EVOLUTION OF CAM AND C$_4$ CARBON-CONCENTRATING MECHANISMS

Jon E. Keeley[1] and Philip W. Rundel

U.S. Geological Survey, Western Ecological Research Center, Sequoia–Kings Canyon Field Station, Three Rivers, California 93271, U.S.A.; and Department of Organismic Biology, Ecology, and Evolution, University of California, Los Angeles, California 90095-1606, U.S.A.

Mechanisms for concentrating carbon around the Rubisco enzyme, which drives the carbon-reducing steps in photosynthesis, are widespread in plants; in vascular plants they are known as crassulacean acid metabolism (CAM) and C$_4$ photosynthesis. CAM is common in desert succulents, tropical epiphytes, and aquatic plants and is characterized by nighttime fixation of CO$_2$. The proximal selective factor driving the evolution of this CO$_2$-concentrating pathway is low daytime CO$_2$, which results from the unusual reverse stomatal behavior of terrestrial CAM species or from patterns of ambient CO$_2$ availability for aquatic CAM species. In terrestrials the ultimate selective factor is water stress that has selected for increased water use efficiency. In aquatics the ultimate selective factor is diel fluctuations in CO$_2$ availability for palustrine species and extreme oligotrophic conditions for lacustrine species. C$_4$ photosynthesis is based on similar biochemistry but carboxylation steps are spatially separated in the leaf rather than temporally as in CAM. This biochemical pathway is most commonly associated with a specialized leaf anatomy known as Kranz anatomy; however, there are exceptions. The ultimate selective factor driving the evolution of this pathway is excessively high photorespiration that inhibits normal C$_3$ photosynthesis under high light and high temperature in both terrestrial and aquatic habitats. CAM is an ancient pathway that likely has been present since the Paleozoic era in aquatic species from shallow-water palustrine habitats. While atmospheric CO$_2$ levels have undoubtedly affected the evolution of terrestrial plant carbon-concentrating mechanisms, there is reason to believe that past atmospheric changes have not played as important a selective role in the aquatic milieu since palustrine habitats today are not generally carbon sinks, and the selective factors driving aquatic CAM are autogenic. Terrestrial CAM, in contrast, is of increasing selective value under extreme water deficits, and undoubtedly, high Mesozoic CO$_2$ levels reduced the amount of landscape perceived by plants as water limited. Late Tertiary and Quaternary reductions in atmospheric CO$_2$, coupled with increasing seasonality, were probably times of substantial species radiation and ecological expansion for CAM plants. C$_4$ photosynthesis occurs in only about half as many families as CAM, and three-fourths of C$_4$ species are either grasses or sedges. Molecular phylogenies indicate C$_4$ is a more recent innovation than CAM and that it originated in the mid-Tertiary, 20–30 Ma, although some data support an earlier origin. While the timing of the origin of C$_4$ remains controversial, the nearly explosive increase in C$_4$ species is clearly documented in the late Miocene, 4–7 Ma. Increasing seasonality has been widely suggested as an important climatic stimulus for this C$_4$ expansion. Alternatively, based on models of photosynthetic quantum yield at different temperatures and CO$_2$ concentration, it has been hypothesized that the late Miocene C$_4$ expansion resulted from declining atmospheric CO$_2$ levels. This model is most appropriate for explaining the transition from C$_3$ grasslands to C$_4$ grasslands but by itself may not be sufficient to explain the more likely scenario of a late Miocene transition from C$_3$ woodland/savanna to C$_4$ grasslands. A largely unexplored hypothesis is that climatic changes in late Miocene altered disturbance regimes, in particular the incidence of fires, which today are often associated with maintenance of C$_4$ grasslands. Oceanic charcoal sediments that appear to represent Aeolian deposits from continental wildfires follow a strikingly similar pattern of explosive increase in late Miocene. Climate, CO$_2$, and disturbance are not mutually exclusive explanations and probably all acted in concert to promote the expansion of C$_4$ grasslands. More recently, late Quaternary changes in CO$_2$ may have been responsible for driving major changes in the landscape distribution of C$_4$ species. The theory is sound; however, many of the studies cited in support of this model are open to alternative interpretations, and none has eliminated climatic factors as important selective agents. CAM and C$_4$ evolution required coupling of biochemical pathways with structural changes in photosynthetic tissues, succulence in CAM and Kranz in C$_4$. This was apparently accomplished by piecemeal evolution beginning with mechanisms for recapturing respiratory CO$_2$, although this need not have been so in aquatic CAM species. It has been proposed that the extreme rarity of both pathways in the same plant results from biochemical and structural incompatibilities (Sage 2002). Equally important is the fact that the selective environments are quite different, with CAM evolution thriving on stressful sites inhospitable to C$_3$ species whereas C$_4$ evolution has selected for rapid growth capable of outcompeting associated C$_3$ plants.

Keywords: aquatic, grasslands, photosynthetic pathways, succulents.

> Thirty years ago I recall standing in front of the chalk board in George Laties' laboratory at UCLA with James Bassham, there experiencing first-hand the pervasive influence of the Benson-Calvin cycle as I discussed evidence for the primary labeling of C-4 in malic acid. (C. B. Osmond 1997)

Introduction

The capture of light energy and storage as reduced carbon compounds is one of the oldest molecular mechanisms on earth.

[1] Author for correspondence and reprints; fax 559-565-3170; e-mail jon_keeley@usgs.gov.

The biochemical steps involved in carbon reduction known as the Calvin-Benson cycle evolved early in the history of life, reflected in its ubiquitous presence in photosynthetic plants (Raven 1998b). Also reflecting this ancient origin is the suboptimal performance under contemporary atmospheric gas composition, and the molecular complexity that has resisted experimental attempts at altering the performance of the main photosynthetic step catalyzed by the enzyme RuBP carboxylase-oxygenase (Rubisco). In present-day atmospheres when temperature, water,

and light are not limiting, the majority of plants on earth are photosynthetically inhibited by the process of photorespiration. This results when internal carbon dioxide levels are low and oxygen outcompetes carbon dioxide at the enzyme's active site. A significant part of plant evolution has been the development of mechanisms for inhibiting photorespiration by concentrating CO_2 around Rubisco. In vascular plants, CO_2-concentrating mechanisms are crassulacean acid metabolism (CAM) and C_4 photosynthesis, while plants lacking any concentrating mechanism are known as C_3. CAM and C_4 are "an addendum to, not a replacement for, the conventional C_3 pathway" (Osmond et al. 1980) and clearly originated from C_3 progenitors.

Photosynthesis and CO2-Concentrating Mechanisms

Crassulacean acid metabolism (CAM), named for the family in which this physiological system was first observed, concentrates CO_2 around Rubisco through the use of dual carboxylation pathways, first by CO_2 fixation with phosphoenolpyruvate carboxylase (PEPCase) and secondarily by Rubisco (fig. 1A). These two processes are separated temporally in the same tissue, with stomata opening at night to take in and fix CO_2 using PEPCase. This carbon is temporarily stored subcellularly in the vacuole, primarily as the organic acid form of the anion malate. During the day, stomatal closure (in terrestrial plants) reduces water loss but results in an internal carbon deficit that is overcome by decarboxylation of the malic acid stores. This process concentrates CO_2 around Rubisco, resulting in daytime internal inorganic carbon levels two to 60 times ambient levels (Lüttge 2002). A significant number of submerged aquatic macrophytes also exhibit CAM, but they lack functional stomata, and nighttime CO_2 uptake is largely controlled by diurnal changes in ambient CO_2 levels in the water (Keeley 1998a). One of the prerequisites for carbon-concentrating mechanisms is the prevention of CO_2 leakage following decarboxylation. In terrestrial CAM species, daytime stomatal closure inhibits leakage. In aquatic species, this is facilitated by the centripetal placement of chloroplasts and the high diffusive resistance of water.

Both terrestrial and aquatic CAM plants exhibit extraordinary plasticity with respect to the contribution of this CO_2-concentrating mechanism to the plant's carbon budget. In many CAM species, dark fixation accounts for nearly all of the carbon gain, while in others (CAM-cycling species) there is little or no net contribution, and the primary role of CAM is in recycling of respiratory carbon (Osmond 1984). This gradient of CAM dependence for carbon uptake has led to the designation of species as being obligate CAM or CAM flexible and involves both a genetic component and phenotypic plasticity, allowing some species to change photosynthetic pathways in response to seasonal changes in their environment.

C_4 photosynthesis is a second major mechanism for concentrating CO_2. C_4 plants also exhibit a double carboxylation, but these steps are not separated temporally as in CAM but rather spatially in different tissues of the leaf (fig. 1B). PEPCase fixation of CO_2 occurs in the outer mesophyll, and malate (or aspartate) are transported to a special sheath of thick-walled chlorophyllous cells known as the bundle sheath cells, where these organic acid carriers are decarboxylated and CO_2 is concentrated around Rubisco. Unlike Rubisco, PEPCase is not inhibited by O_2 and thus is able to able to act as a CO_2 pump from the mesophyll to the only site of Rubisco activity in the bundle sheath cells. This biochemical pathway is usually associated with a leaf structure termed Kranz anatomy, in which the chloroplasts are concentrated in a ring of prominent bundle sheath cells around the veins. Although older literature tended to equate this carbon-concentrating mechanism with Kranz anatomy, there are now clear examples of both aquatic (Bowes and Salvucci 1984; Keeley 1998b) and terrestrial (Voznesenskaya et al. 2001) C_4 species without this structure. In contrast to the flexibility in CAM expression, C_4 species are generally obligate in the use of this metabolic system, except in the case of aquatic C_4.

One powerful tool for studying CO_2-concentrating pathways in plants has been carbon stable isotope ratios ($\delta^{13}C$) that distinguish carbon initially fixed by PEPCase from carbon initially fixed by Rubisco (Ehleringer and Rundel 1989). Thus, relatively simple surveys of CAM and C_4 metabolism can be made using isotopic analyses of small tissue samples, both fresh and dried herbarium specimens. Carbon isotope analyses alone are unable to separate these two CO_2-concentrating mechanisms, but morphological structure and phylogenetic relationships established by past surveys are usually sufficient to separate them. Beyond simple surveys, carbon isotope ratios have been widely used in studies of CAM plants to identify the relative proportions of CAM and C_3 metabolism used by the plants (Rundel et al. 1999). However, in CAM-cycling species, where only respiratory carbon is fixed via the CAM pathway, $\delta^{13}C$ is incapable of distinguishing these from C_3 species (Winter and Holtum 2002). $\delta^{13}C$ ratios in aquatic plants are affected by chemical and physical characteristics of the aquatic milieu, and thus they are not a useful indicator of photosynthetic pathway (Osmond et al. 1981; Raven et al. 1987; Keeley and Sandquist 1992). Stable carbon isotopes also have been an important tool for reconstructing the geological history of C_3- and C_4-dominated ecosystems.

Beyond the use of $\delta^{13}C$ measures, empirical designation of CAM is most commonly made by the demonstration of nocturnal acidification of chlorophyllous tissues with a stoichiometry of 1 malate^{2-} to 2 H$^+$, followed by daytime acidification. Demonstration of nighttime CO_2 uptake that exceeds daytime C_3 uptake is considered indicative of fully expressed CAM (fig. 2); however, there is much variation both between species and in seasonal variation within species in patterns of day and night CO_2 uptake (Winter and Smith 1996b). C_4 species are most commonly recognized by their distinctive Kranz anatomy, although the $\delta^{13}C$ signature and the physiological demonstration of a lack of photorespiration in gas exchange studies are also good indicators. A more definitive measure is to demonstrate that the 4-C organic acids of malate or asparate anions are the initial products of carbon fixation and that they rapidly turn over and release carbon to the Calvin-Benson carbon reduction cycle of photosynthesis (Edwards and Walker 1983).

The two modal patterns for concentrating carbon (CAM and C_4) will be the focus here. However, an array of carbon-concentrating mechanisms exists in single and multicellular organisms from seawater and freshwater habitats. Some of these are based on biophysical pumps that capitalize on the rich store of HCO_3^- in many aquatic habitats and specialized
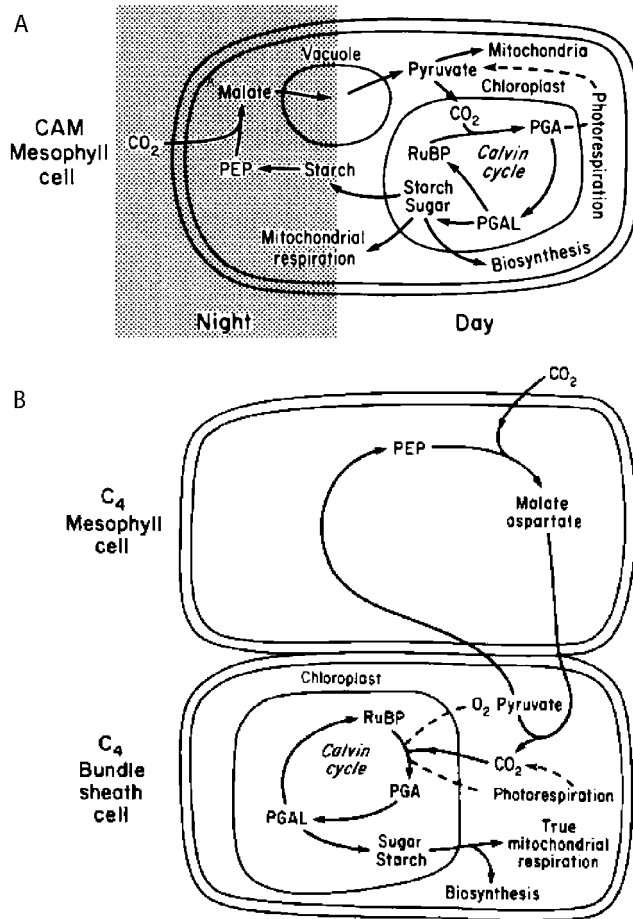
Fig. 1 Schematic depiction of CAM (*A*) and C_4 (*B*) carbon photosynthetic pathways (modified from Rundel and Gibson 1996; reprinted with permission of Cambridge University Press).

organelles known as pyrenoids or carboxysomes (Raven 1985). For a further fascinating discussion of known and hypothetical mechanisms, see Raven and Spicer (1996).

To put this review in historical context, CAM biochemistry was largely worked out in the late 1940s, only shortly after the steps of carbon reduction in the C_3 (Calvin-Benson) cycle were elucidated (Thomas 1949). There was a 25-yr hiatus before the C_4 photosynthetic pathway was discovered and several more years before it was accepted by the scientific community (Osmond 1997).

Phylogenetic Patterns of CAM Photosynthesis

CAM is known only from vascular plants and includes species from the Lycophyta, Pterophyta, Gnetophyta, and Anthophyta divisions. It is known from 33 plant families and because of its wide systematic distribution would appear to have arisen independently a number of times (Moore 1983; Smith and Winter 1996), and this is borne out by the molecular phylogeny of PEPCase, which shows CAM was antecedent to C_4 (Chollet et al. 1996). The vast majority of species are in the Anthophyta and are well represented in both monocotyledons and dicot-

yledons (Smith and Winter 1996). Terrestrial CAM species are typically desert succulents and are prominently represented in the Caryophyllidae (Aizoaceae, Cactaceae, and Portulacaeae), Rosidae (Crassulaceae and Euphorbiaceae), and Asteridae (Asclepiadaceae and Asteraceae). Epiphytic species that inhabit aerial environments are largely monocots from the Lilianae (Orchidaceae) and Bromelianae (Bromeliaceae).

One of the more remarkable examples of evolutionary convergence is selection for CAM photosynthesis in both desert succulents and aquatic macrophytes (Keeley 1981, 1998*a*). The vast majority of aquatic CAM plants are spore-bearing Lycophyta in the genus *Isoëtes*, but there are also five Anthophyta families, including both monocots and dicots. Only one of the six aquatic CAM families (Crassulaceae) shares terrestrial CAM species.

Roughly 6% of the terrestrial (including epiphytes) and 6% of the aquatic flora are estimated to have CAM (Keeley 1998*a*). A less phylogenetically biased estimate is the distribution of CAM in plant families, and it is estimated that 8% of the terrestrial and 9% of the aquatic families have evolved CAM. Thus, it would appear that CAM has had an equal likelihood of evolving in water as on land.

Ecological Distribution of CAM species

Terrestrial CAM Ecology

CAM is best known for desert succulents where daytime high temperatures and low relative humidities lead to stomatal resistance that restricts CO_2 entry. Lower atmospheric water vapor deficits at night have selected for reverse stomatal behavior with the associated increase in water use efficiency (carbon gain per unit water loss) as carbon fixation occurs at lower ambient vapor pressure deficits and high efficiency of carbon fixation by PEPCase. Terrestrial CAM plants are well represented as stem succulents and as suffrutescents or small woody species with succulent leaves but can be found in virtually all terrestrial plant growth forms. Since the primary advantage of this pathway is the ability to continue photosynthetic carbon gain under extreme water stress, summer dormant herbaceous perennials are seldom CAM, although there are small summer-dormant succulents that utilize CAM (Rundel et al. 1999). Under terrestrial conditions, annuals are only rarely CAM, perhaps because the ephemeral growth selects against slow growth rates. A notable exception is the annual species of *Mesembryanthemum* (Aizoaceae), which switches developmentally from C_3 to CAM metabolism as they experience water stress (Edwards et al. 1996). In the Cactaceae, seedlings are typically C_3 and later switch to CAM (Altesor et al. 1992); however, it is unclear whether this is due to stress or a programmed developmental change or both.

A significant cost to CAM is that the level of daytime carbon fixation is constrained by the total storage capacity of the vacuole for the nighttime fixation product malic acid, a 4-carbon organic acid. Consequently, relative growth rates are generally low, and thus as aridity decreases these plants are displaced by C_3 or C_4 species with higher growth rates. However, CAM plants grown under optimal conditions of water availability exhibit 24-h CO_2 uptake from both CAM and C_3 metabolism and exhibit extraordinarily high growth rates,

both for terrestrial (Nobel 1996) and aquatic species (Keeley and Busch 1984).

