# 25 • Evolution of photosynthesis II: evolution and expansion of CAM and $C_4$ photosynthetic types

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## 25.1. INTRODUCTION

The evolutionary traits described in the previous chapter are common to all photosynthetic types, and evolved originally in $C_3$-like species. 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 in $C_3$ plants. Consequently, a very 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 CAM and $C_4$ photosynthesis, while all other plants are referred to as $C_3$. Other $CO_2$-concentrating mechanisms exist in some algae and cyanobacteria (Kaplan and Reinhold, 1999; Raven *et al.*, 2008), but these will not be discussed here.

The evolutionary origins of CAM and $C_4$ are presumably tied to changes in paleoclimates and atmospheres, particularly to historic variations in $CO_2$ and $O_2$, and locally warm climates (Fig. 24.2 in previous chapter). Under higher $CO_2$ partial pressure and/or lower temperature the $C_3$ pathway fixation does not exhibit limitations that would put a premium on coupling it with a CAM or $C_4$ pathway. The origins of vascular plants date to around the mid-Silurian (~440 MA, Fig. 24.1 in previous chapter) and these plants were likely $C_3$, although the astomatous CAM plant *Stylites andicola* has been suggested as a possible model of early plant evolution (Keeley *et al.*, 1984). Coupling the $C_3$ pathway with one of the $CO_2$-concentrating mechanisms, CAM or $C_4$, occurred subsequent to the emergence of vascular plants and possibly arose more than once over the past 400 million years.

## 25.2. EVOLUTION OF CAM

CAM is a pathway specialised for the capture and storage of $CO_2$ at night. The $CO_2$ is fixed to a 3-C 'acceptor' molecule by the enzyme phosphoenolpyruvate carboxylase (PEPC) and stored as the 4-C compound malic acid in the large central vacuole of CAM cells. During the subsequent day, the malic acid is decarboxylated, freeing the $CO_2$ internally, and the cell utilises light energy to produce sugar through traditional $C_3$ photosynthesis. Two ecological conditions are known to select for CAM. Most well known is that of persistent water limitation, which has led to the most conventional form of CAM by which plants close stomata during the day to conserve water (see Gibson and Rundel, Chapter 28) and open them at night when the atmospheric water deficit is lower. Another condition occurs in aquatic freshwater environments where $CO_2$ is rapidly depleted during the day owing to high photosynthetic demand by algae and other aquatic plants, and the high diffusive resistance of water hinders the replenishment of $CO_2$ from the atmosphere. Aquatic CAM plants lack functional stomata and are able to take advantage of the higher night-time availability of $CO_2$ and lower water temperatures. The distant phylogenetic relationship of terrestrial and aquatic CAM plants suggests that the pathway arose in these taxa through independent but convergent evolution (Keeley and Rundel, 2003).

### 25.2.1. Terrestrial CAM-plant origins

In the case of terrestrial CAM plants, arid microhabitats selecting for CAM have undoubtedly been present throughout the Earth's history, although changes in paleoatmospheric conditions would have amplified or muted the selective value of CAM at different points in time (Raven and Spicer, 1996). High $CO_2$ and/or low $O_2$ partial pressures

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will increase net $CO_2$ fixation for a given stomatal conductance and thus increase carbon gain per unit of water loss. Under these conditions one might expect a less selective value of CAM, and such would have been the case during early vascular plant evolution. The late Carboniferous period (~300 MA) marks the first time vascular plants were exposed to extremes of low $CO_2$ and high $O_2$ that might have put a premium on $CO_2$-concentrating mechanisms such as CAM. However, none of the current CAM taxa are from clades that date to this period. Although atmospheric conditions during the Mesozoic era were not strongly conducive to the advantages of CAM, local selective environments such as those with topographic rain shadows or those caused by unique plant-growth forms, such as occurs with the epiphytic growth habitat, could have favoured the emergence of CAM. Indeed, this seems likely considering experimental results that show photosynthesis of CAM plants comparable with that of $C_3$ plants under doubled current atmospheric $CO_2$, (Drennan and Nobel, 2000). However, we lack clear models predicting the relative changes in selective value of CAM under changing atmospheric conditions, temperature and water availability and thus the early stages of CAM evolution, and associated forcing variables are largely a matter of speculation. As these parameters vary we might expect some conditions favouring strict night-time uptake and others a weaker CAM expression.

Since the biochemical steps behind CAM are evident in $C_3$ stomatal guard cells it is probable that the origins of CAM were founded on altered patterns of gene expression in new cell types. Genomic studies show CAM induction in $C_3$-CAM facultative plants is largely a matter of transcriptional activation (Cushman, 2001). It has been postulated that the precursor to fully expressed CAM was CAM-like cycling of $CO_2$ between stored organic acids and freed $CO_2$ in otherwise $C_3$ plants (Monson, 1999).

Succulence is an obvious structural trait that typically occurs in terrestrial CAM species from arid climate zones to facilitate storage of organic acids formed with nocturnal carbon fixation. Recent studies have examined functional constraints of CAM leaf anatomy on the level of CAM function as seen in weak and strong modes of nocturnal carbon fixation (Silvera *et al.*, 2005). Plants with strong modes of CAM function have been defined as those with more than 70% of their carbon fixed in nocturnal uptake, whereas weak modes of CAM function, often termed CAM flexibility, are those species where less than one-third of carbon is fixed using nocturnal CAM (Nelson and Sage, 2008). Such facultative CAM species only utilise the CAM pathway when induced by stress, typically water stress (Cushman and Borland, 2002). Two traits often associated with CAM function, cell size and degree of succulence, were not found to be related to the degree of CAM function, whereas reduced intercellular air space in photosynthetic tissues and a reduction in the surface of mesophyll cells exposed to intercellular air space were positively related to CAM function and negatively related to $C_3$ function (Nelson and Sage, 2005, 2008). Both of these anatomical traits act to reduce internal $CO_2$ conductance owing to an enhancement of carbon economy, and thus appear to have been a strong selective force in evolving the efficiency of CAM function (Griffiths, 1989; Maxwell *et al.*, 1997).

