 1% of all other ferns possess such an ability in their sporophyte stage (Proctor and Pence, 2002) although it is widely exhibited in the gametophyte stage (Watkins Jr. *et al.*, 2007). In higher plants, desiccation tolerance is restricted to seeds, with some exceptions like the genus *Craterostigma*, which shows vegetative desiccation tolerance (Bartels and Salamini, 2001).

24.3.1. Cuticles

Although we understand a great deal about stomatal evolution, much less is known about the evolution of plant cuticles. Kerstiens (2006) pointed out that our understanding of the totality of evolutionary pressures acting on the formation of the cuticle, in past and present environments, is poor. It is likely that cuticles had early roles in defence against parasites and grazers in reflecting UV-B and in water repellency, in addition to preserving plants from desiccation (Raven, 2002a). Regardless of its function, there is evidence that early land plants had cuticles with a similar thickness to present-day plants (Edwards *et al.*, 1998). The water permeability of cuticles in extant plants ranges 1000-fold, from 0.1 to 100 m $s^{-1} \times 10^5$ (Kerstiens, 1996). It is still unclear whether these differences arise from differences in cuticle thickness, composition and/or the number and size of aqueous pores (Kerstiens, 2006).

Such pores occur in many plant cuticles, and they are normally small – 1 to 2 nm in diameter – although they can reach much larger sizes, such as the 'giant' cuticular pores in the Proteaceae *Eidothea zoexylocarya* that can reach diameters of 1 μm (Carpenter *et al.*, 2007). Using the bryophyte gametophyte as the best model of ancestrally astomatous land plants to model photosynthesis, Raven (2002a) suggested that these pores may have permitted higher photosynthetic rates per unit ground area in early land plants under high CO_2 conditions.

Although values of cuticular water permeability in different species are relatively scarce, apparently there are no significant differences in the range of permeability between different evolutionary groups (Kerstiens, 1996, 2006). This suggests that cuticular evolution may have responded mostly to stress conditions, which is supported by comparisons of parental species with different habitat, showing that cuticular waxes (Pearce *et al.*, 2006), hypodermis and cuticular pores (Carpenter *et al.*, 2007) are more evenly distributed in leaf abaxial and adaxial sides in species inhabiting drier habitats. Some well-preserved fossil records from the Jurassic (Wang *et al.*, 2005) and the Cretaceous (Upchurch, 1984; Yang *et al.*, 2009) demonstrate that cuticles of some Ginkgophyta, Coniferophyta and extinct angiosperms already possessed secretory cells comparable with modern-plant oil cells, waxes, fibrils, trichomes and stomatal types (see below), i.e., similar characteristics to those found in extant basal angiosperms (Carpenter, 2006).

24.3.2. Stomata

Contrary to cuticles, much is known about the evolution of stomata. Functional considerations suggest that stomata evolved from pores in the epidermis, and cladistic analyses are consistent with a unique origin of stomata (Edwards *et al.*, 1998; Raven, 2002a). Stomata are absent in liverworts and in the gametophyte phase of hornworts and mosses, but present in the sporophyte phase of the latter two groups and in all known groups of vascular plants, including the early extinct Rhyniopsids. This implies later loss of stomata in the evolution of some lycopsids such as *Stylites*, and ferns such as *Hymenophyllum* (Keeley *et al.*, 1984; Kessler *et al.*, 2007; see Chapter 6), as well as in many submerged aquatic vascular plants (Raven, 2002a; see Chapter 6). The proposed functional roles of stomata include maintenance of hydration, restricting the occurrence of xylem embolism, optimising carbon fixation per unit water lost, cooling leaves and transporting nutrients from roots to shoots (Raven, 2002a). Since their early appearance in land plants, stomata have suffered substantial evolution concerning their numbers and density, morphology, position and function (Hetherington and Woodward, 2003). Stomatal densities range from between 5 and 1000 mm^{-2} of epidermis, depending on species and environmental conditions (Hetherington and Woodward, 2003). It is well known that stomatal density decreases with increasing atmospheric CO_2 and vice-versa (Woodward, 1987a; Woodward and Kelly, 1995; Beerling and Woodward, 1996; Franks and Beerling, 2009); a response already present in the so-called 'living fossil' *Ginkgo biloba* (Beerling *et al.*, 1998) and other gymnosperms (Woodward and Kelly, 1995). This stomatal tracking of CO_2 has been utilised as a proxy signal for paleo-atmospheric changes (Royer *et al.* 2001).

CO_2 perception and stomatal development are linked by the *HIC* (high carbon dioxide) gene-signalling pathway (Gray *et al.*, 2000), although other CO_2-independent genes, such as *STOMAGEN*, are involved in regulating

stomatal density during leaf development (Sugano *et al.*, 2010). Similarly, stomatal densities in a particular species may increase with elevation and aridity (Quarrie and Jones, 1977; Franks and Farquhar, 2001; Kessler *et al.*, 2007; Xu and Zhou, 2008). These changes have important implications for photosynthesis, as higher stomatal densities are often associated with higher maximum stomatal conductance, net photosynthesis and WUE, as well as with better stomatal regulation in response to water stress (Pearce *et al.*, 2006; Galmés *et al.*, 2007c; Xu and Zhou, 2008). Moreover, decreasing CO_2 in the atmosphere in the late Devonian may have allowed the development of large stomatal densities, triggering the evolution of megaphylls (Beerling, 2005, see Section 24.4.2.1) and small stomatal sizes, allowing increasing gas-exchange capacity (Franks and Beerling, 2009).