Vacuolar storage demands of CAM metabolism require a tight evolutionary coupling of this pathway with succulent tissues, a linkage present in the majority of Cactaceae, Crassulaceae, succulent *Euphorbia*, and monocot genera such as *Agave* and *Aloe*. However, this form/function linkage cannot always be assumed, even among desert plants. Tall stem succulents with deciduous leaves are typically C_3, including species of *Fouquieria* (Fouquieriaceae), *Pachycormus* (Anacardiaceae), and *Bursera* (Burseraceae) in the deserts and dry forests of Mexico. In the succulent karoo of South Africa, there is a huge number of succulent species of Aizoaceae, Portulacaceae, Asteraceae, Crassulaceae, Zygophyllaceae, and Asphodelaceae, all families in which CAM has been documented (Cowling et al. 1999). However, carbon isotope studies have shown that CAM, CAM-flexible, and C_3 forms of metabolism are all represented among these leaf-succulent taxa (Rundel et al. 1999) because succulent leaves provide the most energetically efficient means of storing water (Rundel et al. 1998).

CAM plants do not exhibit the same predictability of distribution with latitude and elevation as C_4 plants (Boutton et al. 1980). This results from their occupation of azonal conditions of extreme soil aridity, such as shallow soils or rock outcrops. CAM plants commonly increase in diversity and dominance along aridity gradients, although there is a marked interaction with temperature. They decline with decreases in temperature, but examples are known up to 4700 m (Keeley and Keeley 1989) and at high latitudes (Nobel 1981). CAM also declines when nighttime temperatures are too high (Mooney et al. 1974). In the Crassulaceae, there is evidence of latitudinal patterns of CAM expression, where southern taxa rely on nighttime carbon uptake and northern taxa are CAM cyclers (Teeri 1982).

Epiphyte CAM Ecology

In Neotropical lowland forests, epiphytes may comprise 35%–50% of canopy leaf biomass and more than a third of the flora (Gentry and Dodson 1987). CAM metabolism is widespread in these tropical forest epiphytes, selected largely because of physiological drought associated with their aerial growth habit (Lüttge 1989; Benzing 1990; Martin 1994). Families well represented with CAM epiphytes include monocots such as Bromeliaceae (1144 epiphytic species, and an estimated 50% are CAM) and Orchidaceae (20,000 species, 60% CAM) and dicots Asclepiadaceae (135 species, 90% CAM) and Cactaceae (120 species, 100% CAM) (Griffiths 1989). Elevational gradients are evident in epiphytic CAM, being most common in lowland tropics where fully expressed CAM is commonplace, but decline in higher montane forests where they are more commonly present as CAM cycling species (Earnshaw et al. 1987).

Aquatic CAM Ecology

Aquatic CAM species are well represented in *Isoëtes* (Lycophyta: Isoetaceae) and *Crassula* (Crassulaceae), with many species found worldwide in both palustrine (marshy, shallow, often seasonally wet) and lacustrine (deep-water lake) habitats (Keeley 1998a). Selection for CAM has been driven by daytime CO_2 limitation. In palustrine habitats (fig. 3A), extreme diel fluctuations in CO_2 are created by biogenic processes, whereas
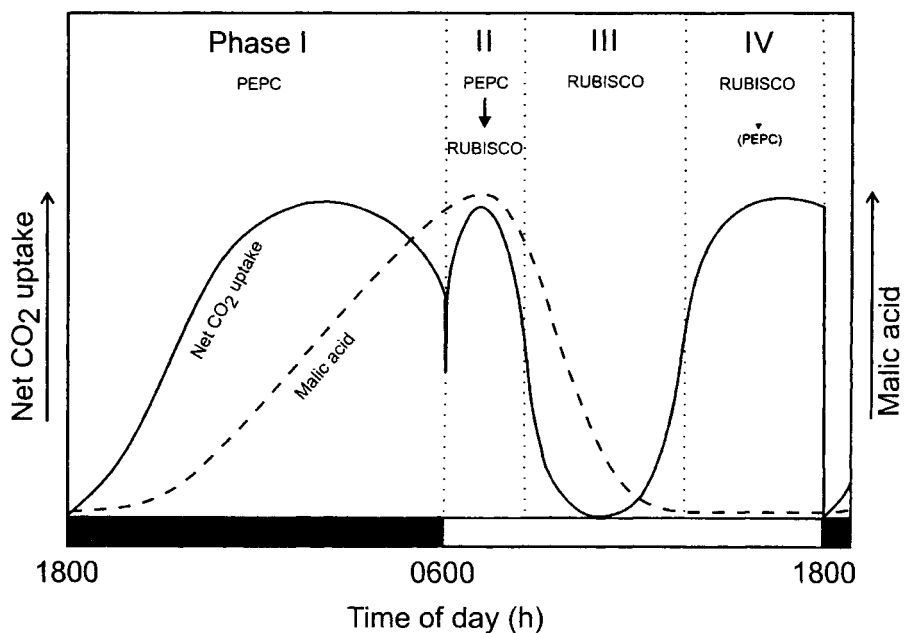
Fig. 2 Day/night cycle of CO_2 fixation and malic acid content in fully expressed CAM. Phase I comprises nighttime PEPC-driven carbon uptake and acid accumulation; phase II is a transitional phase with an accelerated burst of CO_2 uptake due to both PEPC-mediated and Rubisco-mediated CO_2 fixation; in phase III CO_2 uptake is diminished and decarboxylation of malic acid stores generates internal CO_2 for Rubisco-mediated CO_2 fixation; phase IV may include CO_2 uptake that is fixed directly by Rubisco, not unlike daytime uptake by C_3 plants (from Winter and Smith 1996a; reprinted with permission of Springer).
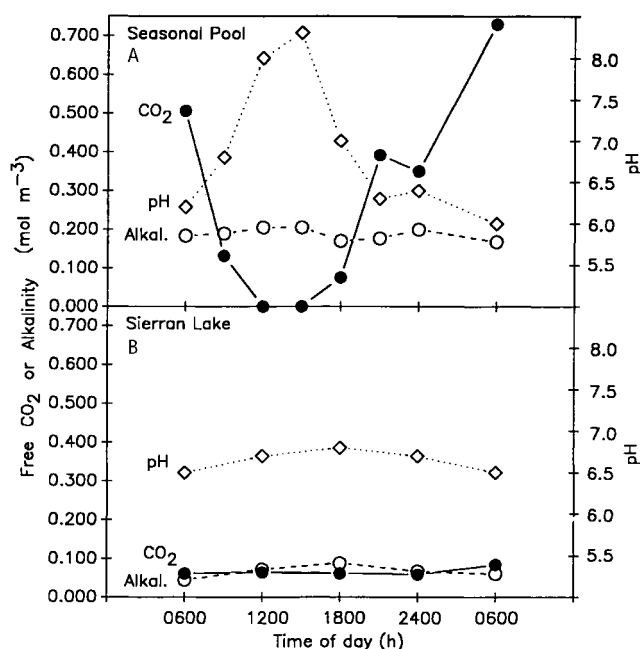
Fig. 3 Diel changes in water chemistry in a seasonal pool palustrine habitat (*A*) and a high-elevation oligotrophic lacustrine habitat (*B*) (from Keeley 1996; reprinted with permission of Springer).

oligotrophic lacustrine habitats (fig. 3*B*) result in continuously low CO_2 levels (Keeley 1998*a*; Madsen et al. 2002).

The extreme carbon deficiency of oligotrophic lacustrine habitats has also selected for extensive root systems that capture the copious CO_2 evolved from organic-rich sediments (Raven et al. 1988). Thus, there is a steady stream of sediment-derived carbon diffusing from the roots, and CAM captures this stream at night; thus, in these species, nighttime uptake may depend heavily on sediment carbon.

There are a substantial number of reports of dark fixation of CO_2 by algae from both saltwater and freshwater. Most of these have not been studied in sufficient detail to adequately conclude they are using the CAM pathway, and indeed there are a variety of metabolic functions that dark fixation may play. Some have been shown to lack the key CAM characteristic of overnight acid storage and daytime transfer of carbon to Calvin-Benson cycle phosphorylated sugars, suggesting other functions for dark fixation in these species (Keeley 1998*a*).

Evolution of CAM

Terrestrial CAM Plants

Since water use efficiency is a function of atmospheric CO_2 (Raven and Spicer 1996), as CO_2 levels decrease, one should expect an expansion of habitats selecting for CAM, and as CO_2 increases, those habitats shrink. Thus, constraining the timing of terrestrial and aerial CAM evolution requires examination of paleoatmospheric changes. Direct measures of the partial pressure of carbon dioxide (PCO_2) in bubbles from ice cores have provided valuable insights into Quaternary (ta-

ble 1) atmospheric CO_2 levels (Petit et al. 1999), but we have no direct measures for earlier periods. However, from the widespread interest in global carbon cycles, much attention has been given to geochemical mass balance models of past atmospheric CO_2 levels (Berner 1998). Models (fig. 4) predict extraordinarily high levels (>2000 ppmv) prior to 350 Ma. The Paleozoic rise of land plants through direct consumption and indirectly through increased soil weathering drew down CO_2 so that by 300 Ma levels were comparable to contemporary atmospheres (Raven 1998*a*). Over the subsequent 150 Ma, CO_2 increased to levels four to five times higher than the current atmosphere (fig. 4). Beginning in the mid-Cretaceous period (ca. 100 Ma), there was a gradual drop in CO_2, and from the early Miocene, levels have been below 600 ppmv and reached their lowest levels (180–200 ppmv) during full glacial episodes in the Pleistocene. These patterns are supported by proxy measures of carbon isotopes from paleosol carbonates (Cerling 1991; Mora et al. 1996), boron isotopes from marine plankton fossils (Pearson and Palmer 2000), and stomatal density (McElwain 1998; Retallack 2001), and these measures indicate considerably finer scale temporal variation than illustrated in figure 4.

Relative to CAM evolution, analysis of paleoatmospheres indicates that glacial episodes during the Pleistocene would have produced global atmospheric conditions more favorable to CAM species than atmospheric conditions during the previous 100 million years (Raven and Spicer 1996). However, since microsite aridity is the ultimate factor selecting for terrestrial CAM, examining patterns of paleoclimates (fig. 4) cannot completely constrain the timing of terrestrial CAM origins but can only broadly indicate the geological periods when selection would have been most intense for CAM evolution and radiation. Even under doubled current atmospheric CO_2, photosynthetic responses of CAM species are comparable to C_3 plants (Drennan and Nobel 2000). Considering the limitations to growth imposed by other nutrients on stressful oligotrophic sites, there may have been many earlier opportunities for the evolution of CAM.

The essential steps of the CAM biochemical pathway are present in stomatal guard cells of C_3 species, and thus it has been proposed that the origin of CAM was a simple matter

Table 1

Stratigraphic Classification of the Relevant Geologic Timetable

Era	Period	Epoch	Beginning (ca. Ma)
Cenozoic	Quaternary	Holocene	0.01
		Pleistocene	1.6
	Tertiary	Pliocene	5.2
		Miocene	22.5
		Oligocene	35.4
		Eocene	56.5
		Paleocene	65.0
Mesozoic	Cretaceous		145
	Jurassic		195
	Triassic		230
Paleozoic	Permian		280
	Carboniferous		345
	Devonian		395
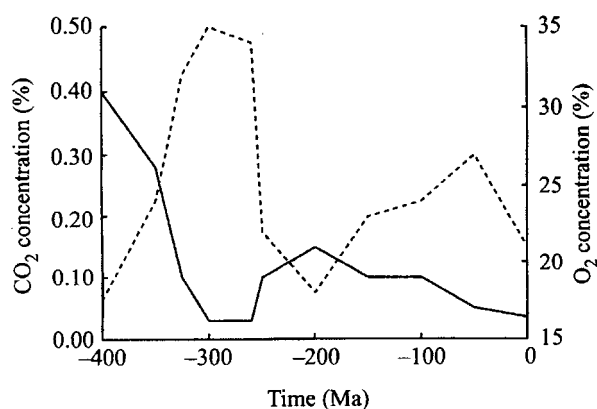
Fig. 4 Variations in atmospheric CO_2 (solid line) and O_2 (dashed line) over the past 400 million years predicted by the geochemical mass balance model (from Beerling et al. 1998; reprinted with permission of the Royal Society of London).

of transferring these reactions to mesophyll cells (Cockburn 1981). Consistent with this model are genomic studies that show CAM induction is controlled primarily by transcriptional activation (Cushman 2001). Certain key enzymes such as PEP-Case have specific isoforms whose expression is controlled by a cascade of signals that are turned on by various factors. These characteristics would provide the capacity for repeated evolution of CAM in different lineages and help explain the extraordinary flexibility evident in CAM expression.

The importance of this flexibility may lie in the proposition that the precursor to fully expressed CAM was CAM cycling (Monson 1989). Perhaps an example of this early form of CAM is *Welwitschia mirabilis*, a morphologically unusual gymnosperm restricted today to the deserts of Namibia and Angola, which exhibits CAM cycling of organic acids but not CAM gas exchange (Ting and Burk 1983). On the basis of a putative fossil-pollen record, it is possible this species dates back to the early Permian (Taylor 1981), when CO_2 levels are estimated to have been extraordinarily low (fig. 4). Also, within the Crassulaceae family, CAM cycling appears to have preceded the evolution of fully developed CAM (Teeri 1982).

Aquatic CAM Plants

CAM photosynthesis is an ancient pathway probably present in early vascular plants that lived in seasonal pools in the Mesozoic era more than 200 million years ago (Keeley 1998a). This would place the origin of CAM during a time when atmospheric CO_2 levels were moderately high (fig. 4). However, atmospheric CO_2 levels were likely less relevant to the evolution of aquatic CAM because diffusional limitations and biogenic processes in the water buffer these systems from atmospheric concentrations; in most surface waters CO_2 supersaturation results in ecosystems that are sources rather than sinks of atmospheric CO_2 (Cole et al. 1994). Contemporary diel changes in CO_2 concentrations in shallow palustrine habitats exceed the estimated changes in atmospheric CO_2 since the Paleozoic era (600 Ma) and thus there is reason to believe Mesozoic pools were selective environments for aquatic CAM.

CAM is apparently present in all aquatic species of *Isoëtes*

(over 30% of ca. 125 species have been investigated; Keeley 1998a). This is a relatively ancient taxon known to occupy palustrine habitats as far back as the early Triassic period (>230 Ma) (Retallack 1997a), and part of the success of this taxon is thought to be tied to its CAM physiology that allowed it to capitalize on open high-light marshy environments following the Permian-Triassic biotic crisis (Looy et al. 2000). The nearly ubiquitous presence of CAM in *Isoëtes* indicates that CAM has originated just once in the Isoëtaceae, which represents the oldest clade of CAM plants. There is cladistic evidence for loss of CAM in several *Isoëtes* lineages that lead to the secondary evolution of terrestrial C_3 species (Keeley 1998a).

Whereas the CO_2 levels in palustrine habitats are driven by autogenic processes within the pools, such is not the case for the oligotrophic lacustrine (lake) habitats. Thus, the latter may have been influenced to a much greater degree by past atmospheric changes, and potentially, *Isoëtes* have invaded these habitats more recently as atmospheric CO_2 levels dropped during Pleistocene glacial episodes (fig. 4). Cladistic analysis supports this scenario. Based on the absence of many plesiomorphic traits in *Isoëtes* from lacustrine habitats, it appears that these species are more recently derived from palustrine ancestors (Hickey 1986; Taylor and Hickey 1992).

Cladistic analyses further show that species from both palustrine and lacustrine habitats have given rise to terrestrial *Isoëtes* taxa, which represent two distinct patterns, the terrestrial C_3 syndrome and the terrestrial CAM syndrome (Keeley 1998a). In the former, CAM has been lost, functional stomatal are present, and photosynthesis is strictly through the C_3 pathway. These are all temperate zone geophytes that go dormant during stressful periods. In tropical alpine habitats there are some rare evergreen terrestrial *Isoëtes* that have retained CAM (Keeley et al. 1984, 1994). These appear to be derived from lacustrine species and have also retained extensive root systems necessary for the sediment-based nutrition typical of species from these oligotrophic habitats. They lack functional stomata and obtain most of their carbon from the sediment, and CAM plays a role in recycling respiratory CO_2 as well as extending the carbon fixation period to 24 h.

The second globally most widespread aquatic CAM taxon is *Crassula* (Keeley 1998a). This is the nominant genus for crassulacean acid metabolism, and it includes an amazing diversity of terrestrial CAM species. The majority of terrestrial *Crassula* are CAM-obligate perennials (Rundel et al. 1999) restricted to South Africa, but there are a number of annual CAM-flexible aquatic species distributed worldwide (Tolken 1977). These aquatic CAM species (sometimes placed in the genus *Tillaea*) are distributed in both palustrine and lacustrine habitats on four continents and are distantly related to their perennial congeners (Tolken 1977; Van Ham 1995). In this family, as well as the Isoetaceae, CAM apparently has had but a single origin. It appears that CAM has been lost several times in *Crassula* but only in terrestrial annuals that are likely derived from aquatic ancestors (Keeley 1998a).

Phylogenetic Patterns of C_4 Photosynthesis

C_4 photosynthesis occurs in 18 families, half as many as in CAM, and in only one-third as many species as CAM (Sage

et al. 1999*a*). It is restricted to flowering plants and is a much more recent innovation than CAM (Chollet et al. 1996). Specieswise, it is primarily a grass and sedge phenomenon; 61% of all C_4 species are Poaceae (comprising nearly half of the grass family) and 18% Cyperaceae. By comparison, C_4 is far less common in dicots, comprising about 21% of the total C_4 flora and less than 1% of all dicots. The majority of these are in the order Caryophyllidae, which includes the Chenopodiaceae, Amaranthaceae, and Polygonaceae with 550, 250, and 80 species, respectively. Two other dicot orders with C_4 are the Rosidae (Euphorbiaceae) and Asteridae (Acanthaceae, Asteraceae).

The C_4 pathway for concentrating carbon occurs in submerged aquatic angiosperms, both monocots and dicots. The former is only known from one very small tribe of Poaceae, the Orcuttieae (Keeley 1998*b*), and the latter from two genera in the Hydrocharitaceae (Reiskind et al. 1997; Lara et al. 2002).