Perhaps an example of this early evolution of CAM is the unusual *Welwitschia mirabilis*, a species long known to utilise CAM cycling but now established to fix nocturnal carbon to a limited extent in the field (von Willert *et al.*, 2005). This desert plant in the Gnetophyta (sister group to the angiosperms) has origins that date to the Cretaceous (Crane, 1996). Traits in the Gnetophyta are thought to be the result of seasonally extreme environments (Doyle, 1996), further suggesting conditions early in the Mesozoic era may have been conducive to CAM. Some of these early plants may have been herbaceous species with poor fossil records (Crane, 1996), but the distribution of *Welwtschia* suggests evolutionary origins in open environments where plants might have been subjected to higher temperatures and drought stress leading to selection for CAM as a means to scavenge respiratory $CO_2$. Despite this evolutionary history in primitive vascular plants, modern lineages of CAM species appear to have largely evolved in the past 35 MA.

Since the mid-Cretaceous the partial pressure of $CO_2$ has dropped from over 1000 ppm to below 600 ppm through the later half of the Miocene. This decline became very marked during the Pleistocene with full glacial episodes having levels below 200 ppm, significantly below pre-industrial levels and representing a time of strong selection for $CO_2$-concentrating mechanisms such as CAM (Raven and Spicer, 1996).

CAM is extremely widespread both phylogenetically and geographically, occurring in more than 30 diverse plant families and about 20,000 species of vascular plants, indicating that CAM has evolved independently multiple times (Winter and Smith, 1996; Sayed, 2001). Aridity has clearly been one of the major selective factors in this evolution, taking advantage of the relatively high WUE of CAM species. The iconic CAM habitat would be the succulent dominated deserts of the American Southwest or the Succulent Karoo

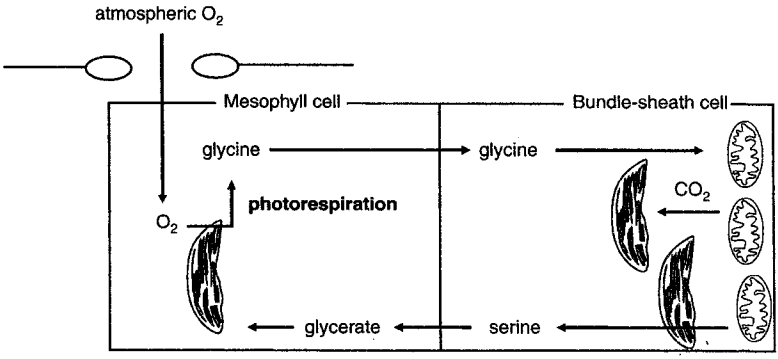


Fig. 25.1. Schematic showing the fundamental steps of the glycine decarboxylase, $CO_2$-recycling system in $C_3$-$C_4$ intermediate species. Glycine decarboxylase activity is isolated in bundle sheath cells. A gradient in the photorespiratory metabolite, glycine, forces $CO_2$ from photorespiration to be released by mitochondria in the bundle sheath cells. When rates of photorespiration are greater than the rate of glycine decarboxylation and/or in response to diffusive resistance of $CO_2$ leakage from the bundle sheath cells, the bundle sheath $CO_2$ concentration can increase; this improves the carboxylation capacity of Rubisco in chloroplasts of the bundle sheath cells.

the angiosperms (Sun *et al.*, 2002), the independent origin of a similar set of traits so many times provides a remarkable example of evolutionary convergence (Christin *et al.*, 2007). This becomes even more significant when one considers the fact that $C_4$ photosynthesis is a complex, integrated set of morphological, biochemical and physiological traits, probably requiring the structural conversion and/or genomic rewiring of tens to hundreds of genes (e.g., Wyrich *et al.*, 1998). How and why did this set of traits emerge so many times independently in such a short span of geological time? What do the lessons of $C_4$ tell us about the interaction of terrestrial plant photosynthesis and environmental change? What lessons can we glean from the $C_4$ story with regard to molecular architecture, evolvability of specific lineages and the nature of selection? These are fundamental questions within the broad topic of plant evolution.

### 25.3.1. Molecular evolution

$C_4$ photosynthesis has not arisen *de novo* from newly transcribed evolving genes but rather from the duplication of non-photosynthetic $C_3$ genes and modification of their expression (Monson, 2003). Presumably the biochemical steps in $C_4$ were widespread in angiosperms long before this pathway was fully expressed. Some distantly related $C_3$ species exhibit $C_4$ carbon assimilation from vascular stem cells and this may be interpreted as one precursor to the evolution of fully expressed $C_4$ (Raven, 2002b), and some genetic evidence has been interpreted as evidence of such a piecemeal evolution of the pathway (Taylor *et al.*, 1997). Of particular significance here is the fact that some aquatic species exhibit $C_4$ photosynthesis without the presence of Kranz anatomy, and the $CO_2$-concentrating mechanism appears to be tied to diffusional constraints characteristic of the aquatic milieu (Keeley, 1998b).