Morphologically, stomata differ in size (from about 10 to 80 μm in length), shape and cellular composition (Hetherington and Woodward, 2003). There is a trade-off between guard-cell length and stomatal density, and hence differences in size are mostly owing to acclimation responses, as described for stomatal density. In contrast, stomatal morphologies have clear evolutionary trends (Hetherington and Woodward, 2003; Carpenter, 2005; Franks and Farquhar, 2007). Stomata in early land plants (Silurian and Devonian fossils) presented lower densities, they were 'kidney shaped' (more or less circular or elongate) and positioned superficially over leaves (Phanerostomata), and they were anomocytic, i.e., they presented no anatomically distinct subsidiary cells (Edwards *et al.*, 1998; Hetherington and Woodward, 2003). The cuticle was generally thinner over the outer periclinal walls of guard cells, but thicker elsewhere. Notably, these plants lacked an extensive substomatal cavity, but instead the stomatal pore led into a narrow canal formed by hypodermal cells with thickened walls, opening into a deep chamber occupied by parenchyma (Edwards *et al.*, 1998). Despite the fact that early land plants are often considered to have inhabited shady and humid environments, because of rapid drainage and little organic matter in soils these habitats were often water-stressed. Consistently, this unusual tissue playing the role of a substomatal cavity has been interpreted as an adaptation to increase WUE, by creating a large internal surface area per unit biomass that would increase efficiency of CO_2 transport to carboxylation sites (Edwards *et al.*, 1998). However, this explanation is not clear, as it has been suggested that large kidney shaped stomata with low densities, such as those encountered in extant ferns from deep-shade environments, may be an important feature of plants of humid and deep shade conditions, but unfavourable under dry conditions (Hetherington and Woodward, 2003).

Anomocytic stomata, such as those described for extinct early land plants, are present in extant lycophytes and ferns, as well as in some basal angiosperms (Carpenter, 2005; Franks and Farquhar, 2007). In Lycopods (Fig. 24.4A), anomocytic stomata are characterised by big guard cells with a small stomatal pore, and present minimal lateral movement or swelling during their aperture. In ferns (Fig. 24.4B) stomata are also anomocytic, but guard cells are smaller and the stomatal pore wider. Some basal angiosperms also have anomocytic stomata, but most have stephanocytic (a more or less well-defined rosette of four or more weakly specialised subsidiary cells), laterocytic (three or more subsidiary cells) or paracytic (one or two lateral subsidiary cells oriented parallel to guard cells) stomata (Carpenter, 2005). Paracytic stomata seem to represent the most evolved form (Carpenter, 2005). Therefore, most angiosperms have 'kidney shaped' stomata but with subsidiary cells clearly defined (Fig. 24.4C).

Two important evolutionary events concerning stomata occurred in the Cenozoic era (~70–35 MA), when there were profound changes in the global climate and terrestrial flora and fauna (Hetherington and Woodward, 2003). These consisted in the evolution of encrypted stomata in the Proteaceae and the evolution of 'dumbell-shaped' stomata in the Poaceae. For species of *Banksia* and *Dryandra* in the Proteaceae of Australia, two clades of species are currently recognised by differences in stomatal distributions: Phanerostomata and Cryptostomata. In the latter, stomata occur in shallow pits or in crypts, whereas the Phanerostomata have a more superficial distribution. Species of the clade Cryptostomata occur in much drier climates and probably diverged from the Phanerostomata clade 55–35 MA ago, at a time when the climate was becoming more arid (Mast and Givnish, 2002). A recent study with a large number of Proteaceae species (Jordan *et al.*, 2008) has shown that deep encryption of stomata evolved at least 11 times, always in very dry environments, whereas other forms of stomatal protection (sunken but not closely encrypted stomata, papillae and layers of hairs covering the stomata) also evolved repeatedly, but had no systematic association with dry climates. It has been recently shown that stomatal encryption rather than creating a favourable humid microenvironment around stomata, facilitates diffusion of CO_2 to adaxial mesophyll cells in thick sclerophylls, i.e., it improves photosynthesis and photosynthetic WUE by means of increasing mesophyll conductance to CO_2 (Hassiotou *et al.*,

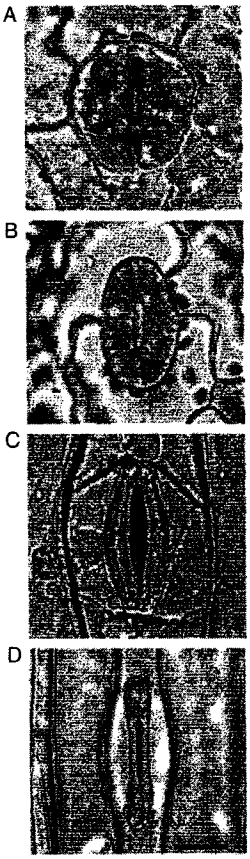

Fig. 24.4. Stomata from extant plants representative of stomatal evolution. (A) Anomocytic 'kidney shaped' stomata of the lycopod *Huperzia prolifera* with big guard cells and small stomatal pore. (B) Anomocytic 'kidney shaped' stomata of the fern *Nephlorepis exaltata* with smaller guard cells and larger pore. (C) Paracytic 'kidney shaped' stomata of the herbaceous angiosperm *Tradescantia virginiana*. (D) Paracytic 'dumbell-shaped' stomata of the grass *Triticum aeastivum*. Fig. taken from Franks and Farquhar (2007).