Multiple origins of C_4 are readily apparent based on systematic considerations (Moore 1983), and recent molecular phylogenies reveal 31 origins, with multiple origins within several families, in particular, the Poaceae and Asteraceae (Monson 1996; Kellogg 1999). One key to the multiple origins is the fact that there are three known biochemical subtypes that probably had independent origins. Biochemical pathway diversity coupled with extraordinary anatomical diversity illustrates that C_4 is actually a composite of many different independently evolved photosynthetic modes (Kellogg 1999).

Ecological Distribution of C_4 Species

Terrestrial C_4 Ecology

The C_4 pathway has an added energetic cost compared to C_3 species (Ehleringer and Monson 1993); therefore, only in a subset of environments are the benefits of this CO_2-concentrating mechanism sufficient to outweigh the costs. The primary benefits are an ability to eliminate photorespiration, which can lead to substantially higher photosynthetic rates and an ability to maintain high rates of water use efficiency. C_4 habitats are most commonly environments where light and temperature are not highly limiting to carbon uptake (Ehleringer and Monson 1993). Terrestrial C_4 dominance increases as growing season temperature increases and declines with cooler growing seasons and shading (Sage et al. 1999*b*). Due to a lower investment of nitrogen in Rubisco, C_4 plants have higher nitrogen use efficiency (nitrogen per unit carbon fixed), but there are no clear-cut correlations between C_4 distribution and soil nitrogen content.

Tropical and subtropical grasslands are dominated by rhizomatous perennial C_4 grasses and sedges. For example, in East Africa, the Kenya grass flora (Ibrahim and Kabuye 1988, based on Watson and Dallwitz 1992) records 606 species (including subspecies); 62% are C_4 perennials and 27% are C_4 annuals. In terms of sedges (Hesla et al. 1982; Haines and Lye 1983), Kenya has 220 species, of which 55% are C_4 perennials and 10% are C_4 annuals. In these summer rainfall environments, the high growing season temperatures give C_4 grasses a clear advantage over C_3 species. This advantage changes with elevation, and temperature appears to be the primary factor.

Along one tropical transect, the balance between C_3 and C_4 species was at the elevation where the warmest month averaged 21°C (Rundel 1980). In central African grasslands, C_4 species comprise 99% of the grass flora below 2000 m and are absent above 3000 m (Tieszen et al. 1979). Similar patterns are evident in subtropical latitudes except that the crossover point between C_3 and C_4 occurs at lower elevations (Cabido et al. 1997). Exceptions to these broad patterns are present, however. Extensive areas of tropical savannas in Southeast Asia are commonly dominated by C_3 bamboo under climate regimes considered favorable to C_4 (P. W. Rundel, personal observation). At the other extreme, a C_4 grass species in the genus *Muhlenbergia* grows well at 3900 m (Sage and Sage 2002). Special azonal habitats may also favor C_4 species. For example, while C_4 grasses comprise only 9% of the grassland flora in the mediterranean climate of California, they are substantially more important where water accumulates in roadside ditches, agricultural fields, or vernal pools; e.g., in the latter habitat 63% of the grass flora is C_4 (Keeley 1998*b*). This is consistent with broader patterns because in this summer drought climate, temporary wetlands extend the growing season and thus increase the mean growing season temperature.

C_4 annual plants are a conspicuous component of the summer flora of desert regions with summer rains (Mulroy and Rundel 1977). These annuals commonly include species of Amaranthaceae, Asteraceae, Euphorbiaceae, Nyctaginaceae, and Poaceae, and they are able to achieve high rates of photosynthesis because of the absence of photorespiration in C_4 metabolism. C_4 also represents an important ecological strategy in certain desert shrubs, most notably species of *Atriplex* (Chenopodiaceae), particularly in saline soils. In these shrubs, the key adaptation is the ability to maintain growth under high summer temperatures and drought conditions at a time when C_3 shrubs are dormant (Caldwell et al. 1977), thus reducing interspecific competition for water. The maximal rates of photosynthesis in these desert C_4 shrubs are generally no higher than that of co-occurring C_3 shrubs, but the water use efficiency is far greater.

C_4 metabolism in trees is rare. This is to be expected since the tree growth form is ultimately a response to light competition. Such habitats would not be sites of C_4 evolution since the major photosynthetic advantage of higher quantum efficiency in C_4 species is only expressed in high light environments. The only known C_4 trees were apparently derived from ancestors indigenous to high light environments. One example comes from the Hawaiian islands, where ancient colonization events by herbaceous C_4 Euphorbiaceae led to an evolutionary lineage of C_4 woody shrubs and a C_4 tree (*Euphorbia forbesii*) whose habitat is relatively open forests (Pearcy et al. 1987). Frequent sun flecks in this habitat are sufficient to recoup some of the cost of C_4 but only enough to break even with the associated C_3 tree species. Other C_4 trees include species of *Haloxylon* (Chenopodiaceae) and *Calligonum* (Polygonaceae) from deserts of middle Asia, which are variously described as trees or large shrubs from saline habitats (Winter 1981; Pyankov et al. 2000).

Aquatic C_4 Ecology

A significant number of emergent aquatics include C_4 grasses (e.g., *Spartina* spp) and sedges (e.g., *Cyperus* spp), in both

fresh- and saltwater wetlands (Long 1999). Enhanced nitrogen use efficiency has been advocated as the primary selective factor for C_4 in these habitats (Jones 1987; Li et al. 1999). However, many of these wetlands are frequently flushed with nutrients, and because forests are excluded from these water-logged soils, C_4 species apparently flourish due to their much greater photosynthetic efficiency in the absence of both nutrient and water limitations (Long 1999). Certain Cyperaceae species exist in wetlands both as emergent and submerged aquatics, and when submerged, switch from C_4 to C_3, both in anatomy and carbon assimilation (Ueno 1996).

Somewhat surprising are submerged aquatic species that have fully functional C_4 carbon assimilation (Salvucci and Bowes 1983; Keeley 1998b). Like their terrestrial counterparts, these aquatic macrophytes occupy high light and high temperature environments. They appear to lack Kranz anatomy, but nonetheless exhibit reduced photorespiration. For such a C_4 pump to be effective and avoid a futile cycling of carbon, it is necessary that there be a disequilibrium between the CO_2 pool at the active site of Rubisco and the CO_2 pool in the ambient environment (Monson 1989). This is accomplished by intracellular separation of the two carboxylating enzymes (Reiskind et al. 1997), maintenance of high P_{CO_2} in lacunae, and the high diffusional resistances of water that inhibit CO_2 leakage (Keeley 1998b).

Evidence for the C_4 pathway in unicellular algae has recently been reported by Reinfelder et al. (2000), but serious questions have been raised by Johnston et al. (2001). While this report does show C_4 fixation into malate and turnover into phosphorylated sugars (the sine qua non for the C_4-concentrating mechanism), their extraordinarily long 60-s pulse of ^{14}C prior to the ^{12}C chase makes it hard to interpret how much of the malate is indeed due to primary PEPCase fixation. Secondary labeling of malate is considerable with multicellular tissues exposed to ^{14}C pulses far shorter than 60 s and is likely to be an even more acute problem with unicellular organisms.

Evolution of C_4

C_4 genes are homologous with ancestral C_3 genes; thus, the modifications required to produce the C_4-concentrating mechanism involved changes in gene expression, such that cues turn on and turn off expression, dependent upon location within the leaf (Monson 1999). In short, C_4 photosynthesis has not arisen de novo from the evolution of newly transcribed gene regions but rather from duplication of nonphotosynthetic C_3 genes and modification of the expression of those genes for a photosynthetic function (Westhoff et al. 1997; Monson 2003). This indicates that the biochemical steps involved in C_4 were widespread in flowering plants long before this pathway was fully expressed. Further evidence of this is the demonstration by Hibberd and Quick (2002) of C_4 carbon assimilation from vascular stem cells of two distantly related C_3 species, Nicotiana tabacum (Solanaceae) and Apium graveolens (Apiaceae). Although all carbon gain in these species is through C_3 photosynthesis, the C_4 machinery appears to play a role in recapturing respiratory CO_2. Such metabolic adaptations were possibly the precursor to fully expressed C_4 (Raven 2002), and the diverse mechanisms controlling gene expression support

this and other "piecemeal" pathways of C_4 evolution (Taylor et al. 1997).

An alternative model is that C_4 carbon assimilation was a later stage in the evolution of C_4 photosynthesis, arising only after the origin of enhanced Rubisco activity in bundle sheath cells (Ehleringer and Monson 1993; Monson 1999). This model requires early evolution of a carbon-concentrating mechanism driven by enhanced glycine decarboxylase activity in bundle sheath cells, an enzyme that recaptures CO_2 from products of photorespiration. Such a model depends upon mechanisms for intercellular movement of metabolites, and one model is a glycine-serine shuttle between mesophyll and bundle sheath cells (Leegood and von Caemmerer 1994). It is hypothesized that subsequent steps in the further evolution of C_4 included the thickening of bundle sheath cells to prevent leakage of glycine decarboxylase–generated CO_2 and other anatomical changes in leaf structure, the addition of PEPCase capture of CO_2, and the restriction of Rubisco to bundle sheath cells. One of the strengths of this model is that it avoids the potential futile cycling of CO_2 between carboxylation and decarboxylation if C_4 fixation were to first arise in the same cells as the Rubisco enzyme (Monson 1989). But work on the submerged aquatic Hydrilla is instructive in that apparently C_4 fixation can act as an efficient CO_2 pump even though it operates within the same cell as Rubisco (Bowes and Salvucci 1984; Reiskind et al. 1997). Edwards et al. (2001) have expressed doubt that relocation of the photorespiratory CO_2-evolving apparatus into bundle sheath cells would be an effective precursor to a CO_2-concentrating mechanism. One reason for this skepticism is that the glycine decarboxylase model is fundamentally different from the mechanism for reducing photorespiration in C_4 photosynthesis (Monson 1989). To some it seems doubtful a mechanism that initially concentrates carbon through up-regulation of glycine decarboxylase would evolve into functional C_4, which abolishes that mechanism (C. B. Osmond, personal communication).

Studies of nearly two dozen monocot and dicot species that combine physiological, anatomical, and biochemical traits of C_3 and C_4 have been used to provide support for this model (Monson and Moore 1989; Ku et al. 1991). These C_3-C_4 intermediates all exhibit a high concentration of chloroplasts, mitochondria, and peroxisomes in the bundle sheath cells, and most also exhibit reduced photorespiration, but only some exhibit C_4 carbon assimilation. Flaveria (Asteraceae), which has radiated into open sites on alkaline or gypseous soils in the New World subtropics, is particularly important because it contains C_3, C_4, and C_3-C_4 intermediates. Monson (1996) has mapped these photosynthetic pathways onto a tentative phylogenetic tree, providing insight into the evolutionary pattern in this functional plant trait (fig. 5). It appears that C_4 has arisen independently at least two times, and both were apparently preceded by the C_3-C_4 intermediates. These intermediates are often interpreted as taxa that have not yet succeeded to the stage of fully expressed C_4 (Kellogg 1999; Bläsing et al. 2000). Alternatively, the presence of distinctive features in some C_3-C_4 intermediates not found in either C_3 or C_4 relatives (Leegood and von Caemmerer 1994; Teese 1995), coupled with the apparent evolutionary lability of photosynthetic pathway in Flaveria (Monson 1996), suggests the hypothesis that some of these C_3-C_4 intermediates represent a fully func-
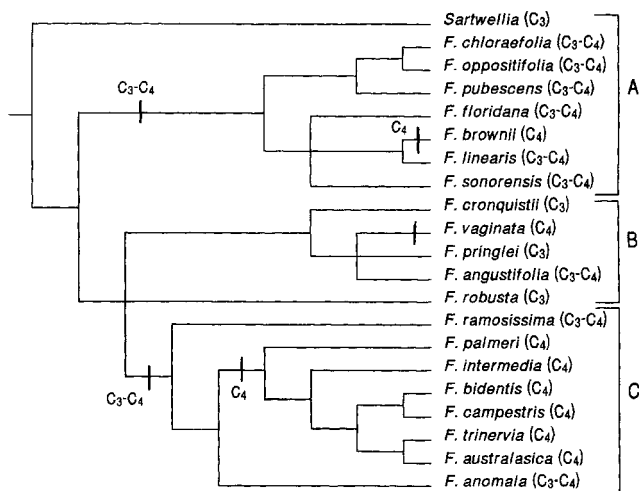
Fig. 5 Phylogenetic tree for *Flaveria* (Asteraceae) based on 15 morphological characters (photosynthetic pathway in parentheses). Path of greatest parsimony indicates that C_4 evolved independently more than once and was preceded by C_3-C_4 intermediates (from Monson 1996; reprinted with permission of the Missouri Botanical Garden).

tional alternative carbon-concentrating mechanism and may not be intermediates to some better future state.

Terrestrial Origins

Models predict that the C_4-concentrating mechanism is unlikely to evolve except under CO_2-limiting conditions (Ehleringer et al. 1991, 1997). In terrestrial environments, atmospheric conditions would not have been conducive to C_4 until late Carboniferous (fig. 4), when models predict the feasibility of the C_4 pathway (Beerling and Woodward 2001). Cerling et al. (1997) also propose that during the late Carboniferous to Permian glaciation some plants may have evolved the C_4 pathway, but they contend that it would have been lost in the Mesozoic when CO_2 levels were once again high (>1000 ppmv). Several lines of evidence have been offered to support the speculation that the C_4 pathway persisted through the Mesozoic. Empirical studies of contemporary species' responses to conservative estimates of hypothetical late Cretaceous atmospheric conditions indicate that despite high CO_2 levels, the elevated O_2 levels (fig. 4) were a potential driver in the evolution and maintenance of C_4 physiology (Gale et al. 2001). While contemporary modeling studies of O_2 versus CO_2 impacts on Rubisco activity may not fully support these speculations, elevated atmospheric oxygen could have had indirect impacts relative to the competition of C_3 versus C_4 (Rachmilevitch et al. 1999). C_4-like Cretaceous $\delta^{13}C$ values from marine carbonates (Kuypers et al. 1999), terrestrial sediments (Wright and Vanstone 1991), dinosaur collagen, and wood fragments (Bocherens et al. 1993) have all been offered as evidence of a C_4 or CAM presence during this period. As with many proxy measures, these have been questioned as to whether or not they are truly indicative of a C_4 origin (Cerling 1999). Another potential period of C_4 evolution would have been the early Tertiary (65 Ma), when CO_2 concentrations dropped to near current levels (Ehleringer et al. 1991). Increased temperatures

and open high light environments following the Cretaceous/Tertiary catastrophic event at 65 Ma (Tschudy et al. 1984; Wolfe 1990) would have further intensified selection for C_4. Despite this window of opportunity for C_4 origins, the subsequent CO_2 increase is thought to have suppressed C_4 throughout much of the Tertiary (Ehleringer et al. 1991).

The dominant C_4 family, the Poaceae, appears to have had a Cretaceous origin in South America or Australia (Bremer 2002), and pollen records place the family back at least to 70 Ma (Linder 1987), although the earliest macrofossils date only to the early Eocene (Jacobs et al. 1999). In North America, the earliest fossils assignable to Poaceae subfamilies are late Eocene–early Oligocene and belong to the Pooideae (Stebbins 1981), an entirely C_3 subfamily of grasses. The earliest fossil evidence of a C_4 grass, based on Kranz anatomy (Thomasson et al. 1986), dates from the mid-Miocene, 12.5 Ma (Jacobs et al. 1999). However, these records cannot rule out an earlier origin of C_4 grasses, particularly because the fossil record is highly biased against recording macrofossils from the types of arid habitats where grass evolution is thought to have begun (Cerling 1999). On the basis of soil carbonate $\delta^{13}C$, the first hint of C_4 is at ca. 15 Ma (Morgan et al. 1994); however, this represents the point where C_4 biomass is sufficient to override the C_3 signal. Considering the range of values indicative of C_3 and C_4 plants, it is feasible that as much as a quarter or more of the biomass could be C_4 and still generate a paleosol $\delta^{13}C$ value within the C_3 range. Molecular clock estimates indicate the origin of one C_4 clade, the Andropogoneae in the Poaceae, dates to at least the late Oligocene, more than 25 million years ago (Kellogg 1999).

Reasonable bounds on the origins of C_4 lineages in the Poaceae are between 15 and 30 million years ago. Since this was long after the breakup of the continents, the global distribution of large, entirely C_4 grass genera, e.g., *Andropogon*, *Chloris*, *Eragrostis*, *Ischaemum*, *Hyparrhenia*, *Muhlenbergia*, *Paspalum*, *Pennisetum*, *Setaria*, and *Sporobolus* (Watson and Dallwitz 1992) indicates dispersal rather than vicariance accounts for the global distribution of many C_4 grass genera. By the late Miocene, C_4 grasses had a global distribution (Cerling 1999).