An alternative view is suggested by the study of $C_3$–$C_4$ intermediate species that strongly suggest $C_4$ evolution has involved coordinated changes in morphological, biochemical and genetics (Monson, 1999, 2003). More than 30 species of terrestrial plants in seven different families, both monocot and eudicot, have been reported as having photosynthetic phenotypes intermediate in one way or another to those of fully expressed $C_3$ or $C_4$ species (Sage *et al.*, 1999). All of the intermediates described to date share the same glycine decarboxylase $CO_2$-recycling system illustrated in Fig. 25.1. It is important to note that this recycling system is fundamentally different in many of its biochemical and anatomical features from the fully expressed $C_4$ pathway, though it appears to share the same advantage of the $C_4$ pathway in providing elevated $CO_2$ concentrations at the active site of Rubisco; in the intermediates, however, only a small fraction of the leaf's Rubisco benefits from the elevated $CO_2$ concentration. This $CO_2$-concentrating system of $C_3$–$C_4$ intermediate species is driven by a gradient in one of the key photorespiratory metabolites (Gly), and is therefore dependent on high rates of photorespiration for its maintenance. This insight presents an interesting piece of irony to the story of $C_4$ evolution: while it appears that one of the ultimate benefits of the appearance of fully expressed $C_4$ photosynthesis is a reduced photorespiration rate, high

of South Africa, where there are widespread areas with CAM species as ecosystem dominants (Esler and Rundel, 1999). Environmental selection for CAM function, however, clearly has phylogenetic limitations. Extensive areas of arid lands in the world, such as, for example, those of central Australia, are relatively lacking in CAM species. Although it has been hypothesised that nutrient poverty and intense fires have selected against CAM species in central Australia (Orians and Milewski, 2007), there is little to support this idea. Moreover, introduced *Opuntia stricta* thrived over millions of hectares of arid Australia rangeland before being brought under biological control (Parsons and Cuthberson, 2001).

Tropical mesic environments present epiphytic microhabitats where aridity exists and CAM plants are adaptive because of physiological drought associated with their aerial growth habit (Lüttge, 1989; Benzing, 1990; Martin, 1994). Such a condition can be seen in wet and dry Neotropical forests where CAM plants may be abundant. Epiphytes may comprise up to 35–50% of canopy leaf biomass and taxonomically represent more than one-third of the flora in such forests (Gentry and Dodson, 1987), with CAM epiphytes common in Bromeliaceae (1144 epiphytic species and an estimated 50% are CAM), Cactaceae (120 species, 100% CAM), and Orchidaceae (>10,000 epiphyte species, approximately 60% CAM) (Griffiths, 1989). Asian tropical forests have abundant epiphytes but CAM is less common, where it is best represented in epiphytic Asclepiadaceae (135 species, 90% CAM) and a few epiphytic ferns have been found to have CAM (Holtum and Winter, 1999).

The Bromeliaceae represent an excellent example of adaptive radiation into multiple terrestrial and epiphytic habitats, with CAM as one of the key innovations that has allowed diversification. Both the epiphytic growth habit and CAM photosynthesis have evolved at least three times from an ancestral terrestrial $C_3$ mesophyte, likely in response to increasing aridity and other climate changes in the late Tertiary. The great majority of CAM epiphytes in the Bromeliaceae occur in the subfamilies Tillansioideae and Bromelioideae. In the former, $C_3$ photosynthesis was the ancestral state and CAM evolved later in extreme habitats, while in the latter, CAM and a terrestrial growth habit predated the evolution of the epiphytic growth habit (Crayn *et al.*, 2004). Subsequent radiation of the Bromelioideae into more mesic habitats led to a reversion to $C_3$ photosynthesis.

Although both CAM and $C_4$ metabolic pathways, the latter as described below, have evolved independently in many lineages, it is interesting to note that both pathways have evolved in four plant families. These are the Aizoaceae, Asteraceae, Euphorbiaceae and Portulacaceae. Within families containing many CAM representatives there remain open questions in some groups as to whether CAM has multiple origins or a single origin that has been switched on and off in various lineages (Reinert *et al.*, 2003).

### 25.2.2. Aquatic CAM-plant origins

Aquatic CAM is restricted to the Tracheophyta and the earliest appearing taxa are in the genus *Isoetes* (Keeley, 1998a), with fossils dated to the Triassic (>230 Ma) (Wang, 1996; Retallack, 1997). These early representatives of *Isoetes* and related taxa in the Lycophyta were amphibious species restricted to shallow seasonal pools that would have undergone sharp daytime $CO_2$ depletion even if atmospheric $CO_2$ levels were high (Keeley, 1998a). This is because of the high demand for $CO_2$ by the pool flora, coupled with the diffusional limitations of water, both of which inhibit rapid equilibrium with atmospheric $CO_2$ levels. In addition, high Triassic temperatures (Spicer, 1993; McElwain *et al.*, 1999) would have exacerbated the daytime depletion of $CO_2$ availability in these pools, as $CO_2$ is substantially less soluble in warm water compared with cool water.

In summary, considering both terrestrial and aquatic CAM species, the CAM pathway likely evolved earliest in aquatic plants and has persisted in particular lineages that show preference for shallow seasonal pools and oligotriphic lakes where carbon in the water column is low (Keeley, 1998a). Certainly, of extant CAM species, the aquatic genus *Isoetes* is representative of the earliest origins of CAM.

Angiosperms that are aquatic CAM plants include monocot species in the Alismataceae (Sagittaria spp.), dicots in the Crassulaceae (annual species of *Crassula*) and Plantaginaceae (*Littorela* spp.) (Keeley, 1998a). Other than the *Crassula* species, aquatic CAM plants share a common morphology known as the isoetid growth form that includes a short rosette of cylindrical leaves. In light of this it would be most interesting to investigate what is considered the most basal angiosperm family, Hydatellaceae, comprising annual aquatic isoetids in Australia and New Zealand (Rudall *et al.*, 2007; Friedman, 2008).