2009a,b, 2010; Roth-Nebelsick *et al.*, 2009). The environmental correlates of the differences in stomatal distribution seen for the Proteaceae are also supported by observations in *Cistus incanus*, for which similar differences in stomatal distribution occur, but between the summer and winter in the mediterranean climate (Aronne and De Micco, 2001). Leaves produced in winter are large and flat with abundant stomata on the adaxial leaf surface, whereas leaves developed in the hot and dry summer are crimped and partially rolled, forming a crypt on the abaxial surface where stomata are located.

The Poaceae or grasses comprise a group of about 10,000 species that originated between about 55 and 70 MA, leading to lineages that were understory plants of tropical forests (Kellogg, 2001). The 'dumbell-shaped' stomata of grasses (Fig. 24.4D) are generally believed to represent a more evolutionary advanced form than the 'kidney shaped' stomata, and indeed during development, grass guard cells adopt a transient 'kidney shaped' phase before assuming their mature 'dumbell-shaped' aspect.

Regarding stomatal functioning, it has been demonstrated that functional improvement parallels morphological evolution from anomocytic 'kidney shaped' (Fig. 24.4A) to paracytic 'dumbell-shaped' (Fig. 24.4B) stomata (Franks and Farquhar, 2007). The anomocytic stomata with large guard cells found in Lycopods (Fig. 24.4A) present a small open-stomata pore area and a small ratio of open pore-to-total-stomata area. Pore area and pore-to-stomata ratios are about double in the anomocytic stomata, with smaller guard cells found in ferns (Fig. 24.4B), but these are still much lower than those found in the paracytic 'kidney shaped' stomata of herbs (Fig. 24.4C) and especially in the paracytic 'dumbell-shaped' stomata of grasses (Fig. 24.4D). This means that for a given leaf area, species with paracytic 'kidney shaped' stomata and especially grasses with 'dumbell-shaped' stomata can have larger total pore areas than anomocytic stomata. This leads to higher maximum stomatal conductance and photosynthesis. However, stomatal apertures are virtually unaffected by subsidiary cell pressure in anomocytic stomata, but substantially influenced in paracytic stomata, which is known as the mechanical advantage of epidermal cells over guard cells. This massive mechanical counteraction is a side effect arising from the need for greater lateral displacement of guard cells to create a larger stomatal pore and could potentially eliminate much of the gain from a more mobile guard-cell pair. However, it is suggested that subsidiary cell turgor is much lower during maximum stomatal aperture, which may be achieved by an active osmotic mechanism that seems to operate better in grasses than in any other species (Franks and Farquhar, 2007). Therefore, the 'dumbell' design magnifies small changes in width to cause large openings, and maximises the potential of stomata to track changes in environmental conditions. Smaller changes in guard and subsidiary cell turgor lead to greater increases in stomatal aperture in the 'dumbell-shaped' stomata than in 'kidney shaped' stomata. This efficiency and speed of stomatal opening in grasses enhances photosynthesis and WUE compared with non-grass species (Grantz and Assmann, 1991). A rapid stomatal response to blue light

augments photosynthesis in early morning and under intermittent sunlight, in which light has an enhanced blue-light content and that would have characterised the understory environment during the early evolution of grasses (Grantz and Assmann, 1991). In view of the functional advantages provided by 'dumbell-shaped' stomata, with the capability to respond quicker and more efficiently to the enhanced light conditions of newly open habitats, but with the capacity to avoid the increased likelihood of drought, grasses may have enhanced their spread and diversification of grasses during global aridification, 30–45 MA (Hetherington and Woodward, 2003; Franks and Farquhar, 2007).

Besides these differences among stomatal types in response to guard-cell pressure, most known stomata close in response to darkness and increasing VPD, CO_2 or ABA, and open in response to increasing light intensity and blue light. However, there are doubts as to the functionality of stomata in some bryophyte sporophytes, including all hornworts, but the stomata of the moss *Funaria hygrometrica* respond normally to light, dark and ABA (Raven, 2002a). Despite showing some ABA responsiveness, stomata of these plants are not considered to have a strong regulatory function (Hartung, 2010). Some ferns, such as *Adiantum capillus-veneris*, lack the blue-light-specific opening and CO_2 response, but respond normally to red light (Doi and Shimazaki, 2008). Recently, Brodribb *et al.* (2009) have shown that stomatal responsiveness to low CO_2 has increased from ferns and lycopodes to conifers, and from these to angiosperms. Only the stomata of the latter close in response to high CO_2, which lead them to increased WUE under conditions that depress photosynthesis increasing C_i, such as photoinhibition and fluctuating light and temperature. More recently, Brodribb and McAdam (2011) have analysed in depth stomata responses to leaf excision, ABA, increasing VPD and darkness. They found that lycophytes and ferns generally lacked ABA responses and presented a slower stomatal closure upon transfer to darkness than angiosperms and conifers, but plants of all groups responded similarly to leaf excision and increased VPD. All together, these evidences suggest that some functional attributes, including the response to light/dark, VPD and desiccation, appeared early in the evolution of stomata, whereas other responses such as the blue-light-specific opening and the CO_2 response, may have appeared later, although specifically designed experiments are required to obtain conclusive evidence as for the evolution of stomatal functional attributes. The appearance of ABA responses remains more controversial.