The marked evolutionary success of C_4 metabolism in two monocot families, the Poaceae and Cyperaceae, is not matched by an equal dominance or adaptive radiation of C_4 metabolism among dicots and there is no consensus on the causes behind this phylogenetic disparity. It appears that constraints imposed by the dicot leaf anatomy lead to lower quantum yields at most temperatures for C_4 dicots relative to C_4 monocots (fig. 6) (but see Sage 2001). Based on this observation, it has been hypothesized that CO_2 levels sufficiently low to favor C_4 in monocots may still be too high to greatly favor C_4 in dicots (Ehleringer et al. 1997). Further, it has been hypothesized that CO_2 levels low enough to favor C_4 dicots were absent prior to the glacial episodes of the Pleistocene when CO_2 dropped below 200 ppmv. Thus, this presumed recency of C_4 in dicots is a possible factor accounting for limited evolutionary radiation of C_4 in the group (Ehleringer et al. 1997). Such a recent origin may be inferred from the patterns of C_3-C_4 intermediates in *Flaveria* (fig. 5), and biogeographic patterns support a Pleistocene radiation for the genus (Powell 1978). However, the phytogeographic patterns of C_4 in Chenopodiaceae suggest
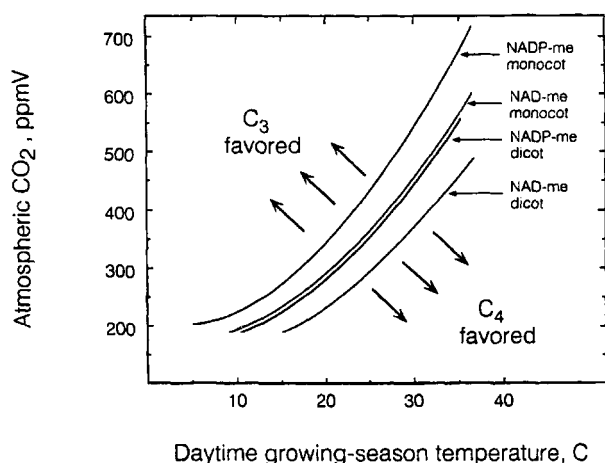
Fig. 6 Modeled crossover temperatures of the quantum yield (defined as the leaf level ratio of photosynthetic carbon gain to photons absorbed) as a function of atmospheric CO_2 concentrations and daytime growing season temperature under saturating light conditions. Boundary conditions vary between monocots and dicots and between different decarboxylase subtypes and are defined as the temperature at which a particular CO_2 results in quantum yields for CO_2 uptake that are equivalent between C_3 and C_4 species (from Ehleringer et al. 1997; reprinted with permission of Springer).

otherwise; C_4 genera such as *Atriplex* are found worldwide in both temperate and tropical saline or alkaline habitats. Also, in this family, in the tribe Salsoleae, C_4 apparently arose just once and radiated in central Asia and then subsequently dispersed to Africa and Europe (Pyankov et al. 2001). In *Atriplex* and other Chenopodiaceae, the origin of C_4 appears to predate the Pleistocene, although there is evidence of more recent radiation in some C_4 lineages (Osmond et al. 1980; Akhani et al. 1997).

Other potential historical constraints on C_4 dicot evolution include that of genetic limitations; specifically, it has been proposed that life history characteristics of dicots have made them less prone to gene duplication and subsequent neofunctionalization that is considered the precursor to C_4 evolution (Monson 2003). This model hypothesizes that large population sizes and rapid turnover of generations are most conducive to the evolution of gene duplication and innovative new gene functions. While this is likely true, there are reasons to question whether or not it adequately accounts for the disparity in radiation of the C_4 pathway between monocots and dicots. To be sure, on a proportionate basis, these life history characteristics achieve greater dominance in monocots than in dicots; however, in terms of absolute numbers, it is not apparent from either tropical or subtropical floras that dicots are inferior to monocots in terms of numbers of annuals and other short-lived herbaceous species. Consider, for example, the eastern Mojave Desert of California and Nevada with ca. 275 annuals, 96% of which are dicots, and C_4 dicots outnumber C_4 monocots 4 to 1 (based on data from Thorne et al. 1981). It is our impression that in most tropical floras the annual life form comprises as many, if not more, dicots as monocots.

We propose an alternative hypothesis tied to different ecological traits associated with monocots and dicots. These two groups have modal growth form distributions that have specialized on different adaptive landscapes, and these vary in their selective value for C_4 photosynthesis. The two prominent C_4 monocot families, Poaceae and Cyperaceae, have attributes that favor them in high light environments, which is a necessary prerequisite for C_4. Both have basal intercalary meristems that give them a distinct advantage when exposed to intense grazing pressure or to the grazers' primary competitor, fire. Intense grazing and browsing or fire are all correlated with a reduction in biomass, and more important, a reduction of plant stature associated with elimination of woody growth forms (Bond and van Wilgen 1996), leading to a relatively permanent and uniform high light environment. Another contributor to tree-free high light environments is predictable severe drought, and basal meristems provide a decided advantage, allowing for more rapid recovery than is the case for dicots. Many Cyperaceae are wetland species where grazing is of lesser importance; however, waterlogged soils are another means of reducing woody plant growth and thus ensuring a high light environment. Dicots comprise a much greater diversity of forms that are structurally more complex than monocots. A substantial proportion of dicots are woody, reflecting competition in more light-limited environments where C_4 would be of less advantage, illustrated by the nearly complete absence of C_4 in trees and vines. Dicots that have specialized on stressful saline or alkaline habitats are ensured a high light environment free of forest shading, and, not surprisingly, halophytic families such as Chenopodiaceae exhibit the greatest radiation of C_4 known for dicots (Winter 1981; Pyankov et al. 2000).

Aquatic Origins

The evolution of aquatic macrophyte C_4 species does not appear to be tied to geological patterns of atmospheric CO_2. *Hydrilla verticillata* switches between C_4 and C_3 dependent on changes in CO_2 in the water, and these are driven by biogenic depletion of carbon in dense mats of vegetation (Salvucci and Bowes 1983). Thus, it is more problematical to temporally constrain the origin of aquatic C_4 by inspection of the atmospheric history.

Different aquatic C_4 species have had very different origins. The presence of C_4 in *Hydrilla* and *Egeria*, which comprise separate lineages of the Hydrocharitaceae, indicate more than one origin of C_4 within the family. All taxa in the family are aquatic, and C_4 is absent from the nearest sister groups (Kellogg 1999), supporting an independent aquatic origin for C_4. However, the presence of C_4 photosynthesis in aquatic foliage of species in the grass tribe Orcuttieae shows a very different origin, one from terrestrial C_4 ancestors (Keeley 1998b). The Orcuttieae comprises three genera and nine species endemic to shallow rain-filled seasonal pools in California. It is one of four tribes in the Chloridoideae, a subfamily of ca. 1400 species, all but one with C_4 photosynthesis; thus, Orcuttieae likely were derived from upland C_4 ancestors, and monophyly of the Orcuttieae is supported by shared synapomorphies not found in any other grass. Cladistic analysis supports a basal position for the monotypic *Neostapfia* and more recent origin for the six *Orcuttia* species (fig. 7). These phylogenetic patterns parallel the relative degree of specialization to the aquatic milieu;
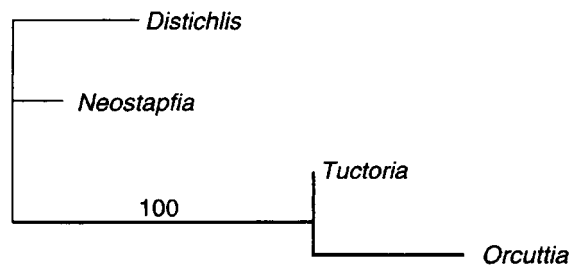
Fig. 7 Phylogenetic tree for aquatic grasses in the Orcuttieae tribe and sister genus *Distichlis*. Separation of *Tuctoria* and *Orcuttia* from *Neostapfia* is supported 100% by bootstrap analysis with 1000 replicates (from Keeley 1998*b*; reprinted with permission of Springer).

on a relative scale, 0.28, 0.40, and 1.00 for *Neostapfia*, *Tuctoria*, and *Orcuttia*. Aquatic foliage of *Neostapfia* (fig. 8*B*, 8*C*) retains full expression of Kranz anatomy, *Tuctoria* (fig. 8*E*, 8*F*) partial, and some *Orcuttia* species (fig. 8*H*, 8*I*) exhibit an apparent loss of Kranz anatomy. However, enzymology and ^{14}C pulse chase data show clear retention of the C_4 carbon assimilation in Orcuttia (fig. 9). These patterns indicate a terrestrial origin for C_4 in the group, partial or complete loss of Kranz anatomy with increasing specialization to the aquatic environment, but retention of the C_4 pathway under aquatic conditions.

Expansion of Terrestrial C_4

From both pollen and macrofossils it appears that extensive grasslands were uncommon until mid to late Miocene (Wolfe 1985; Jacobs et al. 1999). Despite the paucity of a fossil record on the development of C_4 grasslands, there are numerous studies utilizing the proxy signal of $\delta^{13}C$. One of the best records of C_4 grassland expansion is from sediments in a region of northern Pakistan known as the Siwaliks (summarized in Barry et al. 2002). Based on selected paleosols (fossil soils) with carbonate deposits, it has been possible to develop a chronology of changes in stable isotopic content through the mid to late Miocene. With the implicit assumption that CAM plants were not part of this flora, the isotope signatures have been calibrated to reflect the contribution of C_3 and C_4 plant species (Cerling 1991). Late Miocene increases in $\delta^{13}C$ values from soil carbonates have been interpreted as reflective of an increasing importance of C_4 grasses, and after 7 Ma, dominance by C_4 (fig. 10). Despite this apparent dominance of C_4 on carbonates, it is quite likely that C_3 vegetation persisted on many paleosols lacking carbonates necessary for $\delta^{13}C$ determination (Barry et al. 2002). This late Miocene switch appears to have been the result of community type conversion from C_3 woodland and forests to C_4 grassland (Quade et al. 1989).

Isotopic signatures from both mammalian and avian fossils from northern Pakistan also reflect a switch from C_3 to C_4 plants in their diet; however, this began earlier, between 8 and 9 Ma (Morgan et al. 1994; Stern et al. 1994). This difference in apparent "emergence" of C_4 is not surprising since these two techniques have different attributes (Barry et al. 2002). Paleosol data provide a long-term time-averaged $\delta^{13}C$ signal of plant composition on a limited number of soil types. The

fossil teeth enamel isotope data are not a good sampling of plant composition because of selective feeding; however, they may provide a broader picture of plant availability on more soil types.

Late Miocene expansion of C_4 grasslands was a global phenomenon documented for many tropical, subtropical, and even temperate sites, but at slightly different times. In East Africa, $\delta^{13}C$ from fossil tooth enamel indicates the presence of C_4 grasses more than 15 Ma (Morgan et al. 1994), but paleosol carbonates do not reflect the presence of C_4 until 8–9 Ma and dominance not until 3–4 Ma (fig. 11) (Cerling 1992). In South America, based on $\delta^{13}C$ values of paleosols and fossil teeth, C_4 grasses first appear somewhat later than in either Pakistan or East Africa, ca. 7 Ma, and like the latter, did not dominate until 4 Ma (fig. 12) (MacFadden et al. 1996; Latorre et al. 1997). Many paleosols from South America reveal only a very weak C_4 signal in the late Miocene, presumably because Andean uplift resulted in lower temperatures at sites between 1000 and 2000 m, which diminished the photosynthetic advantage of C_4 (MacFadden et al. 1994). In North America, $\delta^{13}C$ of fossil teeth enamel (fig. 12) shows C_4-dominated grasslands beginning between 5 and 6 Ma (Wang et al. 1994).

While C_4 grasslands expanded in many parts of the globe between 4 and 8 Ma, the ecological courses may have followed different successional trajectories. Evidence from the Siwaliks in South Asia is consistent with a model of woodland/forest conversion to grassland during late Miocene. The positive correlation between the $\delta^{13}C$ and $\delta^{18}O$ value of carbonate in many paleosols has been interpreted as evidence of a reduction in forest cover (Cerling et al. 1993), as does the shift in fossil leaf and log deposition (Quade et al. 1995). In addition, the paleoecology of Neogene mammalian faunas supports the idea that the C_4 grassland expansion was at the expense of woodlands (Gunnell et al. 1995), as does the sudden appearance of grassland type soils in the late Miocene (Retallack 1991). In addition, both African and South American C_4 grassland expansions appear to have resulted from the replacement of woodlands and forests (Retallack et al. 1990; Morgan et al. 1994; Latorre et al. 1997).

In apparent contrast, the North America radiation in early Miocene horses with hypsodont teeth designed for grazing indicates that (C_3) grasslands were widespread prior to the C_4 expansion (Simpson 1951; MacFadden and Hulbert 1988; but Prothero 1999). In addition, there are paleosol data (Retallack 1997*b*; Retallack et al. 2002) and soil phytolith evidence (Stromberg 2002) to support this early origin of grasslands. Also, the early development of carnivore locomotor adaptations suggests prey that inhabited open grassland habitats (Van Valkenburgh 1999). However, faunal assemblages argue for a mixture of woodland and grassland in the mid-Miocene plains region of North America. At this time not only were grazers radiating but several different lineages of browsers exhibited species radiations as well (Janis et al. 2000, 2002). Coincident with the expansion of C_4 was the beginning of a sharp decline in equid diversity (Cerling et al. 1998*b*), which would be consistent with a shift from a more complex C_3-dominated woodland/grassland mosaic to a more monotonous C_4 prairie.

In terms of the driving forces behind the late Miocene expansion of C_4 grasslands, three factors need to be considered: (1) climate change, (2) atmospheric changes in CO_2, and (3)
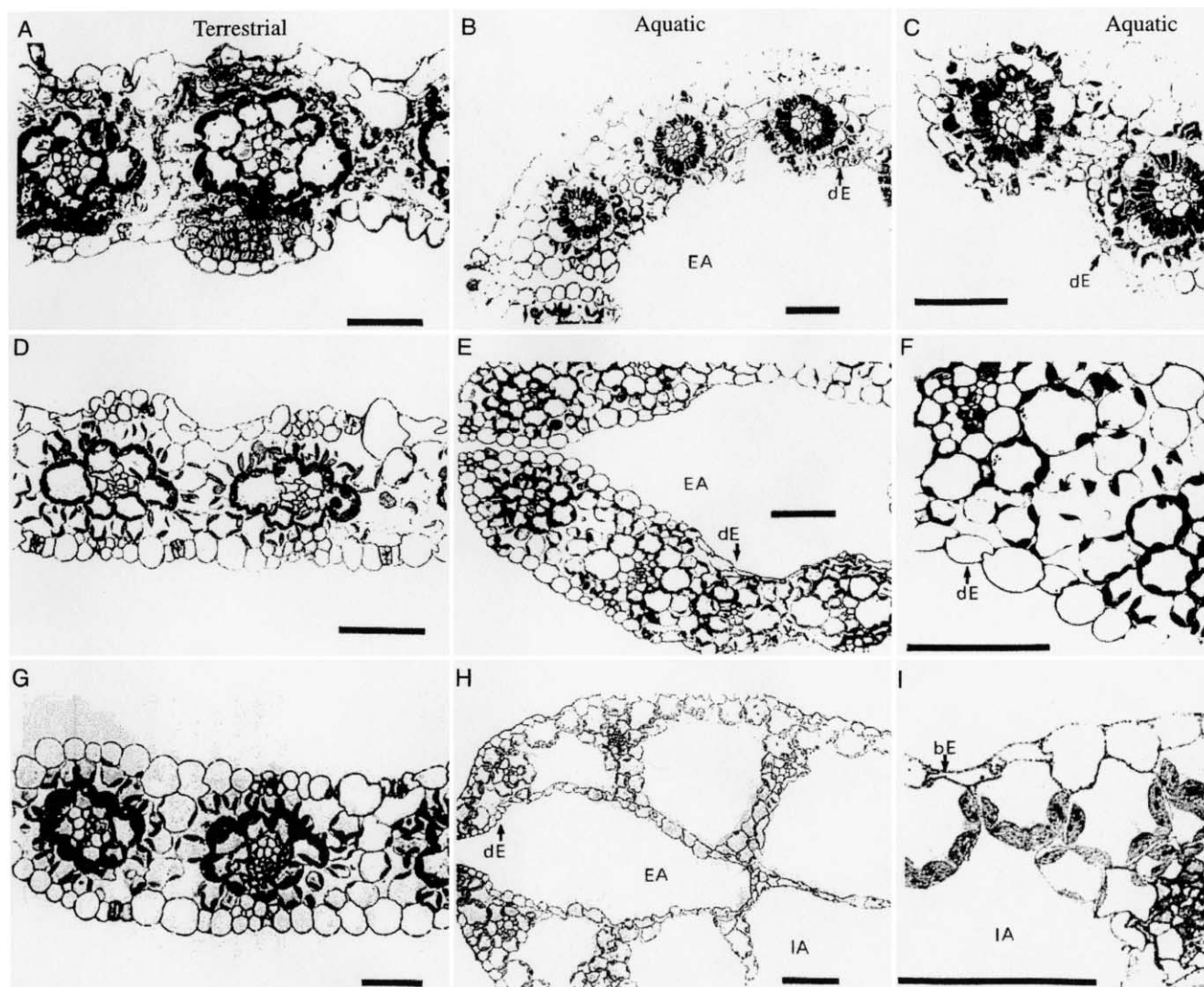
Fig. 8 Transverse sections of terrestrial (*A, D, G*) and aquatic (*B, C, E, F, H, I*) leaves of Orcuttieae taxa (bar = 50 μm, *EA* = extracellular airspace, *IA* = intracellular airspace, *bE* = abaxial epidermis, *dE* = adaxial epidermis). Top row (*A–C*) is *Neostapfia colusana*, middle row (*D–F*) is *Tuctoria greenei*, and bottom row (*G–I*) is *Orcuttia viscida*. Folding of aquatic leaves (*B, E, H*) was due to a greater proliferation of cells on the abaxial than on the adaxial surface. Aquatic leaves differed from terrestrial leaves as follows: in the species least specialized to the aquatic habitat, *N. colusana*, chloroplasts lost grana and were centripetal in more elongate and thinner-walled bundle sheath cells; in the species most specialized to the aquatic habit, *O. viscida*, lacunal airspace comprised 75% of cross-sectional area, Kranz anatomy was lost, chloroplasts were centripetally concentrated in mesophyll cells (similar to the pattern described for a terrestrial C_4, which lacks Kranz anatomy; Voznesenskaya et al. 2002), vascular bundles were greatly reduced, and there was a several-fold increase in the number of mesophyll cells between veins (from Keeley 1998*b*; reprinted with permission of Springer). *Orcuttia* exhibits unusual structural variation as the arrangement of chloroplasts in terrestrial leaves of *O. viscida* is centrifugal (*G*), but it is centripetal in *Orcuttia tenuis* (Sage et al. 1999*a*).

changes in ecological equilibria induced by fire. In all likelihood, each of these factors has played a role, although the relative importance of these is a matter of debate.

Climatic changes. The $\delta^{18}O$ of carbonates in the Siwalik paleosols shift toward more positive values in the late Miocene, and a similar but delayed pattern is evident from East Africa. Changes in these isotope signatures are generally interpreted to indicate changes in climate (Cerling 1992), but we are a long way from understanding their full meaning since paleosol $\delta^{18}O$ values are a function of both temperature and the isotopic signature of meteoric water. To some, these changes in $\delta^{18}O$

are considered to represent greater aridity, and this, coupled with a shift toward increasing summer precipitation in the late Miocene, is thought to be sufficient explanation for the C_4 grassland expansion (Pagani et al. 1999*b*; Huang et al. 2001).