## 25.3. $C_4$ EVOLUTION

$C_4$ photosynthesis has evolved approximately 45 times independently in 19 families and nine orders of the angiosperms (Sage, 2004). With an estimated age of 125 million years for

rates of photorespiration were required to sustain the earliest forms of the $C_4$ pathway.

The biochemical and anatomical features of $C_3$–$C_4$ intermediates have been covered in recent reviews (e.g., Monson, 1999; Monson and Rawsthorne, 2000). In this chapter, we take the opportunity to expand the discussion beyond the specific traits associated with $C_3$–$C_4$ intermediacy, taking up the broader evolutionary issues of evolvability and predisposition to $C_4$ evolution. In other words, what are the genotypic, phenotypic, climatic and ecological traits that might allow us to predict whether $C_4$ photosynthesis will emerge in a specific taxonomic line?

Although the ecologic and climatic contexts for $C_4$ evolution have been actively debated in recent years, the genetic and life-history traits that might have favoured $C_4$ evolution have been less discussed. It is clear from the several studies that have been conducted on gene families that code for those enzymes important in $C_4$ photosynthesis, that $C_4$-specific genes have most commonly evolved from pre-existing $C_3$-specific genes through duplication and subsequent neofunctionalisation (Monson, 2003). In some cases, such as that for NADP MDH in the $C_4$ species, *Flaveria trinervia*, a single $C_4$ isoform is present without $C_3$-type isoforms (McGonigle and Nelson, 1995). This may indicate evolutionary modification of a single-copy gene or it may be the result of past duplication and subsequent loss of the $C_3$ progenitor gene from the $C_4$ genome. Nonetheless, most $C_4$ genes belong to larger gene families that include $C_3$-specific forms. Several studies conducted in Peter Westhoff's laboratory in Dusseldorf have suggested that the genetic modifications required to produce $C_4$-specific genes from $C_3$-specific genes are relatively minor for those genes that code for several key enzymes. In the genus *Flaveria* (Asteraceae), the key $C_4$ enzyme PEPC is coded by three genes, *ppcA*, *ppcB* and *ppcC*, with the latter two sharing common sequences in both $C_3$ and $C_4$ species (Bläsing *et al.*, 2002). It is the *ppcA* gene that, while being present in both $C_3$ and $C_4$ species, has been modified for specific $C_4$ function (Westhoff and Gowik, 2004). One of the key events that occurred in the evolution of $C_4$ *ppcA* was the modification of cis-regulatory elements in the gene promoter providing for enhanced expression in mesophyll cells (Gowik *et al.*, 2004). A functional analysis of the *ppcA* promoter in $C_3$ and $C_4$ *Flaveria* species has revealed that a 41 base-pair segment (MEM1) in the $C_4$ promoter is responsible for mesophyll-specific expression. This segment exhibits three key differences between the $C_3$ and $C_4$ forms: (1) the $C_3$ MEM1 sequence includes a segment of approximately 100 base pairs in the middle of the sequence, which is missing in the $C_4$ gene; (2) the first nucleotide in the sequence is guanine in the $C_4$ gene, but adenine in the $C_3$ gene; and (3) the $C_4$ gene contains a four-base CACT sequence in one part of the MEM1, which is missing in the $C_3$ gene. Thus, the totality of genetic modifications possibly required for the conversion of a $C_3$-type promoter to a $C_4$-type promoter may be as little as a deletion of a 100-base-pair segment, a single-point mutation and addition of a four-base segment.

Of even more relevance to the early evolution of $C_4$ photosynthesis is the recent analysis of promoter expression patterns for the gene coding for glycine decarboxylase (*GDC*). As discussed earlier, one of the earliest steps in $C_4$ evolution appears to be a switch in expression patterns of *GDC* from non-specific expression in leaf cells in the $C_3$ state to bundle-sheath-specific expression in the $C_4$ state. Glycine decarboxylase (GDC) is a multi-subunit enzyme, and the differential expression of GDC activity in $C_3$–$C_4$ intermediate and $C_4$ plants is owing to the P-subunit, which carries the decarboxylase active site (Rawsthorne *et al.*, 1988, 1998). Thus, the key evolutionary innovation in this case appears to involve a change in cellular expression pattern (from all cells to just BSC) and potentially addition of an expression enhancer (in BSC). Recently, Englemann *et al.* (2008) conducted a functional analysis of the promoter region of the active P-subunit gene for glycine decarboxylase (*GLDPA*) focusing on expression patterns in the $C_4$ species *Flaveria bidentis* and the $C_3$ species *Arabidopsis thaliana*. It was discovered that the $C_4$ *GLDPA* is expressed in the BSC of *A. thaliana*, indicating that in this $C_3$ species, the transcription factors are present for bundle-sheath-specific expression of GDC. In other words, it appears that evolutionary changes to the cis-regulatory elements in the gene promoter are all that is needed to accomplish the switch in expression pattern from fully expressed $C_3$ to $C_3$–$C_4$ intermediate and ultimately fully expressed $C_4$. Using deletion and recombination analysis, these workers were able to dissect the approximately 1,600-base-pair region of the 5′-flanking region of the *GLDPA* promoter, and show that yet-to-be-identified changes in a 433-base-pair segment at the distal end of the promoter and changes in a 212-base-pair segment in the middle of the promoter cause most of the expression enhancement in the bundle sheath and expression repression in the mesophyll.