24.4. LEAF FORM AND MESOPHYLL COMPONENTS

Since the endosymbiontic event that led to the origin of photosynthetic eukaryotes, the cellular components of mesophyll cells (i.e., cell walls and chloroplasts) have been present in all plants, even before plants colonised the land and far before the appearance of leaves. However, it is likely that some evolution may have occurred since then in the composition, size and distribution of mesophyll elements (Vaughn *et al.*, 1992; Popper and Fry, 2003). Alternatively, leaves have evolved independently in lycophytes and euphyllophytes (Dolan, 2009) and have suffered substantial evolution according to fossil leaf records (Osborne *et al.*, 2004a,b; Feild and Arens, 2007; Royer *et al.*, 2007) and ecophysiological studies in extant plants (Brodribb *et al.*, 2007; Sack *et al.*, 2008).

24.4.1. Cell walls and chloroplasts

Little is known about the evolution of cell walls and chloroplasts. However, recent studies (Popper and Fry, 2003; Popper *et al.*, 2011) showed that major changes in primary cell-wall composition accompanied major evolutionary steps. In this sense, cell walls in charophytes are mostly composed of galacturonic acid and, in smaller amounts, glucuronic acid and mannose, while lacking xyloglucan. Xyloglucan is present in all land-plant lineages, from hornworts to euphyllophytes. Hornworts present the highest content of galacturonic and glucuronic acids among any group, and are the only group containing the unusual glucuronic acid-$\alpha(1\rightarrow3)$-galactose. Mannose is most abundant in liverworts, mosses and lycophytes. Euphyllophytes present the lowest amounts of all these compounds. It is unclear how these differences in cell-wall composition may affect photosynthesis. However, it has been suggested that the resistance to CO_2 diffusion imposed by cell walls (i.e., the inverse of cell-wall conductance to CO_2, see Chapter 12) is directly proportional to mesophyll cell-wall thickness and to the tortuosity of the cell-wall pores, and inversely proportional to the porosity of the wall (Evans *et al.*, 2009; Terashima *et al.*, 2011). Values of cell-wall porosity and the tortuosity of the pores through cell walls are unknown, but likely affected by the composition of the primary cell wall.

Concerning chloroplasts, hornworts, the extant representatives of one of the most ancient groups of land plants, have unique chloroplast features that suggest an advanced evolution of chloroplast numbers and morphologies in

later evolutionary groups (Vaughn *et al.*, 1992). Unlike any other land plant, but similar to many algae, most hornworts have pyrenoids, i.e., the site of accumulation of Rubisco. But unlike most algae, the hornwort pyrenoid is composed of distinct subunits, numbering up to several hundred. Another unique feature of the hornwort chloroplasts is the presence of thylakoids that connect adjacent granal stacks at right angles to the long axis of the granum. It is unclear how these features affect photosynthesis in hornworts owing to the lack of sufficient ecophysiological studies.

Also, similar to some algae (Haupt and Scheuerlein, 1990) and contrary to most vascular plants, liverworts, hornworts and some lycophytes, such as some *Selaginella* species, have a single or a few large chloroplasts per cell (Haupt and Scheuerlein, 1990; Vaughn *et al.*, 1992). It has been suggested that the presence of a large population of small chloroplasts allows for more effective chloroplast movement in response to high light than fewer large chloroplasts (Jeong *et al.*, 2002), although such movements are also present in some algae species having a single chloroplast per cell (Haupt and Scheuerlein, 1990). Chloroplast reorientation in response to high light, i.e., moving from a face pattern under low light to a profile pattern under high light, is a very effective photoprotective mechanism (Kasahara *et al.*, 2002) that is present in many algae and almost all land-plant lineages, including mosses, such as *Funaria hygrometrica* (Haupt and Scheuerlein, 1990), ferns (Agustynowicz and Gabrys, 1999), monocots and dicots (Inoue and Shibata, 1974). In hornworts, however, chloroplast photoactivity is rare, although it has been described in the genus *Megaceros*, possibly being the reason why hornworts within this genus have lost pyrenoids (Vaughn *et al.*, 1992). Although ecophysiological studies comparing photosynthesis in ancestral plants with few large chloroplasts with vascular plants that present many small chloroplasts are scarce, studies with the *arc* mutations of *Arabidopsis* (containing only one to three chloroplasts per mesophyll cell 20-fold larger than wild-type chloroplasts) have shown that mutants have normal phenotype and growth (Pyke *et al.*, 1994), whereas photosynthesis rates are similar to wild-type plants (Austin II and Webber, 2005). Indeed, it has been suggested that few large chloroplasts would result in higher mesophyll conductance to CO_2 (see Chapter 12) and thus higher photosynthesis than plants with many small chloroplasts (Sharkey, personal communication). Moreover, in *Arabidopsis* plants it has been demonstrated that chloroplast movements towards the high-light position results in decreased mesophyll conductance (Tholen *et al.*, 2008). Although mesophyll conductance has yet to be determined in lycophytes or ferns, preliminary measurements in liverworts and hornworts show very reduced g_m rather than an increased one (Meyer *et al.*, 2008). Nevertheless, if an evolutionary trend exists from few large chloroplasts – conferring or not large CO_2 diffusion conductance – towards many smaller chloroplasts facilitating chloroplast movements, this would support the idea that excess irradiance has exerted more selective pressure than low CO_2 availability on land plants.