In general, it appears that the late Miocene was characterized by increasing seasonality, both in tropical (Retallack 1991; Pagani et al. 1999*b*) and temperate regions (Utescher et al. 2000). Quade et al. (1989) provided evidence that late Miocene was marked by a strong intensification of the Asian monsoon beginning ca. 11 Ma. Further evidence of Miocene climate changes is in the alterations in oceanic sedimentation rates,
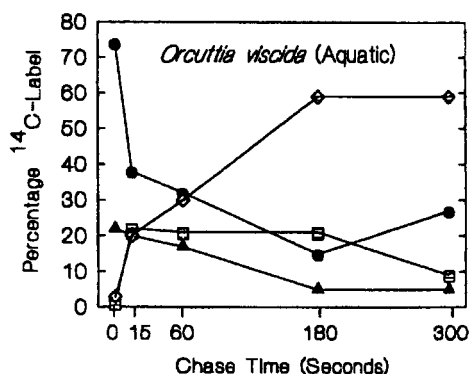
Fig. 9 Pulse chase experiments in the light for aquatic leaves of *Orcuttia viscida*. After a 5-s pulse of ^{14}C, over 95% of label was in C_4 products malate (filled circles) and asparate (filled triangles), and during the ^{14}C-free chase, label was quickly transferred to phosphorylated (open squares) and other (open diamonds) sugars (from Keeley 1998*b*; reprinted with permission of Springer).

which peaked between 5 and 12 Ma (Davies et al. 1977), perhaps reflecting greater continental weathering due to reduced global precipitation. Increased seasonality and associated seasonal droughts have the potential for eliminating woodlands and forests and favoring herbaceous associations.

Paleoatmospheric CO_2. It has been hypothesized that an atmospheric drawdown in CO_2 during the late Miocene was the primary driver causing the rapid expansion of C_4 grasslands (Cerling et al. 1993, 1998*a*), perhaps driven in turn by tectonic activity (Raymo and Ruddiman 1992). It is argued that during the late Miocene, CO_2 levels reached a critical threshold where C_3 species were at a decided competitive disadvantage to C_4 (Cerling et al. 1997; Ehleringer et al. 1997). This conclusion derives from the long-established difference in quantum efficiency (CO_2 fixation per quantum of light) between C_3 and C_4 species and from models of leaf level photosynthetic performance in response to temperature and CO_2. It is predicted that, under high light conditions, C_4 grasses will have a photosynthetic advantage over C_3 grasses at low CO_2 and high temperatures, because of photorespiratory effects (fig. 6). The crossover point corresponding to a shift favoring C_3 versus C_4 is a CO_2/O_2 ratio of less than 0.025 or 500 ppmv CO_2 at 21% O_2 (Ehleringer et al. 1991). Because of the carbon-concentrating ability of C_4 plants, internal CO_2 in the bundle sheath cells is several thousand ppmv, and so photorespiration is virtually absent. In C_3 species, photorespiration increases with temperature, and thus the crossover point favoring C_4 over C_3 grasses is dependent on atmospheric CO_2 and growing season temperature. Collatz et al. (1998) have provided some bounds for these parameters; however, one of the primary limitations in realistic application of the threshold model is the fact that the growing season's midday temperatures may not adequately describe the photosynthetic competitive environment for C_3 and C_4 species. Temperatures vary in ways that could greatly alter the photosynthetic balance not only from month to month but also from hour to hour throughout the day. Field studies conducted between 200 and 500 ppmv CO_2 indicate C_4 responses are more complex than can be encom-

passed in a model that considers only CO_2 and temperature (fig. 6) (Wand et al. 1999; Anderson et al. 2001). Additional ecological information is needed in order to scale from leaves to ecosystems for both past and future environments.

Critical to the threshold model (fig. 6) is the extent of Miocene changes in atmospheric CO_2. Geochemical models and proxy measures using different isotopes and fossil stomata are in general agreement on the broad changes in CO_2 since the Paleozoic (fig. 4). However, Miocene patterns of CO_2, including the period of C_4 grassland expansion in the late Miocene, have been more controversial and do not appear to support the early predictions of a marked decrease in CO_2 correlated with the expansion of C_4 grasslands. Recently, Pearson and Palmer (2000) report very slight changes in CO_2 over the last 20 Ma (between 200 and 300 ppmv) and Pagani et al. (1999*a*, 1999*b*) report a slight rise in CO_2 (fig. 13) through the period of C_4 grassland expansion (from 220 to 320 ppmv). However, such reports have not gone unchallenged, and Cowling (1999*a*) has argued these low CO_2 estimates are unlikely because photosynthetic models predict plant life would not have survived and thrived under such conditions.

More recently, Cerling et al. (1998*a*) have suggested that the rapid expansion of C_4 grasslands need not be accompanied by a large change in atmospheric CO_2; rather, once levels dropped between 400 and 600 ppmv, this range provided sufficient advantage to C_4 species to allow them to dominate. Temperature is a critical part of this equation, and the evidence for cooling during the late Miocene (Zachos et al. 2001) indicates the C_4 threshold would require even lower CO_2 levels (fig. 6). Low temperatures likely account for the lack of C_4 expansion at higher latitudes or higher elevations during the late Miocene (MacFadden et al. 1994; Quade et al. 1994; Franz-Odendaal et al. 2002).

One of the characteristics of the C_3 to C_4 transition that has been held up as evidence for the overriding importance of atmospheric changes in CO_2 is the fact that this transition occurred at the same time on many continents. However, this conclusion is dependent upon the temporal scale of reference since the transitions did occur in different regions over a period of ca. 4 million years. In support of CO_2 changes as the primary driver of global C_4 expansion, it has been suggested that such synchronization could not be climate driven. However, some events, as for example the uplift of the Tibetan plateau, are thought to have had global climatic impacts (Raymo and Ruddiman 1992). Global links in climate change are also apparent from other events during the Pleistocene (Porter and Zhisheng 1995).

Changes in ecological equilibria due to fire. Under a scenario where CO_2 levels are declining during the Miocene, the photosynthetic efficiency model (fig. 6) predicts the late Miocene replacement of C_3 grasslands by C_4 grass species. However, this is a less convincing model if C_4 grasslands owe their origin to type conversion from woodland formations, which seems to have been the case in many parts of the globe. This is not to say that figure 6 does not predict a photosynthetic advantage for C_4 grasses over C_3 trees, but it is an oversimplification for predicting landscape scale vegetation change (Osmond 1987; Field et al. 1992; Henderson et al. 1995). C_3 trees with lower maximum photosynthetic rates are commonly capable of outcompeting C_4 grasses, even at CO_2 levels lower
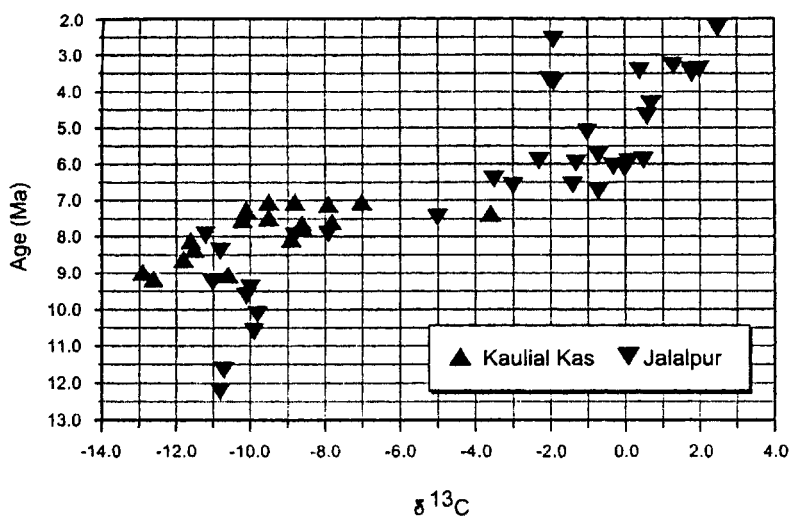
Fig. 10 Stable carbon isotope ratios for paleosol carbonates of different ages from the Siwaliks of northern Pakistan (modified from Quade and Cerling 1995, recalculated and plotted by Barry et al. 2002; reprinted with permission of the Paleontological Society).

than those predicted for the late Miocene. That is because photosynthetic advantage is only one of many factors in the competitive equation, and these must be taken into account in predicting the outcome of competition between different growth forms. The threshold model (fig. 6) is only relevant under high light conditions, and C_3 trees have the capacity to overtop and shade C_4 grasses, thereby reducing light and temperature and thus diminishing the C_4 photosynthetic advantage. Indeed, the fact that C_4 is rare in trees indicates it is not highly relevant in the competition between trees and grasses. This raises the question, What other event took place in the late Miocene to prevent C_3 trees from overtopping the C_4 grasses?

Low CO_2 alone would seem to have limited ability to explain C_4 grassland replacement of woodlands and forests since there is no evidence of a global collapse in tropical C_3 woody diversity during the late Miocene. In general, trees lose out to grasses when seasonally arid conditions produce greater water demand for trees and their seedlings (Goldstein and Sarmiento 1987). Additionally, trees are displaced by grasslands when the ecosystem is challenged with disequilibrial processes such as increased disturbance by fire (Huntley and Walker 1982; Coupland 1993; Goldammer 1993; Knapp and Medina 1999).

We hypothesize a scenario where sharply declining CO_2 levels beginning in the mid-Tertiary spurred the origin and diversification of C_4 grasses. Thus, they were widespread, but of limited importance in tropical and subtropical habitats long before the late Miocene C_4 expansion. By mid-Miocene, a diversity of C_4 grasses adapted to various open woodland microsites were poised for a change in conditions that would reduce tree canopy cover. These woodland/grassland mosaics were probably favored by increasing seasonality but perhaps enhanced by large mammalian herbivores present since early Oligocene (Sage 2001). We suggest that the rapid expansion of C_4 in the late Miocene was due to the greatly increased probability of fires in these increasingly seasonal climates, particularly monsoonal systems dominated by convective storms

with associated lightning. There is widespread agreement that late Miocene climates were becoming more seasonal, meaning that ecosystems were being predictably exposed to a dry season where biomass fuels would be vulnerable to ignition and combustion. In Southeast Asia, increased drying and seasonal character of the late Miocene climate would certainly have made vegetation more prone to fires, and the increasing intensity of the Asian monsoon would have brought a ready source of lightning ignitions at the end of a long dry season, when most aboveground grass biomass is dead (Keeley and Bond 2001). The warm growing season immediately follows the fire season and within months the prefire biomass is restored (Sarmiento 1984), and C_4 metabolism would have had an added benefit under conditions selecting for rapid growth.

Evidence of such a change in fire incidence is the several
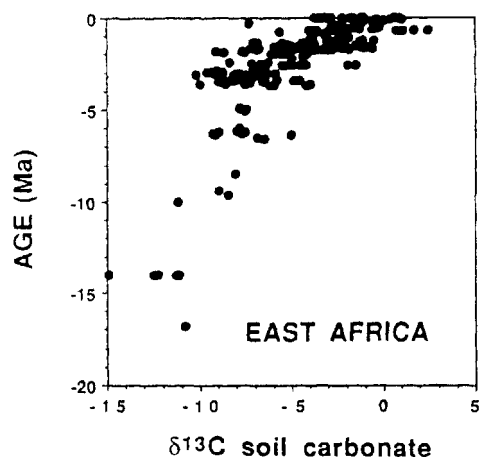
Fig. 11 Stable carbon isotopic composition of paleosol carbonate from East Africa (from Cerling 1992; reprinted with permission of Elsevier Science Publishers).
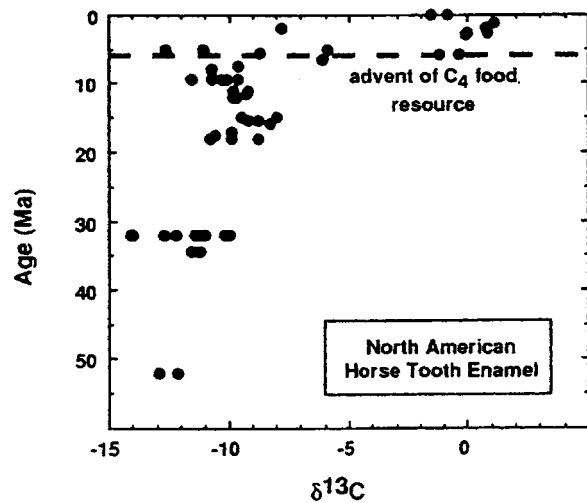
Fig. 12 $\delta^{13}C$ values of horse tooth enamel versus age-showing isotopic shift in the diet of Miocene horses that parallels paleosol shifts from Southeast Asia and East Africa (from Wang et al. 1994; reprinted with permission of Elsevier Science Publishers).

orders of magnitude increase in North Pacific Ocean charcoal deposition, some containing particles similar in morphology to modern grasses (Herring 1985). Those deposition sites in the western Pacific (fig. 14; at similar latitudes and downwind of the Siwaliks likely represent Aeolian deposits) show rapid increases in charcoal that parallel the late Miocene expansion of C_4 grasslands evident from South Asia (fig. 15). Such events may have taken place in many parts of the world. Global synchrony of climate-driven changes in fire regimes is lacking for the late Miocene; however, Quaternary studies show such phenomenon for widely separate regions such as South America and Indonesia (Haberle and Ledru 2001). This model of seasonality driving fire conversion of woodlands to grasslands is even more powerful when accompanied by the "threshold" model (fig. 6). As Bond and Midgley (2000) point out, the fire-driven interaction between savanna trees and C_4 grasslands could have had an important CO_2 component. Tree establishment is often dependent upon rapid regrowth prior to recurring fires, and this would be affected by CO_2 such that lowered Miocene levels could have decreased the regrowth potential of trees during the fire-free interval. Perhaps a time/space compressed demonstration of the Miocene C_4 grassland expansion is the modern fire-driven displacement of Hawaiian C_3 forests by exotic C_4 grasses (D'Antonio and Vitousek 1992). The overriding importance of disturbance is emphasized by the fact that this recent displacement is occurring during a period of increasing global CO_2, a situation opposite of what might be interpreted from the threshold model (fig. 6).

While the late Miocene data coupling climate change and fire disturbance in the $C_3 : C_4$ balance are circumstantial, a more robust demonstration is evident for the Holocene grassland/woodland boundary in North America (Clark et al. 2001) or Mayan forest clearance with fire (Huang et al. 2001). It is certainly clear that contemporary grassland ecosystem dynamics are determined by the interaction between fire, grazing, and climate. For example, tropical savannas in both the Old World

and New World require fire to maintain open C_4 grassland (Huntley and Walker 1982; Sarmiento 1984; Goldammer 1993; Bond et al. 2001). In the southwestern United States, nineteenth-century ponderosa pine savannas with C_4 grasses have been type-converted to dense coniferous forests by the exclusion of fire, and this is reflected in changes in $\delta^{13}C$ of soil organic matter (Kerns et al. 2001).

In summary, climate, CO_2, and disturbance are not mutually exclusive explanations. Probably they all acted in concert during the late Miocene to drive the explosive increase in C_4

Selective Factors Driving Pleistocene Fluctuations of C_4

Periods of Pleistocene glaciation have dominated nearly 90% of the last 1.6 million years (Petit et al. 1999). Glacial episodes not only reduced global temperatures but there was also a substantial drawdown of atmospheric CO_2 from carbon burial and perhaps also a reduction in biomass emissions from greatly reduced wildfire activity (Bird and Cali 1998). Estimates for the last glacial maximum (LGM) at ca. 18,000 ka are 180–200 ppmv CO_2, which increased to 280 ppmv by the late Pleistocene and stabilized at that level until late nineteenth century industrialization drove CO_2 to its current 370 ppmv (Petit et al. 1999). In light of the photosynthetic advantage of C_4 species under low CO_2, it is to be expected that during glacial and interglacial periods, CO_2 changes alone could have resulted in changes in the distribution of C_3 and C_4 species (Ehleringer et al 1997; Cerling et al. 1998a). As discussed below, there are a number of studies that appear to show C_4 dominance on many sites during the LGM, only to be replaced by C_3 vegetation during the Holocene. In many cases, it is hypothesized that increasing CO_2 since the LGM was the primary driver of this change. The apparent anomaly where Pleis-
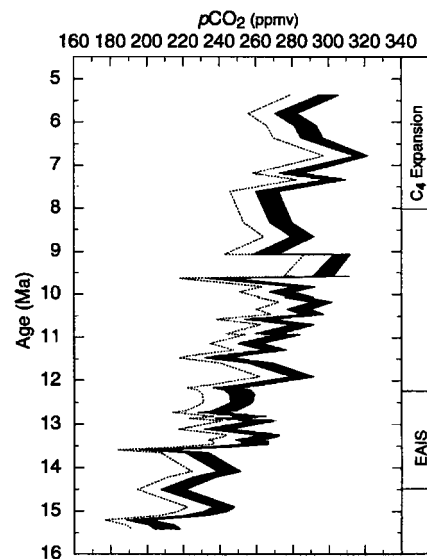

Fig. 13 Maximum $pCO2$ estimates and 95% confidence limits for mid- to late Miocene atmospheres based on carbon isotopic fractionation during marine photosynthetic carbon fixation (from Pagani et al. 1999a; reprinted with permission of American Association for the Advancement of Science).
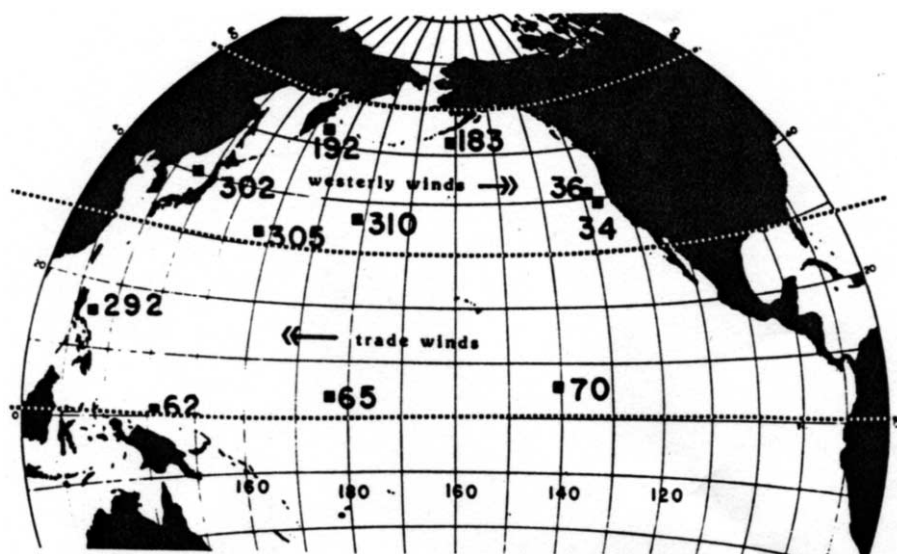
Fig. 14 Deep-sea drilling project site localities from the north Pacific used in Neogene charcoal deposition study. Approximate surface wind zones indicated (from Herring 1985; reprinted with permission of the American Geophysical Union).

tocene C_4 grasses were retreating at a threshold $CO_2 < 280$ ppmv, but Miocene C_4 grasses were advancing at $CO_2 > 400$ ppmv, may be explained by the interaction between temperature and CO_2 (fig. 6). However, temperatures were moving in the opposite direction predicted for these transitions: temperatures were declining in the late Miocene and rising in the late Pleistocene (Zachos et al. 2001).