These studies to date provide evidence that much of the evolutionary change required at the genetic level during the transition from $C_3$ to $C_4$ photosynthesis is owing to modifications to the cis-regulatory elements in gene promoter

sequences. There are some exceptions to this generalisation, such as the cases for differential cell expression of the small subunit for Rubisco (Patel *et al.*, 2006) and NADP-specific MDH (Nomura *et al.*, 2005). However, most cases of $C_4$-specific enzyme isoforms that have been examined point to changes in cis-regulatory elements as the primary agent of $C_4$ evolution (Monson, 1999, 2003). Recent syntheses in the field of evolutionary developmental biology, particularly those dealing with morphological evolution, have concluded that much of the evolutionary novelty that emerges in developmental pathways occurs through changes in the function of cis-regulatory elements (Prud'homme *et al.*, 2007). Processes of gene duplication and neofunctionalisation using pre-existing promoter sequences and transcription factors appear to be the principle processes leading to evolutionary novelty. These generalisations have been criticised (Hoekstra and Coyne, 2007), but at least for the case of $C_4$ evolution, they explain much of the recent analysis of $C_3$ versus $C_4$ trait expression. In the face of these emerging principles we might conclude that the broad and repeating patterns of $C_4$ evolution over the past several million years are in large partly owing to the pre-existence of a narrow set of biochemical options, the potential for cis-regulatory modification to exert large influences on phenotype and strong selection in the face of global climatic and biogeochemical change.

One aspect of $C_4$ evolution that has not been considered in much depth in past discussions concerns life-history traits and the potential for certain types of traits to foster the rates of mutation and genetic neofunctionalisation required to force high rates of photosynthetic diversification. For example, in the genus *Flaveria*, a small taxonomic group with 23 closely related species, diversification from $C_3$ to $C_4$ has occurred up to three times independently and all three possible occurrences appear to be associated with the evolution from perennial to annual lifecycle and tendency toward self-fertilisation (McKown *et al.*, 2005). Similarly, in the genus *Boerhavia* (Nyctaginaceae), where both $C_3$ and $C_4$ species exist, $C_4$ species are correlated with the annual lifecycle, whereas $C_3$ photosynthesis is associated with the perennial lifecycle (Douglas and Manos, 2007). Monson (2003) relied on arguments that gene duplication occurs at the highest rates in large populations, and the coupling between neofunctionalised genes and environmental selection would be tightest in small populations of plants with short generation times. Conventional wisdom suggests that self-fertilisation in a species may actually reduce fitness owing to an increased fixation of deleterious alleles (Charlesworth *et al.*, 1993; Nordborg, 2000; Charlesworth and Wright, 2001). However, it is also possible that the evolution of self-fertilisation reduces genome size and in the process simplifies genetic relations that might otherwise be deleterious (Wright *et al.*, 2008); thus, enhancing fitness in the face of neofunctionalised traits. The ultimate reasons for the correlations among changes in life history, mating system and photosynthetic diversification remain to be determined. However, it is clear that this aspect of $C_4$ evolution has the potential to constrain rates of photosynthetic diversification and may be as important as first-order relations between climate and rates of photosynthetic carbon uptake.

### 25.3.2. Origins of $C_4$ photosynthesis

Central to the question of $C_4$ evolution is the pattern of change in past atmospheric $CO_2$ concentrations. Conventional theories of $C_4$ evolution tend to follow the quantum-efficiency model (Ehleringer *et al.*, 1991), which predicts threshold $CO_2$ concentrations where $C_4$ photosynthesis has advantages over $C_3$ photosynthesis in the moles of $CO_2$ assimilated per mole of solar quanta absorbed. This threshold is constrained by high atmospheric-$CO_2$ concentrations to favour $C_3$ photosynthesis throughout much of the Earth's history. However, windows of opportunity for $C_4$ evolution occurred both early and late in land-plant evolution. Paleoatmospheric models by Beerling and Woodward (2001) predict that significant parts of the globe would have favoured $C_4$ over $C_3$ during the Carboniferous (Devonian to Permian Periods). Isotopically heavy carbon deposited during this period may indicate the presence of a $C_4$-like carbon-concentrating mechanism (Jones, 1994). However, if $C_4$ taxa evolved under these conditions they are not evident from the fossil record and direct descendants are unknown as none of the contemporary $C_4$ taxa bear any close relationship to taxa that were extant at that time.

Throughout the Mesozoic era, atmospheric $CO_2$ values remained relatively high and continued into the Cenozoic. From the middle to late Eocene epoch of the Tertiary period, atmospheric values were between 1000–1500 ppm, but decreased in steps through the Oligocene epoch to modern levels by the mid-Miocene (Pagani *et al.*, 2005). The cyclical pattern of $CO_2$ drawdown during glaciation events of the Oligocene (Pälike *et al.*, 2006) may have led to conditions favouring $C_4$ evolution (Ehleringer *et al.*, 1997). Consistent with this scenario is the inferred phylogenic tree of the Poaceae, which suggests the evolution of $C_4$ photosynthesis occurred in the subfamily Chloridoideae,

32–25 MA in the Oligocene (Christin *et al.*, 2008) and a similar molecular clock date for the origin of the largely $C_4$ Andropogoneae (Kellogg, 1999). Other evidence in support of an Oligocene origin includes isotopic signatures from soil carbonates (Kleinert and Strecker, 2001) and tooth enamel (Passey *et al.*, 2002).

### 25.3.3. $C_4$-grassland expansion

Contemporary tropical and subtropical grasslands are dominated by $C_4$ grasses. Such ecosystems appear to be relatively recent on a geological timescale, dating in most parts of the world to the late Miocene, 8–5 MA (Ehleringer *et al.*, 1991, 1997; Cerling *et al.*, 1993; Cerling, 1999). Much of our knowledge about the paleo-distributions of $C_4$ grasslands is based on carbon isotopic ratios measured in fossils of calcareous soils, tooth enamel and egg shells (Tipple and Pagani, 2008). Although there is general agreement on the timing of $C_4$ grassland dominance, there is far less agreement on the causes.