24.4.2. Leaf form

MICROPHYLLS AND MEGAPHYLLS

Two types of leaves are found in vascular plants: microphylls and macrophylls (Tomescu, 2008; Dolan, 2009; Niklas and Kutschera, 2009a,b). Microphylls are defined as leaves of small size with simple venation (one vein), while megaphylls are generally larger in size and with more complex venation (Tomescu, 2008). Generally, microphylls are leaves of lycophytes, whereas megaphylls are leaves of euphyllophytes, although some 'megaphyll-leaved' species, such as *Equisetum* and several extant and fossil gymnosperms have highly reduced leaves supported by one vein, whereas some 'microphyll-leaved' species have large leaves, such as the extinct lepidodendrales (up to 1 m) or extant *Isoetes* (up to 0.5 m), or complex venation patterns, such as those of some *Selaginella* species (Tomescu, 2008). Despite these exceptions, the terms microphyll and megaphyll are still useful to distinguish two groups of leaves that evolved independently, microphylls about 410 MA, i.e., about 50 MA after land colonisation by plants and approximately 10 MA after the appearance of the first vascular plants (Dolan, 2009), and megaphylls about 360 MA, i.e., about 50 MA after the appearance of microphylls (Beerling, 2005).

Megaphyll leaf photosynthesis allows more efficient light interception and higher rates of photosynthesis than microphylls (Micol, 2009). However, both microphylls and megaphylls are produced by determinate growth of small subpopulations of cells on the flanks of indeterminate shoot apical meristems. Indeterminate growth of the shoot apical meristem is controlled by the class-1 knotted-like homebox (*KNOX*) gene family as well as by BELLRINGER class proteins. *Class-1 KNOX* genes and BELLRINGER are expressed in and around the meristem. As these genes control indeterminate growth, their repression is required for leaf development. This repression is mediated by a Myb transcription factor, which originates Myb proteins

collectively known as ARPs (Tomescu, 2008; Dolan, 2009; Micol, 2009).

Class-I KNOX genes are already present in some algae (e.g., *Acetabularia*), mosses, ferns, gymnosperms and angiosperms, and restricted to the development of the diploid phase of the lifecycle. For instance, in the fern *Ceratopteris richardii*, *Class-I KNOX* genes are expressed in the meristem, leaf primordia, vascular bundles and leaf margins of the sporophyte, but not in the gametophyte. In other words, the pattern of *Class-I KNOX* gene expression is similar in lycophytes and euphyllophytes. Given that microphylls and megaphylls evolved independently, it might have been expected that different mechanisms of *KNOX* repression would have evolved in each group. Contrary to that expectation, *ARP* genes are similarly expressed in the lateral primordia of both lycophytes and euphyllophytes. *Class-III HD-ZIP* genes control the initiation of lateral appendages, the differentiation of the top from the bottom of the leaf and the development of vasculature. Moreover, these genes are present in both microphyll and megaphyll species, but their patterns of gene expression in the developing leaf and vasculature are very different in lycophytes and euphyllophytes, suggesting that these genes may have different roles in each group, possibly leading to the observed differences between microphylls and megaphylls (Dolan, 2009). Moreover, it has been suggested that differences may also arise from different *KNOW-ARP* interactions in the different groups (Tomescu, 2008).

Despite the fact that all land plants share most of the genes required for developing either microphylls or megaphylls, the latter did not appear until approximately 50 MA after the appearance of microphylls (Osborne *et al.*, 2004a,b). The appearance of megaphylls in the late Devonian (360 MA) coincides with a large drop of atmospheric CO_2 concentration (Fig. 24.2). Beerling *et al.* (2001) proposed that this CO_2 drop was indeed the requisite for megaphyll formation. The theory is based on the observation that stomatal density is inversely proportional to CO_2 concentration (see Section 24.3.2) and a leaf energy balance model. In plants, energy dissipation takes place mostly through convection (that decreases with increasing leaf size as friction across the surface slows the passage of air and the transfer of heat) and transpiration (whose capacity depends on maximum stomatal conductance, which in turn is directly proportional to stomatal density). Even without invoking a higher global temperature (as a consequence of the 'greenhouse effect' owing to high CO_2), it was calculated that the low stomatal densities found prior to the late Devonian as a consequence of high CO_2 would not allow for the high transpiration rates needed to cool a megaphyll below a lethal temperature threshold. This may have restricted the abundance of megaphyll to a few isolated examples, such as the rare early Devonian plant *Eophyllophyton bellum* (Beerling, 2005). The large fall in CO_2 corresponded with a marked rise in stomatal density, from 5–10 mm^{-2} on early vascular plants (Edwards *et al.*, 1998) to 30–40 mm^{-2} on late Devonian (Osborne *et al.*, 2004a,b) and 800–1000 mm^{-2} on late Carboniferous megaphylls (McElwain and Chaloner, 1995). The rise in stomatal density permitted greater evaporative cooling and alleviated the requirement for convective loss, allowing the spread of megaphylls.