Evidence of CO_2-driven changes in C_4 dominance was presented by Cole and Monger (1994), who showed a marked decline in $\delta^{13}C$ from soil carbonates in the Chihuahuan Desert of New Mexico between the end of the Pleistocene and early Holocene, 7–9 ka. They interpreted this as a replacement of C_4 grasslands, which they presumed had dominated sites since the LGM, by C_3 shrublands during the Holocene. Since soil carbonate $\delta^{13}C$ is unable to distinguish between C_4 grassland and C_4 *Atriplex* shrublands, LGM vegetation is not entirely resolved. However, Pleistocene packrat midden studies do not generally report *Atriplex* this far east (Van Devender and Spaulding 1979). Regardless, the increased C_4 flora during the LGM was hypothesized to have been the result of low atmospheric CO_2 because the very limited changes in $\delta^{18}O$ over this period were interpreted as showing little change in precipitation regimes. It was concluded that during the Holocene the increasing CO_2 reached a threshold where C_4 grasses were no longer competitive with C_3 shrubs. This explanation, however, is unwarranted since CO_2 levels were close to preindustrial levels long before the early Holocene switch from C_4 to C_3. Also, Boutton et al. (1994) have outlined reasons why climate cannot be ruled out in this C_4 to C_3 transition, and Nordt et al. (1994) have reported opposite shifts, from C_3 to C_4, in plant biomass over the last 15 ka in central Texas.

Other studies have reported Pleistocene to Holocene changes in organic matter $\delta^{13}C$ from sediments in small, shallow tropical lakes (in both Africa and South America) that have been interpreted as reflecting changes in the photosynthetic pathways of terrestrial biomass in the surrounding watersheds

(Street-Perrott et al. 1997; Huang et al. 1999; Boom et al. 2001). These studies found $\delta^{13}C$ values were 8–12 per mil higher at the LGM than were more modern lake sediments, although there were often other (unexplained) peaks during the Holocene. These changes in $\delta^{13}C$ were interpreted as a decline in terrestrial C_4 grasslands since the LGM, and it was hypothesized that they were displaced because higher Holocene atmospheric CO_2 increased the competitive ability of C_3 species (fig. 6).

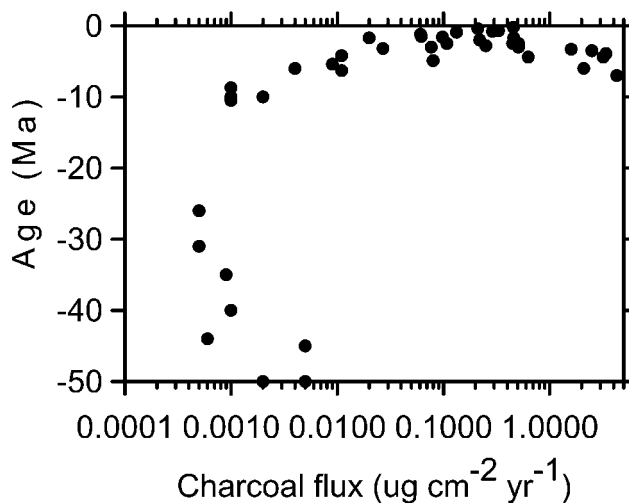Caution is warranted in accepting these studies as evidence

Fig. 15 Estimated charcoal flux measured for the three sites (302, 305, 310) directly downwind from Southeast Asia sites with demonstrated late Miocene grassland expansion (e.g., fig. 5) (redrawn from Herring 1985). That these deposits result from aeolian transport of continental origin is supported by studies of Holocene (Bird and Cali 1998) and recent (Smith et al. 1973) fires.
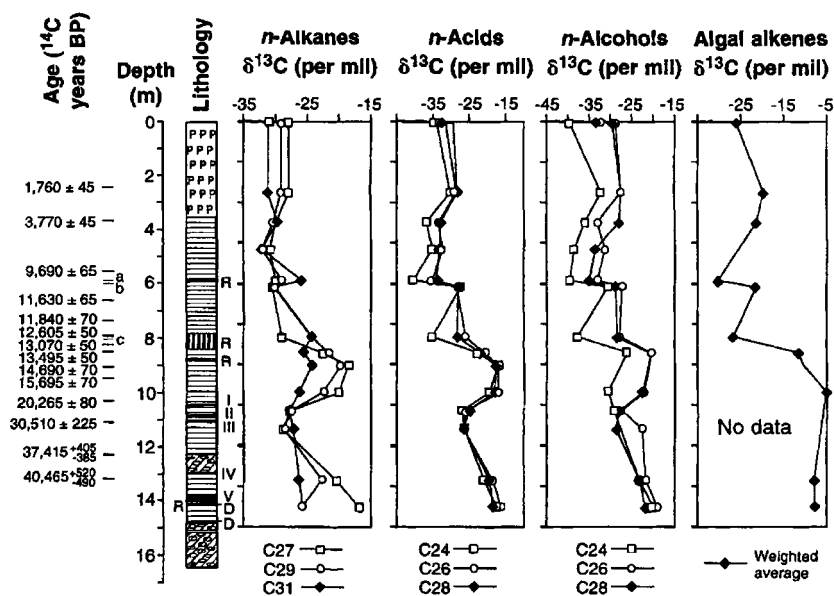
Fig. 16 Carbon isotope stratigraphy for one core from Sacred Lake, Mount Kenya, showing carbon isotope values of putative terrestrial leaves (*n*-alkanes, *n*-acids, *n*-alcohols) and algal alkenes (from Street-Perrott et al. 1997; reprinted with permission of American Association for the Advancement of Science).

of C_4 grassland expansion during glacial periods because assumptions about the source of the lake sediments were never adequately tested. Palynologists often use lake sediments to study terrestrial vegetation changes where identification of pollen microfossils provides clear evidence of their terrestrial origin. There is little evidence that the organic sediments of these small tropical closed basin lakes were derived from terrestrial sources, and plenty of reason to suspect these sediments are not largely allochthonous in nature (Richardson and Richardson 1972; Dieffenbacher-Krall and Halteman 2000). For example, Carpenter's (1981) Lake Wingra study of a small, shallow lake reported that the sediment carbon contribution from net annual production of submerged macrophytes was about three times greater than terrestrial inputs. Small, shallow mid-elevation tropical lakes are often dominated by aquatic CAM species (Keeley 1998a; J. E. Keeley, personal observations). Aquatic macrophytes have $\delta^{13}C$ signals that vary from C_3-like to C_4-like as a function of not just photosynthetic pathway but also HCO_3^- use, basin lithology, diffusional resistances, and physical mixing patterns that influence leaf boundary layers (Osmond et al. 1981; Raven et al. 1987; Keeley and Sandquist 1992). In addition, decreases in the ratio of precipitation/evaporation can markedly increase $\delta^{13}C$ values of the dissolved inorganic carbon substrate for submerged aquatic plant photosynthesis (Talbot and Johannessen 1992).

Certain of these lake studies have attempted to deal with the fact that sediments are a complex mixture of aquatic and terrestrial signals by focusing on $\delta^{13}C$ values from presumed terrestrial biomarkers, most commonly long chain leaf waxes (alkanes $\geq n$-C_{27}). One of the more detailed investigations is that of a small shallow tropical montane lake, Sacred Lake on Mount Kenya (Street-Perrott et al. 1997; Huang et al. 1999, 2001). In these studies, the putative terrestrial biomarkers (*n*-C_{27}–C_{31} alkanes) showed about a 10-mil drop in $\delta^{13}C$ from the

LGM to the present (fig. 16), which was interpreted as demonstrating a decline in terrestrial C_4 grasslands since the LGM. However, these studies failed to demonstrate the validity of these biomarkers, and their assumptions about the origins of these waxes are contradicted by other studies (Amaral et al. 1990; Ficken et al. 2000) that demonstrate both submerged and emergent aquatic macrophytes producing these same waxes (fig. 17). Thus, in light of the likelihood that the bulk of organic matter deposited in these shallow lakes is from the aquatic flora, it is probable that the changes observed in C_{27}, C_{29}, C_{31} alkane $\delta^{13}C$ were largely from both submerged and emergent aquatic macrophytes. Consistent with this interpretation is the observation that the temporal changes in $\delta^{13}C$ of these waxes closely parallel the total organic matter in these sediments. Without a clearer understanding of the origin of the lake sediments, these studies cannot be used as evidence of terrestrial C_4 grassland expansion during the LGM.

A possible clue to the interpretation of these changes in $\delta^{13}C$ is a comparison of lake sediments in two Mesoamerica sites that experienced contrasting moisture regimes since the LGM (Huang et al. 2001). One lake that dried down ca. 10 ka showed an increase in $\delta^{13}C$, and a lake that remained filled showed a decrease in $\delta^{13}C$ values. These patterns were interpreted as evidence that regional climate was far more important than atmospheric CO_2 levels in determining the success of C_3 and C_4 species. However, these results have relevance to all of the lake sediment studies, particularly those from small shallow lakes. They raise the possibility that the glacial to interglacial patterns in $\delta^{13}C$ may be driven by lake basins altering between terrestrial and aquatic conditions. Indeed, the littoral zone surrounding many tropical and subtropical lakes are dominated by C_4 sedges (Cyperaceae; Haines and Lye 1983) and C_4 grasses. The Kenya grass flora (Ibrahim and Kabuye 1988) lists over 20 C_4 species restricted to such wet-
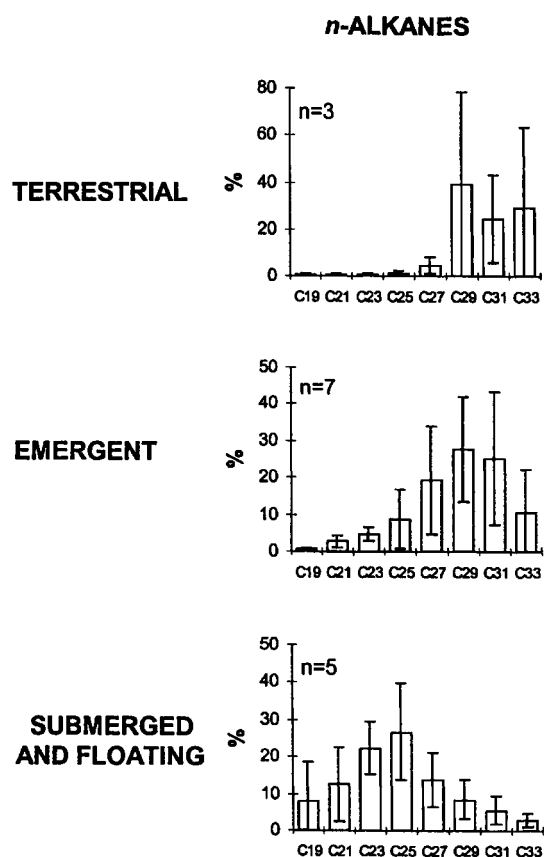
Fig. 17 Size distribution of the *n*-alkyl lipids for terrestrial and aquatic (emergent and submerged plus floating) species (modified from Ficken et al. 2000; reprinted with permission of Elsevier Science Publishers).

lands. Altered lake levels before and after the LGM could have altered the biomass contribution of these emergent aquatic C_4 grasses and sedges (Jones 1987; Hillaire-Marcel et al. 1989). Relevant to the Mount Kenya Sacred Lake studies discussed above is the fact that Mount Kenya was 25% drier at the LGM (Hostetler and Clark 2000), and lake levels throughout East Africa were greatly reduced (Beuning et al. 1997), which could have expanded the littoral zone. Consistent with this scenario is the marked drop in C : N ratios (indicative of declining emergent or terrestrial biomass) that parallels the decline in $\delta^{13}C$ during the Pleistocene–Holocene transition (Street-Perrott et al. 1997).

Alternative signals for Pleistocene to Holocene changes in terrestrial C_4 vegetation may hold promise. Ficken et al. (2002) reported a peak in the ratio of $C_4 : C_3$ grass cuticle deposition from a lake on Mount Kenya during the LGM and suggested that this demonstrated an increase in C_4 grassland. However, inconsistent with that interpretation is the demonstration that this was also the period of the lowest deposition of grass cuticle. In addition, $\delta^{13}C$ from lake sediment organic matter failed to demonstrate any clear evidence of a C_4 signal nor did changes in isotope levels link up well with peaks in C_4 grass cuticle deposition.

The one lake sediment study that almost certainly avoided aquatic macrophyte contamination of the $\delta^{13}C$ values was by Clark et al. (2001), who examined $\delta^{13}C$ of charcoal particles. Their study included only the Holocene, during a period of relatively constant atmospheric CO_2 and provided evidence of aridity driving changes in C_4 grass distribution in the northern Great Plains of North America. Similar conclusions have been reached using $\delta^{13}C$ of paleosols in this region (Kelly et al. 1993). Likewise, longer-term studies of C_4 abundance in Australian Pleistocene deposits shows no obvious correlation with CO_2 but a strong relationship to climatic patterns, in particular the strength of the Australian monsoon (Johnson et al 1999).

To summarize the Pleistocene patterns, glacial episodes have dominated much of the past 1.6 million years and were associated with lower temperatures and lower CO_2. These periods would have dropped the vertical coordinate on the threshold model (fig. 6) in a direction favoring C_4 and moved the horizontal coordinate to the left, in a direction favoring C_3. The net effect would have been site specific and varied with other parameters such as total and seasonal precipitation patterns, soil nutrients, etc. One lesson to be drawn from modeling studies of photosynthetic response is that during glacial periods, the lower CO_2 may very well have translated into lower aridity tolerance for C_3 species than what is presently observed (Cowling 1999*b*). The impact of changes in stomatal density and growth rates on this effect are not clear; however, there is some empirical evidence for it (Polley et al. 2002). As a consequence, plant distributions during glacial episodes might not be directly interpretable in terms of contemporary climates (Cowling and Sykes 1999; but cf. Williams et al. 2000).

Acknowledgments

Literature Cited

Akhani H, P Trimborn, H Ziegler 1997 Photosynthetic pathways in Chenopodiaceae from Africa, Asia and Europe with their ecological, phytogeographical and taxonomical importance. Plant Syst Evol 206:187–221.

Altesor A, E Ezcurra, C Silva 1992 Changes in the photosynthetic metabolism during the early ontogeny of four cactus species. Acta Oecol 13:777–785.

Amaral MCE, AJR DaSilva, A Salatino 1990 Alkanes of surface waxes from eight species of aquatic angiosperms. Aquat Bot 36:281–286.

Anderson LJ, H Maherali, HB Johnson, HW Polley, RB Jackson 2001 Gas exchange and photosynthetic acclimation over subambient to elevated CO_2 in a C_3-C_4 grassland. Global Change Biol 7:693–707.

Barry JC, ME Morgan, LJ Flynn, D Pilbeam, AK Behrensmeyer, SM Raza, IA Khan, C Badgley, J Hicks, J Kelley 2002 Faunal and environmental change in the late Miocene Siwaliks of northern Pakistan. Paleobiol Mem 28:1–55.

Beerling DJ, FI Woodward 2001 Vegetation and the terrestrial carbon cycle: modelling the first 400 million years. Cambridge University Press, Cambridge. 405 pp.

Beerling DJ, FI Woodward, MR Lomas, MA Wills, WP Quick, PJ Valdes 1998 The influence of Carboniferous palaeo-atmospheres on plant function: an experimental and modelling assessment. Phil Trans R Soc Lond B Biol Sci 353:131–140.

Benzing DH 1990 Vascular epiphytes: general biology and related biota. Cambridge University Press, Cambridge. 354 pp.

Berner RA 1998 The carbon cycle and CO_2 over Phanerozoic time: the role of land plants. Philos Trans R Soc Lond B Biol Sci 353:75–82.

Beuning KRM, MR Talbot, K Kelts 1997 A revised 30,000-year paleoclimatic and paleohydrologic history of Lake Albert, East Africa. Palaeogeogr Palaeoclimatol Palaeoecol 136:259–279.

Bird MI, JA Cali 1998 A million-year record of fire in sub-Saharan Africa. Nature 394:767–769.

Bläsing OE, P Westhoff, P Svensson 2000 Evolution of C_4 phospho-enolpyruvate carboxylase in *Flaveria*, a conserved serine residue in the carboxyl-terminal part of the enzyme is a major determinant for C_4-specific characteristics. J Biol Chem 275:27917–27923.

Bocherens H, EM Friis, A Mariotti, KR Pedersen 1993 Carbon isotopic abundances in Mesozoic and Cenozoic fossil plants: palaeo-ecological implications. Lethaia 26:347–358.

Bond WJ, GF Midgley 2000 A proposed CO_2-controlled mechanism of woody plant invasion in grasslands and savannas. Global Change Biol 6:865–869.

Bond WJ, K-A Smythe, DA Balfour 2001 *Acacia* species turnover in space and time in an African savanna. J Biogeogr 28:117–128.

Bond WJ, B van Wilgen 1996 Plants and fire. Chapman, New York.