Worldwide, two-thirds of all $C_4$ species are grasses and it is therefore little wonder that so much attention has been focused on $C_4$-grassland expansion during the Miocene period and the associated climate and atmospheric factors that drove this expansion. One thing that is clear is that the timing of $C_4$-grassland expansion is unrelated to the timing of the origin of the $C_4$ pathway in grass clades (Cerling, 1999). This mismatch between the timing of the origin of $C_4$ in grasses and their subsequent expansion argues strongly that environments in the late Miocene changed in ways that provided $C_4$ grasses a significant ecological advantage over other species. Much of the controversy centres on whether this rise in $C_4$ grasslands was owing to a shift in conditions favouring $C_4$ grasses over $C_3$ grasses, or a shift from woodlands (most of which would have been $C_3$) to grasslands.

The earliest hypothesis on the emergence of $C_4$ grasslands was that there was an atmospheric drawdown in $CO_2$ during the late Miocene. Coupled with higher temperatures in tropical and subtropical latitudes, $CO_2$ partial pressures reached a critical threshold where $C_4$ species were at a competitive advantage over $C_3$ species (Cerling *et al.*, 1997; Ehleringer *et al.*, 1997). This idea was based on the greater quantum efficiency for $C_4$ photosynthesis under low $CO_2$ concentrations and high temperature, compared with $C_3$ photosynthesis. The primary support for this hypothesis was that carbon-isotope data suggested a synchronous rise in $C_4$ grasslands on three different continents, which would be expected owing to global atmospheric mixing and consequent global climate change.

One limitation to applying the quantum-efficiency model to $C_4$-grassland expansion is that it considers only one of many competitive interactions and thus is most appropriately applied to interactions between $C_3$ and $C_4$ species of similar growth form. For example, under $CO_2$ partial pressures within the range of those estimated for the much of the Miocene, $C_3$ trees commonly out-compete $C_4$ grasses, and once a tree has overtopped the grass canopy light levels and temperature are changed to favour $C_3$ photosynthesis in the understory (Belsky *et al.*, 1989). These considerations are important because numerous lines of evidence show that the rise in $C_4$ dominance was coupled with a major vegetation-type conversion from woodland to grassland; this appears to have been the case in Asia, Africa, South America and North America (Morgan *et al.*, 1994; Quade *et al.*, 1995; Hoorn *et al.*, 2000; Janis *et al.*, 2000; Retallack, 2001; Fox and Koch, 2003).

However, Bond and others (Bond and Midgley, 2000; Bond *et al.*, 2003) have suggested the quantum-efficiency model would still be a viable explanation if coupled with fire. They argued that even in a mixed woodland and grassland setting, the impact of fire would be greater on $C_3$ woodland species, because under declining $CO_2$ levels $C_3$ species would be unable to grow quickly enough to shade out their $C_4$ grass competitors. In addition, Retallack (2001) and Sage (2001) suggested that the combination of reduced $CO_2$, fire and drought all combined to favour $C_4$ grasses during the late Miocene.

Despite the important contributions of the quantum-efficiency model (Ehleringer and Bjorkman, 1977), paleoatmospheric $CO_2$ and temperatures during the late Miocene cast considerable doubt on the model as an explanation for $C_4$-grassland expansion (Keeley and Rundel, 2003, 2005; Osborne and Beerling, 2006). The primary reason is that recent estimates based on numerous approaches have failed to detect a $CO_2$ drawdown during the late Miocene (Pagani *et al.*, 1999, 2005; Demicco, 2003). Additionally, average temperatures appear to have declined over that period (Zachos *et al.*, 2001), further weakening the explanatory power of this model.

In place of declining $CO_2$ levels, drought has been proposed as the major factor in this late Miocene $C_4$-grassland expansion (Sage, 2001; Edwards and Still, 2008). This derives largely from the greater WUE of $C_4$ over $C_3$ species of similar growth form. However, if drought alone were driving this phenomenon, modelling studies of late

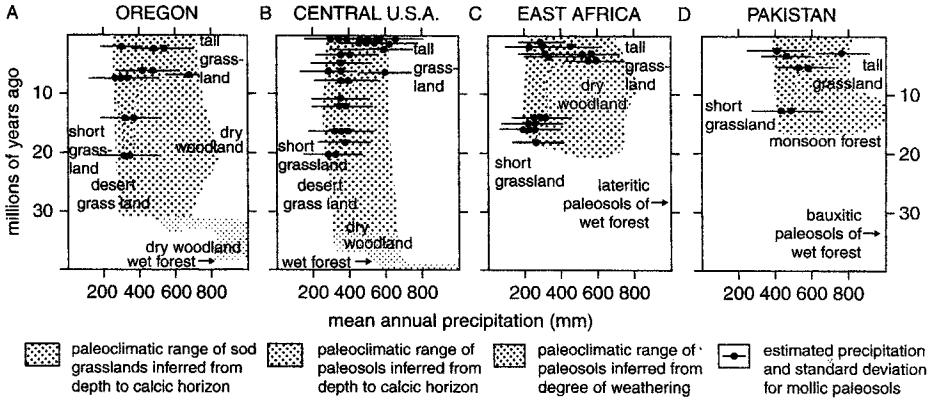


Fig. 25.2. Climatic expansion of sod grasslands through the Miocene in: (A) Oregon; (B) North American Great Plains; (C) East Africa; and (D) Pakistan, based on mollic epipedons and depth to calcic horizon of paleosols. A late Miocene advent of tall grasslands is inferred for each area and replaced preexisting dry woodlands (from Retallack, 2001).