Following a similar but reverse argument, it has been proposed that the later fourfold increase in CO_2 concentration around 200 MA in the Triassic-Jurassic boundary (Fig. 24.2) resulted in a 3° to 4°C 'greenhouse' warming that may have induced another evolutionary constraint on megaphylls (McElwain *et al.*, 1999). Based on the leaf energy balance, these environmental conditions are calculated to have raised leaf temperatures above a lethal threshold, possibly contributing to the observed massive extinction and more than 95% species turnover of the Triassic-Jurasic megaflora. For instance, large leaves (>3 to 4 cm) in full sunlight of the upper canopy (e.g., Ginkgo) or plants in open habitats could have reached noon temperatures at least 10°C above air temperature, which in a time of global warming (summer temperatures >30°C) may have resulted in lethal temperatures. Indeed, fossil records indicate that this transition favoured highly dissected and/or narrow leaves over large entire leaves (McElwain *et al.*, 1999). It was after massive plant extinctions in the Triassic-Jurassic boundary that angiosperms radiated into a riot of unprecedented variation in foliar morphological diversity (Feild and Arens, 2007). Leaf anatomy of basal angiosperms reveals features linked to high performance in wet, shady habitats. For example, a mesophyll dominated by spongy parenchyma tissue (i.e., no palisade layers) is ancestral among angiosperms. Other features of early angiosperms are stephanocytic stomata with hypostomy and low stomatal densities (15–80 mm^{-2}), low photosynthetic capacities and low light-saturation points (around 20% of full sunlight). Feild and Arens (2007) have suggested that the damp, dark and disturbed ancestral habitat of angiosperms allowed the origin and refinement of ecophysiological traits, such as vessels and broad, net-veined leaves, that permitted them expanding in understory habitats. These traits may have later been co-opted or modified as angiosperms and broke out into more demanding

environments. For instance, to break into brighter and more evaporative terrestrial habitats, leaves must have evolved palisade mesophyll and amphistomy to increase photosynthetic rate per A_{leaf} (Smith *et al.*, 1997), while evolution of smaller stomata and more precise guard-cell regulation may have increased WUE (Sack *et al.*, 2003). In addition, it has been suggested that coordinated shifts in hydraulic and photosynthetic performances are necessary to make a substantial movement out of wet habitats (Sack *et al.*, 2003; Feild and Arens, 2007).

LEAF SCLEROPHYLLY

In agreement with the above considerations, plants differ largely in LMA, leaf thickness and density (Beerling and Kelly, 1996; Niinemets, 1999, 2001; Wright *et al.*, 2004). LMA is indeed the product of leaf density and leaf thickness (Niinemets, 1999, 2001). Increasing the complexity of leaf mesophyll may ultimately result in sclerophylly (thick, rigid leaves with high LMA and thick cuticles). This has been debated as to whether it does or does not represent an evolutionary character (Salleo *et al.*, 1997; Salleo and Nardini, 2000; Jordan *et al.*, 2005). The earliest fossil records of sclerophyll leaves date from the late Permian (250 MA), i.e., around 100 MA after the appearance of megaphylls. They are found in Antarctica, South Africa and Texas (Retallack, 2005). Other sclerophyll-rich fossil floras are found in the Triassic-Jurassic boundary (McElwain *et al.*, 1999) and in some Eocene beds (Royer *et al.*, 2007). All these floras correspond with dry and warm (Retallack, 2005; Royer *et al.*, 2007) or warm and high-CO_2 (McElwain *et al.*, 1999) environments.

However, studies in extant plants have questioned the drought-adaptive value of sclerophylly. First, sclerophyll leaves are found in all biomes on Earth, not just in dry environments (Reich *et al.*, 2003; Wright *et al.*, 2004). Second, sclerophyll leaves do not present better water-relation traits than non-sclerophyll leaves (Salleo *et al.*, 1997; Salleo and Nardini, 2000; Galmés *et al.*, 2007c), although other authors have found positive correlations between LMA, leaf density and leaf thickness and the bulk modulus of elasticity (Niinemets, 2001; Corcuera *et al.*, 2002), which is often considered as a water-saving strategy. In addition to water scarcity, other possible determinants of leaf sclerophylly include shortage of nutrients (Loveless, 1962; Wright *et al.*, 2004), increased attack by herbivores or fungi (Turner, 1994; Royer *et al.*, 2007), increased heat stress and increased exposure to the sun (Beerling and Kelly, 1996; Smith *et al.*, 1997; Jordan *et al.*, 2005). For instance, Royer *et al.* (2007) found in Eocene floras a negative correlation between LMA and indices of insect damage to leaves. Alternatively, tests of correlated evolution based on molecular phylogenies suggested that scleromorphic leaf anatomies in Proteaceae have evolved many times, always in association with open vegetation (i.e., high irradiance), but not with dry habitats (Jordan *et al.*, 2005). A positive effect of high irradiance on LMA would also explain why LMA tends to increase with elevation (Beerling and Kelly, 1996; Dunbar-Co *et al.*, 2009). Global analysis shows that LMA is negatively correlated with precipitation and positively correlated with both mean temperature and mean solar radiation (Niinemets, 2001). Interestingly, leaf density correlates better with precipitation, whereas thickness correlates better with temperature and radiation, suggesting that the two components of LMA may respond to different selection pressures (Niinemets, 2001).