Boom A, G Mora, AM Cleef, H Hooghiemstra 2001 High altitude C_4 grasslands in the northern Andes: relicts from glacial conditions? Rev Palaeobot Palynol 115:147–160.

Boutton TW, SR Archer, LC Nordt 1994 Climate, CO_2 and plant abundance. Nature 372:625–626.

Boutton TW, AT Harrison, BN Smith 1980 Distribution of biomass of species differing in photosynthetic pathway along an altitudinal transect in southeastern Wyoming grassland. Oecologia 45:287–298.

Bowes G, ME Salvucci 1984 Hydrilla: inducible C_4-type photosynthesis without Kranz anatomy. Pages 829–832 *in* C Sybesma, ed. Advances in photosynthesis research. Vol 3. Junk, The Hague.

Bremer K 2002 Gondwanan evolution of the grass alliance of families (Poales). Evolution 56:1374–1387.

Cabido M, N Ateca, ME Astegiano, AM Anton 1997 Distribution of C_3 and C_4 grasses along an altitudinal gradient in central Argentina. J Biogeogr 24:197–204.

Caldwell MM, RS White, RT Moore, LB Camp 1977 Carbon balance, productivity, and water use of cold-winter desert shrub communities dominated by C_3 and C_4 species. Oecologia 29:275–300.

Carpenter SR 1981 Submersed vegetation: an internal factor in lake ecosystem succession. Am Nat 118:372–383.

Cerling TE 1991 Carbon dioxide in the atmosphere: evidence from Cenozoic and Mesozoic paleosols. Am J Sci 291:377–400.

——— 1992 Development of grasslands and savannas in East Africa during the Neogene. Palaeogeogr Palaeoclimatol Palaeoecol 97:241–247.

——— 1999 Paleorecords of C_4 plants and ecosystems. Pages 445–469 *in* RF Sage, RK Monson, eds. C_4 plant biology. Academic Press, San Diego, Calif.

Cerling TE, JR Ehleringer, JM Harris 1998a Carbon dioxide starvation, the development of C_4 ecosystems, and mammalian evolution. Philos Trans R Soc Lond B Biol Sci 353:159–171.

Cerling TE, JM Harris, BJ McFadden 1998b Carbon isotopes, diets of North American equids, and the evolution of North American C_4 grasslands. Pages 363–379 *in* H Griffiths, ed. Stable isotopes. Bios Scientific, Oxford.

Cerling TE, JM Harris, BJ MacFadden, MG Leakey, J Quade, V Ei-senmann, JR Ehleringer 1997 Global vegetation change through the Miocene/Pliocene boundary. Nature 389:153–158.

Cerling TE, Y Wang, J Quade 1993 Expansion of C_4 ecosystems as an indicator of global ecological change in the late Miocene. Nature 361:344–345.

Chollet R, J Vidal, MH O'Leary 1996 Phosphoenolpyruvate carboxylase: a ubiquitous, highly regulated enzyme in plants. Annu Rev Plant Physiol Plant Mol Biol 47:273–298.

Clark JS, EC Grimm, J Lynch, PG Mueller 2001 Effects of Holocene climate change on the C_4 grassland/woodland boundary in the northern plains, USA. Ecology 82:620–636.

Cockburn W 1981 The evolutionary relationship between stomatal mechanism, crassulacean acid metabolism and C_4 photosynthesis. Plant Cell Environ 4:417–418.

Cole DR, HC Monger 1994 Influence of atmospheric CO_2 on the decline of C_4 plants during the last deglaciation. Nature 368:533–536.

Cole JJ, NF Caraco, GW Kling, TK Kratz 1994 Carbon dioxide supersaturation in the surface waters of lakes. Science 265:1568–1570.

Collatz GJ, JA Berry, JS Clark 1998 Effects of climate and atmospheric CO_2 partial pressure on the global distribution of C_4 grasses: present, past, and future. Oecologia 114:441–454.

Coupland RT 1993 Natural grasslands. Ecosystems of the world 8A, 8B. Elsevier, New York.

Cowling RM, KJ Esler, PW Rundel 1999 Namaqualand, South Africa: an overview of a unique winter-rainfall desert ecosystem. Plant Ecol 142:3–21.

Cowling SA 1999a Plants and temperature: CO_2 uncoupling. Science 285:1500–1501.

——— 1999b Simulated effects of low atmospheric CO_2 on structure and composition of North American vegetation at the last glacial maximum. Global Ecol Biogeogr 8:81–93.

Cowling SA, MT Sykes 1999 Physiological significance of low atmospheric CO_2 for plant-climate interactions. Quat Res 52:237–242.

Cushman JC 2001 Crassulacean acid metabolism: a plastic photosynthetic adaptation to arid environments. Plant Physiol 127:1439–1448.

D'Antonio CM, PM Vitousek 1992 Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annu Rev Ecol Syst 23:63–87.

Davies TA, WW Hay, JR Southam, TR Worsley 1977 Estimates of Cenozoic oceanic sedimentation rates. Science 197:53–55.

Dieffenbacher-Krall AC, WA Halteman 2000 The relationship of modern plant remains to water depth in alkaline lakes in New England, USA. J Paleolimnol 24:213–229.

Drennan PE, PS Nobel 2000 Responses of CAM species to increasing atmospheric CO_2 concentrations. Plant Cell Environ 23:767–781.

Earnshaw MJ, K Winter, H Ziegler, W Stichler, NEG Cruttwell, K Kerenga, PJ Cribb, et al 1987 Altitudinal changes in the incidence of crassulacean acid metabolism in vascular epiphytes and related life forms in Papua New Guinea. Oecologia 73:566–572.

Edwards G, D Walker 1983 C_3, C_4: mechanisms, and cellular and environmental regulation, of photosynthesis. University of California Press, Los Angeles.

Edwards GE, Z Dai, SH Cheng, MSB Ku 1996 Factors affecting the induction of crassulacean acid metabolism in *Mesembryanthemum crystallinum*. Pages 119–134 *in* K Winter, JAC Smith, eds. Crassulacean acid metabolism: biochemistry, ecophysiology and evolution. Springer, New York.

Edwards GE, RT Furbank, MD Hatch, CB Osmond 2001 What does it take to be C_4? lessons from the evolution of C_4 photosynthesis. Plant Physiol 125:46–49.

Ehleringer JR, TE Cerling, BR Helliker 1997 C_4 photosynthesis, atmospheric CO_2, and climate. Oecologia 112:285–299.

Ehleringer JR, RK Monson 1993 Evolutionary and ecological aspects

of photosynthetic pathway variation. Annu Rev Ecol Syst 24: 411–439.

Ehleringer JR, PW Rundel 1989 Stable isotopes: history, units and instrumentation. Pages 1–16 in PW Rundel, JR Ehleringer, KA Nagy, eds. Stable isotopes in ecological research. Springer, New York.

Ehleringer JR, RF Sage, LB Flanagan, RW Pearcy 1991 Climate change and the evolution of C_4 photosynthesis. Trends Ecol Evol 6: 95–99.

Ficken KJ, B Li, DL Swain, G Eglinton 2000 An n-alkane proxy for the sedimentary input of submerged floating freshwater aquatic macrophytes. Org Geochem 31:745–749.

Ficken KJ, MJ Wooller, DL Swain, FA Street-Perrott, G Eglinton 2002 Reconstruction of a subalpine grass-dominated ecosystem, Lake Rutundu, Mount Kenya: a novel multi-proxy approach. Palaeogeogr Palaeoclimatol Palaeoecol 177:137–149.

Field CB, IFS Chapin, PA Matson, HA Mooney 1992 Responses of terrestrial ecosystems to the changing atmosphere: a resource based approach. Annu Rev Ecol Syst 23:201–235.

Franz-Odendaal TA, JA Lee-Thorp, A Chinsamy 2002 New evidence for the lack of C_4 grassland expansions during the early Pliocene at Langebaanweg, South Africa. Paleobiology 28:378–388.

Gale J, S Rachmilevitch, J Reuveni, M Volokita 2001 The high oxygen atmosphere toward the end-Cretaceous; a possible contributing factor to the KT boundary extinctions and to the emergence of C_4 species. J Exp Bot 52:801–809.

Gentry AH, CH Dodson 1987 Diversity and evolution of Neotropical vascular epiphytes. Ann Mo Bot Gard 74:205–233.

Goldammer JG 1993 Historical biogeography of fire: tropical and subtropical. Pages 297–314 in PJ Crutzen, JG Goldammer, eds. The ecological, atmospheric, and climatic importance of vegetation fire. Wiley, New York.

Goldstein G, G Sarmiento 1987 Water relations of trees and grasses and their consequences for the structure of savanna vegetation. Pages 13–38 in BH Walker, ed. Determinations of tropical savannas. IRL, Oxford.

Griffiths H 1989 Carbon dioxide concentrating mechanisms and the evolution of CAM in vascular epiphytes. Pages 42–86 in U Lüttge, ed. Vascular plants as epiphytes: evolution and ecophysiology. Springer, New York.

Gunnell GF, ME Morgan, MC Maas, PD Gingerich 1995 Comparative paleoecology of Paleogene and Neogene mammalian faunas: trophic structure and composition. Palaeogeogr Palaeoclimatol Palaeoecol 115:265–286.

Haberle SG, M-P Ledru 2001 Correlations among charcoal records of fires from the past 16,000 years in Indonesia, Papua New Guinea, and Central and South America. Quat Res 55:97–104.

Haines RW, KA Lye 1983 The sedges and rushes of East Africa. East African Natural History Society, Nairobi.

Henderson S, P Hattersley, S von Caemmerer, CB Osmond 1995 Are C_4 pathway plants threatened by global climatic change? Pages 529–549 in E-D Schulze, MM Caldwell, eds. Ecophysiology of photosynthesis. Springer, New York.

Herring JR 1985 Charcoal fluxes into sediments of the North Pacific Ocean: the Cenozoic record of burning. Pages 419–442 in ET Sundquist, WS Broecker, eds. The carbon cycle and atmospheric CO_2: natural variations Archean to present. American Geophysical Union, Washington, D.C.

Hesla BI, LL Tieszen, SK Imbamba 1982 A systematic survey of C_3 and C_4 photosynthesis in the Cyperaceae of Kenya, East Africa. Photosynthetica 16:196–205.

Hibberd JM, WP Quick 2002 Characteristics of C_4 photosynthesis in stems and petioles of C_3 flowering plants. Nature 415:451–454.

Hickey RJ 1986 The early evolutionary and morphological diversity of Isoëtes, with descriptions of two new Neotropical species. Syst Bot 11:309–321.

Hillaire-Marcel C, A-M Aucour, R Bonnefille, G Riollet, A Vincens,

D Williamson 1989 ^{13}C/palynological evidence of differential residence times of organic carbon prior to its sedimentation in East African Rift lakes and peat bogs. Quat Sci Rev 8:207–212.

Hostetler SW, PU Clark 2000 Tropical climate at the last glacial maximum inferred from glacier mass-balance modeling. Science 290: 1747–1750.

Huang Y, FA Street-Perrott, SE Metcalfe, M Brenner, M Moreland, KH Freeman 2001 Climate change as the dominant control on glacial-interglacial variations in C_3 and C_4 plant abundance. Science 293:1647–1651.

Huang Y, FA Street-Perrott, RA Perrott, P Metzger, G Eglinton 1999 Glacial-interglacial environmental changes inferred from molecular and compound-specific δ^{13}C analyses of sediments from Sacred Lake, Mt. Kenya. Geochim Cosmochim Acta 63:1383–1404.

Huntley BJ, BH Walker 1982 Ecology of tropical savannas. Springer, New York.

Ibrahim KM, CHS Kabuye 1988 An illustrated manual of Kenya grasses. UNFAO, Rome.

Jacobs BF, JD Kingston, LL Jacobs 1999 The origin of grass-dominated ecosystems. Ann Mo Bot Gard 86:590–643.

Janis CM, J Damuth, JM Theodor 2000 Miocene ungulates and terrestrial primary productivity: where have all the browsers gone? Proc Natl Acad Sci USA 97:7899–7904.

——— 2002 The origins and evolution of the North American grassland biome: the story from the hoofed mammals. Palaeogeogr Palaeoclimatol Palaeoecol 177:183–198.

Johnson BJ, GH Miller, ML Fogel, JW Magee, MK Gagan, AR Chivas 1999 65,000 years of vegetation change in central Australia and the Australian summer monsoon. Science 284:1150–1152.

Johnston AM, JA Raven, J Beardall, RC Leegood 2001 Photosynthesis in a marine diatom. Nature 412:40–41.

Jones MB 1987 The photosynthetic characteristics of papyrus in a tropical swamp. Oecologia 71:355–359.

Keeley JE 1981 Isoetes howellii: a submerged aquatic CAM plant? Am J Bot 68:420–424.

——— 1996 Aquatic CAM photosynthesis. Pages 281–295 in K Winter, JAC Smith, eds. Crassulacean acid metabolism: biochemistry, ecophysiology and evolution. Springer, New York.

——— 1998a CAM photosynthesis in submerged aquatic plants. Bot Rev 64:121–175.

——— 1998b C_4 photosynthetic modifications in the evolutionary transition from land to water in aquatic grasses. Oecologia 116: 85–97.

Keeley JE, WJ Bond 2001 On incorporating fire into our thinking about natural ecosystems: a response to Saha and Howe. Am Nat 158:664–670.

Keeley JE, G Busch 1984 Carbon assimilation characteristics of the aquatic CAM plant Isoetes howellii. Plant Physiol 76:525–530.

Keeley JE, DA DeMason, R Gonzalez, KR Markham 1994 Sediment-based carbon nutrition in tropical alpine Isoetes. Pages 167–194 in PW Rundel, AP Smith, FC Meinzer, eds. Tropical alpine environments: plant form and function. Cambridge University Press, Cambridge.

Keeley JE, SC Keeley 1989 CAM in high elevation tropical alpine cacti. Plant Cell Environ 12:331–336.

Keeley JE, CB Osmond, JA Raven 1984. Stylites, a vascular land plant without stomata absorbs CO_2 via its roots. Nature 310:694–695.

Keeley JE, DR Sandquist 1992 Carbon: freshwater plants: commissioned review. Plant Cell Environ 15:1021–1035.

Kellogg EA 1999 Phylogenetic aspects of the evolution of C_4 photosynthesis. Pages 411–444 in RF Sage, RK Monson, eds. C_4 plant biology. Academic Press, San Diego, Calif.

Kelly EF, C Yonker, B Marino 1993 Stable carbon isotope composition of paleosols: an application to Holocene. Pages 233–239 in PK Swart, KC Lohmann, J McKenzie, S Savin, eds. Climate change in

continental isotopic records. Geophysical monograph 78. American Geophysical Union, Washington, D.C.

Kerns BK, MM Moore, SC Hart 2001 Estimating forest-grassland dynamics using soil phytolith assemblages and $\delta^{13}C$ of soil organic matter. Ecoscience 8:478–488.

Knapp AK, E Medina 1999 Success of C_4 photosynthesis in the field: lessons from communities dominated by C_4 plants. Pages 251–283 in RF Sage, RK Monson, eds. C_4 plant biology. Academic Press, San Diego, Calif.

Ku MSB, J Wu, Z Dai, RA Scott, C Chu, GE Edwards 1991 Photosynthetic and photorespiratory characteristics of Flaveria species. Plant Physiol 96:518–528.

Kuypers MMM, RD Pancost, JSS Damste 1999 A large and abrupt fall in atmospheric CO_2 concentration during Cretaceous times. Nature 399:342–345.

Lara MV, P Casati, CS Andreo 2002 CO_2-concentrating mechanisms in Egeria densa, a submersed aquatic plant. Physiol Plant 115: 487–495.

Latorre C, J Quade, WC McIntosh 1997 The expansion of C_4 grasses and global change in the late Miocene: stable isotope evidence from the Americas. Earth Planet Sci Lett 146:83–96.

Leegood RC, S von Caemmerer 1994 Regulation of photosynthetic carbon assimilation in leaves of C_3-C_4 intermediate species of Moricandia and Flaveria. Planta 192:232–238.

Li M-R, DA Wedin, LL Tieszen 1999 C_3 and C_4 photosynthesis in Cyperus (Cyperaceae) in temperate eastern North America. Can J Bot 77:209–218.

Linder HP 1987 The evolutionary history of the Poales/Restionales: a hypothesis. Kew Bull 42:297–318.

Long SP 1999 Environmental responses. Pages 215–249 in RF Sage, RK Monson, eds. C_4 plant biology. Academic Press, San Diego, Calif.

Looy C, JHA van Konijnenburg-van Cittert, H Visscher 2000 On the ecological success of isoetalean lycopsids after the end-Permian ecologic crisis. Pages 63–70 in C Looy, ed. The Permian-Triassic biotic crisis: collapse and recovery of terrestrial ecosystems. LPP Foundation, Utrecht.

Lüttge U 1989 Vascular plants as epiphytes: evolution and ecophysiology. Springer, Berlin. 270 pp.

——— 2002 CO_2-concentrating: consequences in crassulacean acid metabolism. J Exp Bot 53:2131–2142.

MacFadden BJ, TE Cerling, J Prado 1996 Cenozoic terrestrial ecosystem evolution in Argentina: evidence from carbon isotopes of fossil mammal teeth. Palaios 11:319–327.

MacFadden BJ, JRC Hulbert 1988 Explosive speciation at the base of the adaptive radiation of Miocene grazing horses. Nature 336: 466–468.

MacFadden BJ, Y Wang, TE Cerling, F Anaya 1994 South American fossil mammals and carbon isotopes: a 25 million-year sequence from the Bolivian Andes. Palaeogeogr Palaeoclimatol Palaeoecol 107:257–268.

Madsen TV, B Olesen, J Bagger 2002 Carbon acquisition and carbon dynamics by aquatic isoetids. Aquat Bot 73:351–371.

Martin CE 1994 Physiological ecology of the Bromeliaceae. Bot Rev 60:1–82.

McElwain JC 1998 Do fossil plants signal palaeoatmospheric CO_2 concentration in the geological past? Philos Trans R Soc Lond B Biol Sci 353:83–96.

Monson RK 1989 On the evolutionary pathways resulting in C_4 photosynthesis and crassulacean acid metabolism (CAM). Adv Ecol Res 19:57–110.