Oligocene climates predict that $C_4$ grasslands should have expanded far earlier than they did (Lunt *et al.*, 2007).

Keeley and Rundel (2003, 2005) acknowledge the importance of drought to the Oligocene origins of the $C_4$ pathway, but contend that by itself it is inadequate to explain the expansion of grasslands. The primary evidence against drought as a major factor is the fact that $C_4$-grassland expansion involved more than just an increase in the distribution of $C_4$ species, but also a rather significant habitat shift. Paleosol data from Asia, Africa and North America all show a temporal change in grassland distribution from semi-arid to more mesic conditions (Retallack, 2001). Under this reconstruction (Fig. 25.2) the late Oligocene to the early Miocene grasslands comprised desert or shortgrass grasslands in environments of 300–400 mm precipitation per year, which is consistent with other data on the origins of $C_4$ grasses (Cerling, 1999). However, the late Miocene expansion of $C_4$ grasslands involved a change to tallgrass ecosystems and in regions with 600–800 mm precipitation per year. In other words the $C_4$-grassland expansion does not appear to be the result of a global increase in the amount of arid habitat, but rather an expansion of $C_4$ grasses from drier habitats into more mesic habitats.

Both empirical studies and modelling exercises support the conclusion that semi-tropical environments with 600–800 mm precipitation can support dense woodlands (Bond *et al.*, 2005), which implies that the rise of $C_4$ grasslands involved the invasion of woodland habitats. Fossil records of changes in wood samples during the late Miocene also support this conclusion of a shift from woodlands to grasslands (Quade *et al.*, 1995). In addition, a shift from browser- to grazer-dominated faunas further supports this scenario (Gunnell *et al.*, 1995; Barry *et al.*, 2002).

There are two factors that can drive a shift from woodlands to grasslands; herbivory and fire (Bond and Keeley, 2005; Bond, 2005). In the absence of driving forces from these perturbations, woodlands persist, as illustrated on contemporary landscapes where woodlands readily replace grasslands when all disturbance is removed (Walker and Noy Meir, 1982; Changnon *et al.*, 2002; Furley and Metcalfe, 2007).

A model of how fire and herbivory may have acted in concert to bring about the rise in $C_4$ grasslands is proposed in Fig. 25.3. Crucial to fire being a keystone process in this phenomenon is development of a markedly seasonal climate. Fire environments have a wet season producing sufficient biomass, followed by a dry season that converts this biomass to available fuel. In continuously arid environments primary production fails to produce fuels sufficient to spread fire, and in a constantly humid environment potential fuels fail to dry sufficiently to become available fuels. One of the characteristics noted with the late Miocene expansion of $C_4$ grasslands in widely disjunct parts of the globe is increased climatic seasonality. This conclusion is inferred from Paleosol data (Retallack, 2001), geomorphical changes (Barry *et al.*, 2002) and dietary characteristics inferred from carbon and oxygen isotopes of equid teeth (Nelson, 2005). In some regions this seasonality was generated by monsoon climates, but seasonality developed under other climates that underwent a late Miocene rise in $C_4$ grasslands (Retallack, 2001).

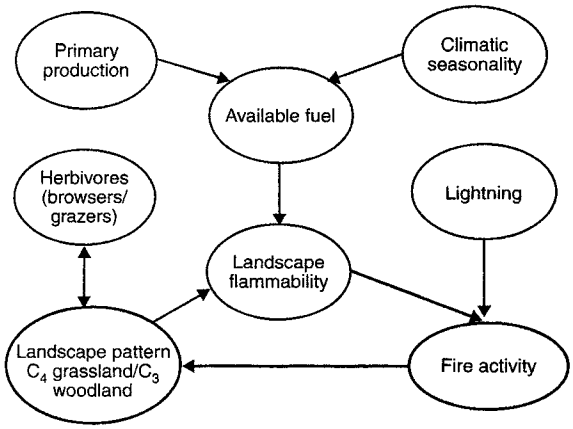


Fig. 25.3. Path model relating factors hypothesised to be the key determinants driving the late Miocene expansion of $C_4$ grasslands in subtropical and tropical regions.

Increased climatic seasonality from the middle Miocene onward set the stage for a major fire-induced shift in vegetation by creating an annual cycle in which biomass dried sufficiently to become available fuels. However, not all vegetation types produce equally flammable fuels. Grasses generate fine fuels that are capable of igniting and spreading fire much more predictably than coarse woody fuels or evergreen leaves with higher moisture as in woodland species. Therefore the proportion of grass biomass to tree biomass and their spatial pattern would affect flammability of the landscape. If one considers a landscape where patches of highly flammable grasses are embedded within a matrix of less-flammable woodland, then the probability of those grassland patches being ignited by lightning would be affected by the size of the patches. As patches increase their extent they in turn cause an ever-increasing probability of burning. Such an effect has been shown empirically on islands, where fire probability is a function of island size because larger islands receive a greater number of lightning strikes than smaller islands (Wardle *et al.*, 2003). A similar model could apply to Miocene savanna 'islands' within less-flammable woodlands.

Herbivores also would have played a role in opening up woodlands and increasing sites for $C_4$-grass establishment, in a manner similar to the contemporary impact of mega-herbivores in African woodlands and savannas (Owen-Smith, 1988). This effect, however, would feed back into impacts on the herbivore community and with increasing grass availability cause a shift from browser-dominated to grazer-dominated communities (Barry, 2002). With increasing size of grassland patches and increasing landscape flammability, fire frequency and fire intensity would increase. This would exacerbate the loss of woodland, and thus feed back into an ever increasing expansion of grasslands until ultimately woodland/savannas were converted to grasslands.