Regardless of its adaptive value, LMA shows a global positive relationship with leaf lifespan and trade-offs with nutrient concentrations and, notably, photosynthetic capacity (Reich *et al.*, 2003; Wright *et al.*, 2004). Increasing mesophyll thickness increases the pathways of light and CO_2 from their sites of interception by leaves to the average site of their use in photosynthesis by mesophyll cells (Smith *et al.*, 1997). It also decreases the proportion of leaf N invested in Rubisco, but not the proportion invested in cell walls (Harrison *et al.*, 2009), although a positive correlation has been found between LMA and cell-wall nitrogen allocation (Hikosaka and Shigeno, 2009), and a trade-off between N investment in Rubisco and cell walls has been suggested (Feng *et al.*, 2009; but see Harrison *et al.*, 2009 and Hikosaka and Shigeno, 2009). However, increasing leaf thickness also increases the amount of mesophyll cells per A_{leaf}, hence increasing the overall photosynthetic capacity per area. Moreover, the optical properties of mesophyll cells also appear to regulate the internal distribution of sunlight for enhanced photosynthesis (Vogelmann *et al.*, 1996; Smith *et al.*, 1997). Indeed, Niinemets (1999) showed in a global analysis of extant woody plants, that leaf thickness was poorly related to photosynthetic capacity. This can be partly explained by the fact that many sclerophyll leaves are heterobaric, and the bundle-sheath extensions have been proposed to increase light-transferring capacity inside leaves, favouring photosynthesis and partly counteracting the negative effects of decreasing the percentage of photosynthetically active leaf area in these leaves (Nikolopoulos *et al.*, 2002). Instead, leaf density was found to be strongly negatively correlated with photosynthesis, which determines the negative

relationship between photosynthesis and LMA (Niinemets, 1999). Recent global (Flexas *et al.*, 2008) or specific surveys in Australian sclerophyll species (Niinemets *et al.*, 2009c) show that this is a result of a reduction of mesophyll conductance to CO_2 (see Chapter 3) as LMA increases.

LEAF FORM AND VENATION

In addition to variations in leaf thickness and density, plants also differ in leaf blade size, form and venation patterns (reviewed in Roth-Nebelsick *et al.*, 2001). The latter includes dendritic open-venation patterns, in which each vein is connected to a single higher-order vein and ramifies into one or more non-interconnected lower-order veins; and anastomosing close patterns with cyclically connected veins (i.e., a single vein can be connected to several different veins of different orders). The dendritic open pattern represents the primitive architecture. In the Devonian and early Carboniferous almost all plants showed this type of venation, whereas anastomosing patterns appeared later in the Upper Carboniferous (Roth-Nebelsick *et al.*, 2001). Both patterns are found in either fossil or extant species of ferns, gymnosperms and angiosperms. In addition, pinnately veined leaves differ from palmate-veined leaves (Sack *et al.*, 2008). The former, which are more common, have a single first-order vein, from which all secondary veins branch. The latter, accounting for up to 30% of regional floras, have multiple first-order veins branching from the petiole. Palmate venation has evolved many times in different lineages. Single-veined leaves or phyllides are more frequent in bryophytes and lycophytes, whereas multiple-veined leaves are more frequent in ferns and angiosperms (Brodribb *et al.*, 2007). Many gymnosperms present single-veined leaves, but with a unique accessory transfusion tissue, consisting of lignified mesophyll cells that greatly increase conductivity of water far from xylem vessels (Brodribb *et al.*, 2007). Different vein-branching patterns, together with many different leaf shapes from lobed to highly dissected (e.g., Nicotra *et al.*, 2008), result in a large variability in total vein density and redundancy in leaf venation.

In a broad study including different evolutionary groups, it has been shown that leaf vein density is positively correlated with leaf maximum photosynthetic rate (Brodribb *et al.*, 2007). This is owing to the fact that increased leaf density increases total leaf hydraulic conductivity by reducing the average hydraulic pathway through the hydraulically inefficient mesophyll tissue (Brodribb *et al.*, 2007). This in turn is intrinsically and genetically linked to stomatal conductance and photosynthesis (Brodribb and Jordan, 2008; Maherali *et al.*, 2008). This will also explain the positive correlations found between a leaf dissection index and photosynthesis in *Pelargonium* species (Nicotra *et al.*, 2008), and the positive correlation between leaf size and leaf hydraulic conductivity in Hawaiian *Plantago* taxa (Dunbar-Co *et al.*, 2009). Moreover, heterogeneity in photosynthesis is observed even within different parts of a single leaf, in coincidence with different vein densities (Nardini *et al.*, 2008).