——— 1996 The use of phylogenetic perspective in comparative plant physiology and developmental biology. Ann Mo Bot Gard 83: 3–16.

——— 1999 The origins of C_4 genes and evolutionary pattern in the C_4 metabolic phenotype. Pages 377–410 in RF Sage, RK Monson, eds. C_4 plant biology. Academic Press, San Diego, Calif.

——— 2003 Gene duplication, neofunctionalization, and the evolution of C_4 photosynthesis. Int J Plant Sci 164(suppl):S43–S54.

Monson RK, BD Moore 1989 On the significance of C_3-C_4 intermediate photosynthesis to the evolution of C_4 photosynthesis. Plant Cell Environ 12:689–699.

Mooney H, JH Troughton, JA Berry 1974 Arid climates and photosynthetic systems. Carnegie Inst Wash Year Book 73:793–805.

Moore PD 1983 Plants and the palaeoatmosphere. J Geol Soc (Lond) 140:13–25.

Mora CI, SG Driese, LA Colarusso 1996 Middle to late Paleozoic atmospheric CO_2 levels from soil carbonate and organic matter. Science 271:1105–1107.

Morgan ME, JD Kingston, BD Marino 1994 Carbon isotopic evidence for the emergence of C_4 plants in the Neogene from Pakistan and Kenya. Nature 367:162–165.

Mulroy TW, PW Rundel 1977 Annual plants: adaptations to desert environments. BioScience 27:109–114.

Nobel PS 1981 Influence of freezing temperatures on a cactus Coryphantha vivipara. Oecologia 48:194–198.

——— 1996 The evolution of crassulacean acid metabolism. Pages 255–265 in K Winter, JAC Smith, eds. Crassulacean acid metabolism: biochemistry, ecophysiology and evolution. Springer, New York.

Nordt LC, TW Boutton, CT Hallmark, MR Waters 1994 Late Quaternary vegetation and climate changes in central Texas based on the isotopic composition of organic carbon. Quat Res 41:109–120.

Osmond CB 1984 CAM: regulated photosynthetic metabolism for all seasons. Pages 557–563 in Sybesma C, ed. Advances in photosynthesis research. Junk, The Hague.

——— 1987 Photosynthesis and carbon economy of plants. New Phytol 106:161–175.

——— 1997 C_4 photosynthesis: thirty or forty years on. Aust J Plant Physiol 24:409–412.

Osmond CB, O Björkman, DJ Anderson 1980 Physiological processes in plant ecology: toward a synthesis with Atriplex. Springer, New York.

Osmond CB, N Valaane, SM Haslam, P Uotila, Z Roksandic 1981 Comparisons of $\delta^{13}C$ values in leaves of aquatic macrophytes from different habitats in Britain and Finland: some implications for photosynthetic processes in aquatic plants. Oecologia 50:117–124.

Pagani M, KH Freeman, MA Arthur 1999a Late Miocene atmospheric CO_2 concentrations and the expansion of C_4 grasses. Science 285:876–879.

——— 1999b Miocene evolution of atmospheric carbon dioxide. Paleoceanography 14:273–292.

Pearcy RW, O Bjorkman, MM Caldwell, JE Keeley, RK Monson, BR Strain 1987 Carbon gain by plants in natural environments. BioScience 37:21–29.

Pearson PN, MR Palmer 2000 Atmospheric carbon dioxide concentrations over the past 60 million years. Nature 406:695–699.

Petit JR, J Jouzel, D Raynaud, NI Barkov, J-M Barnola, M Bender, J Chappellaz, et al 1999 Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antartica. Nature 399: 429–436.

Polley HW, HB Johnson, JD Derner 2002 Soil- and plant-water dynamics in a C_3/C_4 grassland exposed to a subambient to superambient CO_2 gradient. Global Change Biol 8:1118–1129.

Porter SC, A Zhisheng 1995 Correlation between climate events in the North Atlantic and China during the last glaciation. Nature 375: 305–308.

Powell AM 1978 Systematics of Flaveria (Flaveriinae-Asteraceae). Ann Mo Bot Gard 65:590–636.

Prothero DR 1999 Does climatic change drive mammalian evolution? GSA Today 9:1–7.

Pyankov VI, EG Artyusheva, GE Edwards, CC Black Jr, PS Soltis 2001 Phylogenetic analysis of tribe Salsoleae (Chenopodiaceae)

based on ribosomal ITS sequences: implications for the evolution of photosynthesis types. Am J Bot 88:1189–1198.

Pyankov VI, PD Gunin, S Tsoog, CC Black 2000 C_4 plants in the vegetation of Mongolia: their natural occurrence and geographical distribution in relation to climate. Oecologia 123:15–31.

Quade J, JML Cater, TP Ojha, J Adam, TM Harrison 1995 Late Miocene environmental change in Nepal and the northern Indian subcontinent: stable isotopic evidence from paleosols. Geol Soc Am Bull 107:1381–1397.

Quade J, TE Cerling 1995 Expansion of C_4 grasses in the late Miocene of northern Pakistan: evidence from stable isotopes in paleosols. Palaeogeogr Palaeoclimatol Palaeoecol 115:91–116.

Quade J, TE Cerling, JR Bowman 1989 Development of Asian monsoon revealed by marked ecological shift during the latest Miocene in northern Pakistan. Nature 342:163–166.

Quade J, N Solounias, TE Cerling 1994 Stable isotopic evidence from paleosol carbonates and fossil teeth in Greece for forest or woodlands over the past 11 Ma. Palaeogeogr Palaeoclimatol Palaeoecool 108:41–53.

Rachmilevitch S, J Reuveni, RW Pearcy, J Gale 1999 A high level of atmospheric oxygen, as occurred toward the end of the Cretaceous period, increases leaf diffusion conductance. J Exp Bot 50:869–872.

Raven JA 1985 The CO_2 concentrating mechanism. Pages 67–82 in WJ Lucas, JA Berry, eds. Inorganic carbon uptake by aquatic photosynthetic organisms. American Society of Plant Physiologists, Rockville, Md.

——— 1998a Extrapolating feedback processes from the present to the past. Philos Trans R Soc Lond B Biol Sci 353:19–28.

——— 1998b Phylogeny, palaeoatmospheres and the evolution of phototrophy. Pages 323–346 in H Griffiths, ed. Stable isotopes. Bios Scientific, Oxford.

——— 2002 Evolutionary options. Nature 415:375–376.

Raven JA, LL Handley, JJ MacFarlane, S McInroy, L McKenzie, JH Richards, G Samuelson 1988 The role of CO_2 uptake by roots and CAM in acquisition of inorganic C by plants of the isoetid life-form: a review, with new data on *Eriocaulon decangulare* L. New Phytol 108:125–148.

Raven JA, JJ MacFarlane, H Griffiths 1987 The application of carbon isotope discrimination techniques. Pages 129–149 in RMM Crawford, ed. Plant life in aquatic and amphibious habitats. Blackwell Scientific, Oxford.

Raven JA, RA Spicer 1996 The evolution of Crassulacean acid metabolism. Pages 360–385 in K Winter, JAC Smith, eds. Crassulacean acid metabolism: biochemistry, ecophysiology and evolution. Springer, New York.

Raymo ME, WF Ruddiman 1992 Tectonic forcing of late Cenozoic climate. Nature 359:117–122.

Reinfelder JR, AM Kraepiel, FMM Morel 2000 Unicellular C_4 photosynthesis in a marine diatom. Nature 407:996–999.

Reiskind JB, TV Madsen, IC van Ginkel, G Bowes 1997 Evidence that inducible C_4-type photosynthesis is a chloroplastic CO_2-concentrating mechanism in *Hydrilla*, a submersed monocot. Plant Cell Environ 20:211–220.

Retallack GJ 1991 Miocene paleosols and ape habitats of Pakistan and Kenya. Oxford University Press, Oxford. 346 pp.

——— 1997a Earliest Triassic origin of *Isoetes* and quillwort evolutionary radiation. J Paleontol 71:500–521.

——— 1997b Neogene expansion of the North American prairie. Palaios 12:380–390.

——— 2001 A 300-million-year record of atmospheric carbon dioxide from fossil plant cuticles. Nature 411:287–290.

Retallack GJ, DP Dugas, EA Bestland 1990 Fossil soils and grasses of a Middle Miocene East African grassland. Science 247:1325–1328.

Retallack GJ, S Tanaka, T Tate 2002 Late Miocene advent of tall grassland palesols in Oregon. Palaeogeogr Palaeoclimatol Palaecoecol 183:329–354.

Richardson JL, AE Richardson 1972 History of an African rift lake and its climatic implications. Ecol Monogr 42:499–536.

Rundel PW 1980 The ecological distribution of C_4 and C_3 grasses in the Hawaiian Islands. Oecologia 45:354–359.

Rundel PW, KJ Esler, RM Cowling 1999 Ecological and phylogenetic patterns of carbon isotope discrimination in the winter-rainfall flora of the Richtersveld, South Africa. Plant Ecol 142:133–148.

Rundel PW, KJ Esler, G Midgley 1998 Ecophysiological significance of mesemb growth forms and demography. Aloe 35:35–37.

Rundel PW, AC Gibson 1996 Ecological communities and processes in a Mojave Desert ecosystem: Rock Valley, Nevada. Cambridge University Press, Cambridge.

Sage RF 2001 Environmental and evolutionary preconditions for the origin and diversification of the C4 photosynthetic syndrome. Plant Biol 3:202–213.

——— 2002 Are crassulacean acid metabolism and C_4 photosynthetic incompatible? Funct Plant Biol 29:775–785.

Sage RF, M Li, RK Monson 1999a The taxonomic distribution of C_4 photosynthesis. Pages 551–584 in RF Sage, RK Monson, eds. C_4 plant biology. Academic Press, San Diego, Calif.

Sage RF, TL Sage 2002 Microsite characteristics of *Muhlenbergia richardsonis* (Trin.) Rydb., an alpine C_4 grass from the White Mountains, California. Oecologia 132:501–508.

Sage RF, DA Wedin, M Li 1999b The biogeography of C_4 photosynthesis: patterns and controlling factors. Pages 313–373 in RF Sage, RK Monson, eds. C_4 plant biology. Academic Press, San Diego, Calif.

Salvucci ME, G Bowes 1983 Two photosynthetic mechanisms mediating the low photorespiratory state in submersed aquatic angiosperms. Plant Physiol 73:488–496.

Sarmiento G 1984 The ecology of Neotropical savannas. Harvard University Press, Cambridge, Mass. 235 pp.

Simpson GG 1951 Horses: the story of the horse family in the modern world and through sixty million years history. Oxford University Press, Oxford. 247 pp.

Smith DM, JJ Griffin, ED Goldberg 1973 Elemental carbon in marine sediments: a baseline for burning. Nature 241:268–270.

Smith JAC, K Winter 1996 Taxonomic distribution of crassulacean acid metabolism. Pages 427–436 in K Winter, JAC Smith, eds. Crassulacean acid metabolism: biochemistry, ecophysiology and evolution. Springer, New York.

Stebbins GL 1981 Coevolution of grasses and herbivores. Ann Mo Bot Gard 68:75–86.

Stern LA, GD Johnson, CP Chamberlain 1994 Carbon isotope signature of environmental change found in fossil ratite eggshells from a South Asian Neogene sequence. Geology 22:419–422.

Street-Perrott FA, Y Huang, RA Perrott, G Eglinton, P Barker, LB Khelifa, DD Harkness, DO Olago 1997 Impact of lower atmospheric carbon dioxide on tropical mountain ecosystems. Science 278:1422–1426.

Stromberg CAE 2002 The origin and spread of grass-dominated ecosystems in the late Tertiary of North America: preliminary results concerning the evolution of hypsodonty. Palaeogeogr Palaeoclimatol Palaecoecol 177:59–75.

Talbot MR, T Johannessen 1992 A high resolution palaeoclimatic record for the last 27,500 years in tropical West Africa from the carbon and nitrogen isotopic composition of lacustrine organic matter. Earth Planet Sci Lett 110:23–37.

Taylor TN 1981 Paleobotany: an introduction to fossil plant biology. McGraw-Hill, New York. 589 pp.

Taylor WC, RJ Hickey 1992 Habitat, evolution, and speciation in *Isoetes*. Ann Mo Bot Gard 79:613–622.

Taylor WC, E Rosche, JS Marshall, S Ali, CJ Chastain, JA Chitty 1997 Diverse mechanisms regulate the expression of genes coding for C_4 enzymes. Aust J Plant Physiol 24:437–442.

Teeri J 1982 Photosynthetic variation in the Crassulaceae. Pages

244–259 *in* IP Ting, M Gibbs, eds. Crassulacean acid metabolism. American Society of Plant Physiologists, Rockville, Md.

Teese P 1995 Intraspecific variation for CO_2 compensation points and differential growth among variants in a C_3-C_4 intermediate plant. Oecologia 102:371–376.

Thomas M 1949 Physiological studies on acid metabolism in green plants. I. CO_2 fixation and CO_2 liberation in crassulacean acid metabolism. New Phytol 48:390–420.

Thomasson JR, ME Nelson, RJ Zakrzewski 1986 A fossil grass (Gramineae: Chloridoideae) from the Miocene with Kranz anatomy. Science 233:876–878.

Thorne RF, BA Prigge, J Henrickson 1981 A flora of the higher ranges and the Kelso Dunes of the eastern Mojave Desert in California. Aliso 10:71–186.

Tieszen LL, MM Senyimba, SK Imbamba, JH Troughton 1979 The distribution of C_3 and C_4 grasses and carbon isotope discrimination along an altitudinal and moisture gradient in Kenya. Oecologia 37: 337–350.

Ting IP, JH Burk 1983 Aspects of carbon metabolism in *Welwitschia*. Plant Sci Lett 32:279–285.

Tolken HR 1977 A revision of the genus *Crassula* in southern Africa. Bolus Herbarium, Rondebosch, South Africa. 2 pts, 595 pp.

Tschudy RH, CL Pillmore, CJ Orth, JS Gilmore, JD Knight 1984 Disruption of the terrestrial plant ecosystem at the Cretaceous-Tertiary boundary, western interior. Science 225:1030–1032.

Ueno O 1996 Structural characterization of photosynthetic cells in an amphibious sedge, *Eleocharis vivipara*, in relation to C_3 and C_4 metabolism. Planta 199:382–393.

Utescher T, V Mosbrugger, AR Ashraf 2000 Terrestrial climate evolution in northwest Germany over the last 25 million years. Palaios 15:430–449.

Van Devender TR, WG Spaulding 1979 Development of vegetation and climate in the southwestern United States. Science 204:701–710.

Van Ham RCHJ 1995 Phylogenetic relationships in the Crassulaceae inferred from chloroplast DNA variation. Pages 16–29 *in* H Hart, U Eggli, eds. Evolution and systematics of the Crassulaceae. Backhuys, Leiden.

Van Valkenburgh B 1999 Major patterns in the history of carnivorous mammals. Annu Rev Earth Planet Sci 27:463–493.

Voznesenskaya EV, VR Franceschi, O Kiirats, EG Artyusheva, H Freitag, GE Edwards 2002 Proof of C_4 photosynthesis without Kranz anatomy in *Bienertia cycloptera* (Chenopodiaceae). Plant J 31: 649–662.

Voznesenskaya EV, VR Franceschi, O Kiirats, H Freitag, GE Edwards 2001 Kranz anatomy is not essential for terrestrial C_4 plant photosynthesis. Nature 414:543–546.

Wand SJE, GF Midgley, MH Jones, PS Curtis 1999 Responses of wild C_4 and C_3 grass (Poaceae) species to elevated atmospheric CO_2 concentration: a meta-analytic test of current theories and perceptions. Global Change Biol 5:723–741.

Wang Y, TE Cerling, BJ MacFadden 1994 Fossil horses and carbon isotopes: new evidence for Cenozoic dietary, habitat, and ecosystem changes in North America. Palaeogeogr Palaeoclimatol Palaeoecol 107:269–279.

Watson L, MJ Dallwitz 1992 The grass genera of the world. CAB International, Oxon. 1038 pp.

Westhoff P, P Svensson, K Ernst, O Bläsing, J Burscheidt, J Stockhaus 1997 Molecular evolution of C_4 phosphoenolpyruvate carboxylase in the genus *Flaveria*. Aust J Plant Physiol 24:429–436.

Williams JW, T Webb III, BN Shurnan, PJ Barlein 2000 Do low CO_2 concentrations affect pollen-based reconstructions of LGM climates? a response to "physiological significance of low atmospheric CO_2 for plant-climate interactions" by Cowling and Sykes. Quat Res 53:402–404.

Winter K 1981 C_4 plants of high biomass in arid regions of Asia: occurrence of C_4 photosynthesis in Chenopodiaceae and Polygonaceae from the Middle East and USSR. Oecologia 48:100–106.

Winter K, JAM Holtum 2002 How closely do the $\delta^{13}C$ values of crassulacean acid metabolism plants reflect the proportion of CO_2 fixed during day and night? Plant Physiol 129:1843–1851.

Winter K, JAC Smith 1996a Crassulacean acid metabolism: current status. Pages 360–385 *in* K Winter, JAC Smith, eds. Crassulacean acid metabolism: biochemistry, ecophysiology and evolution. Springer, New York.

——— 1996b An introduction to crassulacean acid metabolism: biochemical principles and ecological diversity. Pages 1–13 *in* K Winter, JAC Smith, eds. Crassulacean acid metabolism: biochemistry, ecophysiology and evolution. Springer, New York.

Wolfe JA 1985 Distribution of major vegetational types during the Tertiary. Pages 357–375 *in* ET Sundquist, WS Broecker, eds. The carbon cycle and atmospheric CO_2: natural variations Archean to present. American Geophysical Union, Washington, D.C.

——— 1990 Palaeobotanical evidence for a marked temperature increase following the Cretaceous/Tertiary boundary. Nature 343: 153–156.

Wright VP, SD Vanstone 1991 Assessing the carbon dioxide content of ancient atmospheres using palaeocalcretes: theoretical and empirical constraints. J Geol Soc (Lond) 148:945–947.

Zachos J, M Pagani, L Sloan, E Thomas, K Billups 2001 Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292:686–693.