Consistent with this model (Fig. 25.3) is the increasing evidence that $C_4$-grassland expansion was not globally synchronous; rather, grassland expansion was separated by several million years on different continents (Morgan *et al.*, 1994; Jia *et al.*, 2003; Wang and Deng, 2005; Behrensmeyer *et al.*, 2007; Hopley *et al.*, 2007; Tipple and Pagani, 2007). Our model is consistent with this pattern in that it is not dependent on globally synchronous changes in paleoatmospheric conditions, but rather more local effects of climatic seasonality interacting with geomorphology, which would affect fire spread and faunal changes, all of which would be specific to a region. Also consistent with this model is the structure and rate of $C_4$-grassland expansion within a region. Behrensmeyer *et al.* (2007) have shown that during the late Miocene this expansion was variable at a fine spatial scale, and the complete displacement of $C_3$ woodlands with $C_4$ grasslands occurred gradually over a long period of time.

Consistent with this model (Fig. 25.3) is the observation that faunal composition comprised mixtures of browsers and grazers that persisted through much of the Miocene and only gradually shifted towards dominance by grazers at the end of the epoch (Barry *et al.*, 2002). In Kenya, $C_4$ was a component of herbivore diets as early as 15 MA and shifted to exclusively $C_4$ between 8.5 and 6.5 MA (Jacobs, 2004). In most regions that underwent a shift to $C_4$-grassland dominance, faunal communities comprised mixtures of $C_3$ and $C_4$ diets in the mid-Miocene indicative of woodland and grassland mixes, which ultimately shifted to $C_4$-dominated diets indicative of grasslands by the late Miocene (Morgan *et al.*, 1994; Retallack, 2001; Barry *et al.*, 2002).

Fossil evidence of fires synchronous with $C_4$-grassland expansion is crucial to this hypothesis. There is evidence of an increase in charcoal deposition in late Miocene marine cores in the western Pacific basin (Herring, 1985), but these deposition sites are far removed from sites of grassland expansion and relatively little is known about the area over which this charcoal was drawn. However, in the Niger Delta of west tropical Africa there is a close correlation between charred grass cuticle and grass pollen over the last 8 MA (Germeraad *et al.*, 1967; Morley and Richards, 1993). Also important is the demonstration on the Asian continent of a late Miocene (3 MA) increase in $C_4$ grasses concomitant

with a rise in charred grass from the South China Sea (Jia *et al.*, 2003).

Perhaps the most convincing evidence for the role of fire in $C_4$-grassland expansion is the evidence that contemporary $C_4$ grasslands are heavily dependent on fire for their persistence (Hoffman, 1999; Moreira, 2000; Sankaran *et al.*, 2005; Furley, 2006). When fires are present, woodlands convert to grasslands and when fires are excluded, grasslands shift back to woodlands. D'Antonio (2000) has demonstrated this process of fire-induced $C_4$-grass invasion into less flammable woodlands and ultimately the displacement of woodlands with grasslands in subtropical settings. In these instances grasslands have displaced woodlands on sites that are climatically capable of sustaining woodlands and this has happened in the absence of declining atmospheric $CO_2$ or increasing aridity. The process is driven entirely by fire opening up forest canopies, which in turn causes changes in fuel availability and further increases the probability of subsequent burning, as is observed at forest edges in tropical regions (Cochrane *et al.*, 1999). These empirical studies suggest that the role of fire, as outlined in Fig. 25.3, would have been sufficient to account for the late Miocene $C_4$-grassland expansion.

Alternatively, Beerling and Osborne (2006; also Osborne, 2008) have suggested that fire activity would have increased during the late Miocene owing to atmospheric feedbacks generated by fire itself. Their models contend that fire would have promoted climatic characteristics that reduce regional precipitation thus increasing aridity, and this would further favour fire. Their model is a qualitative description that presents an interesting hypothesis, but lacks sufficient information on many parameters for an analytical evaluation. Although atmospheric feedbacks are potentially important in this global expansion of grasslands, their model predicts that grasslands in the late Miocene were expanding owing to an increase in aridity, which is contradicted by the findings of Retallack *et al.* (2001) that late Miocene grasslands were expanding into regions of higher rainfall, not lower rainfall. In addition, they hypothesised that increase in fire activity would lead to atmospheric changes that decreased rainfall, and they interpreted this as a positive feedback on fire, however, in subtropical savannas fire activity is commonly associated with increased rainfall owing to greater grass biomass production (e.g., Duffin, 2008; Harris *et al.*, 2008). These models, however, raise many interesting feedback processes that need further exploration in refining our thinking about the late Miocene $C_4$-grassland expansion.

## 25.4. CONCLUSIONS

Photosynthetic carbon reduction evolved under atmospheric levels of $O_2$ and $CO_2$ markedly unlike contemporary conditions. This Calvin-Benson cycle is universal in all photosynthetic plants and comprises a complex pathway of tightly coupled reactions that evolved very early in the history of life, and this complexity perhaps has inhibited the ability of plants to alter these reactions as atmospheric conditions became inhibitory during certain periods of the Earth's history. The evolutionary response has been to retain the Calvin-Benson cycle in all photosynthetic cells, but under some conditions certain taxa have evolved various mechanisms for enhancing fitness under atmospheric levels of $O_2$ and $CO_2$ inhibitory to carbon reduction. These carbon concentrating mechanisms are diverse, but much of the variation can be circumscribed under the modes of CAM and $C_4$ photosynthesis, which contrast with the majority of $C_3$ plants. Opportunities for evolution of these alternative pathways varied through the geological record as atmospheric conditions changed and the timing of their origins has likely varied greatly between aquatic and terrestrial representatives of both pathways.