In addition, it has recently been shown that leaf palmate venation confers tolerance to hydraulic disruption as compared with pinnate venation (Sack *et al.*, 2008). It was shown that severing the midrib resulted in large decreases in hydraulic conductivity, stomatal conductance and photosynthesis in pinnately veined leaves, the effect being smaller in palmately veined leaves. These results suggest that evolution of palmately veined leaves may have been an adaptive trait to withstand situations favourable for cavitation, such as drought or cold environments, as well as insectivory (Sack *et al.*, 2008). Indeed, in seven taxa of the Hawaiian *Plantago* radiation, a negative correlation was found between total vein density and the mean annual rainfall in each species habitat (Dunbar-Co *et al.*, 2009).

Altogether, evidence suggests that redundancy in leaf venation and increased leaf density results in higher photosynthesis rates over time, which may have been a key feature for the evolution of high photosynthetic capacities (Sack *et al.*, 2008).

24.5. OTHER EVOLUTIONARY EVENTS AFFECTING PHOTOSYNTHESIS

In the previous sections, the crucial evolutionary aspects of photosynthesis have been covered. However, other events and evolutionary mechanisms also exist that lead to effects on photosynthesis. For instance, there has long been recognised that there is environmentally driven phenotypic selection of photosynthetic traits, leading to adaptive radiation and genetic intra-specific variation of photosynthesis characteristics and WUE in plant natural populations (Dudley, 1996a; Geber and Dawson, 1997; Lauteri *et al.*, 1997; Ares *et al.*, 2000; Heschel *et al.*, 2002; Donovan *et al.*, 2007, 2009; Montgomery and Givnish, 2008) as well as in crops (Koç *et al.*, 2003; Poormohammad Kiani *et al.*, 2007).

Also, gene and genome duplications leading to plant polyploidy have been recognised to have an ancient origin (Adams and Wendel, 2005; Soltis, 2005) and to be of crucial importance for plant evolution (Masterson, 1994; Adams and Wendel, 2005; Moore and Purugganan, 2005).

Polyploidy has extensive effects on gene expression and gene silencing (Adams and Wendel, 2005) and sometimes results in gene redundancy that may be of adaptive value (Moore and Purugganan, 2005). Polyploidy also has effects on photosynthesis. For instance, there are strong positive relationships between the number of chromosomes (Masterson, 1994) or the genome size (Lomax *et al.*, 2009) and the stomatal size, so that polyploid plants or plants with large genomes may have a greater gas-exchange capacity. It has indeed long been recognised that polyploids often present higher growth and photosynthesis rates than diploids (the so-called 'hybrid vigour'), which is related to increased Rubisco, chl., chloroplast numbers and stomatal and mesophyll conductance to CO_2 (Warner *et al.*, 1987; Warner and Edwards, 1989, 1993; Vyas *et al.*, 2007).

Alternatively more recent evolutionary events have taken place in angiosperms only that lead to reduced photosynthetic capacity. One of these is represented by the evolution of carnivorous plants. Carnivory has evolved independently at least six times in five angiosperms orders (Ellison and Gotelli, 2009). This represents an adaptation to particularly nutrient-poor environments, but it has a cost in terms of plant energetics. Particularly, it has been shown that carnivorous plants have lower photosynthesis capacity and photosynthetic NUE than their non-carnivorous relatives (Farnsworth and Ellison, 2008; Ellison and Gotelli, 2009; Bruzzese *et al.*, 2010), and they fall out of the general relationships between photosynthesis and LMA or nitrogen content described as part of the 'worldwide leaf-economics spectrum' (Wright *et al.*, 2004). Low photosynthesis in carnivorous plants appears associated to replacement of chl.-containing cells with digestive glands, low chl. content, low stomatal density and a compacted mesophyll with a small proportion of intercellular spaces, probably resulting in low mesophyll conductance to CO_2 (Pavlovic *et al.*, 2007). An extreme case of loss of chloroplast and photosynthetic function is that of parasitic plants (Bungard, 2004), which have been covered in Chapter 7.

24.6. CONCLUDING REMARKS

In summary, the evolution of photosynthesis has been crucial for plant colonisation of terrestrial ecosystems. This evolution has implied a multitude of adaptations having led to important changes not only in the function but also in the morphology of vascular plants, and resulted in an extraordinary diversity of forms in the plant kingdom. Hence, in addition to variations in the basic photosynthetic elements already present in unicellular algae and early forms of pluricellular aquatic plants, such as Rubisco or photosynthetic pigments, the evolution of photosynthesis on land has comported the development and diversification of plant-atmosphere interface structures, such as the cuticle and stomata, a complex vascular system and the unique structure known as the leaf, which in turn has evolved and diversified a multitude of traits, structures, etc. Still, perhaps the most specific features concerning the evolution of photosynthesis itself are the appearance of photosynthetic types different to the so-called C_3 metabolism, namely C_4 and CAM, whose evolution is covered in the next chapter.