

The Early Origins of Terrestrial C₄ Photosynthesis

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Abstract

The C₄ photosynthetic pathway is a series of structural and biochemical modifications around the more primitive C₃ pathway that improve the photosynthetic efficiency under specific climatic conditions. Hence, the origin and subsequent geographical expansion of the C₄ plants likely reflects a record of climate change. Multiple paleoatmospheric *p*CO₂ proxies indicate a critical CO₂ threshold was breached ~30 Ma, that potentially selected for CO₂-concentrating mechanisms to overcome photorespiratory stresses imposed on the basic C₃ pathway. Details of the C₄ pathway's earliest origins remain enigmatic given the paucity of the geologic record. Nonetheless, δ¹³C proxy records from paleosol carbonates, ungulate teeth, and plant-derived compounds indicate C₄ plants likely represented an important component of plant biomass by the Early Miocene. Low CO₂ levels appear to be a precondition for the development of the C₄ photosynthetic pathway; however, comparisons of δ¹³C proxy records indicate that the timing of C₄ geographical expansion was not globally synchronous, and thus point toward more regional controls on the development of C₄-dominated ecosystems. Terrestrial and marine records indicate that continental aridity and wind strength increased during the Late Miocene. These conditions would have likely increased fire occurrence and fire intensity leading to the clearing of large tree stands and the expansion of C₄ grasses in warm-season precipitation regimes.

Rubisco:

ribulose-1,5-bisphosphate
carboxylase/oxygenase

RuBP:

ribulose
1,5-bisphosphate

G3P:

glyceraldehydes-3-
phosphate

INTRODUCTION

The photosynthetic process of converting carbon dioxide and water into complex organic molecules has persisted for at least 2.5 billion years (Summons et al. 1999). The antiquity of the Calvin-Benson cycle—the chemical reactions that convert inorganic carbon to simple organic molecules—places its origin during a time when the atmosphere was characterized by very high CO₂ levels and much lower O₂ concentrations relative to modern values (Kasting 1987, Rye et al. 1995, Berner & Kothavala 2001, Bekker et al. 2004). Not surprisingly, under these high CO₂/O₂ conditions the efficacy of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the enzyme used to fix CO₂, is enhanced (Jordan & Ogren 1984, Sharkey 1988) and the thermodynamic efficiency of the Calvin-Benson cycle is maximized (Raven et al. 2003). In response to changing climatic conditions over Earth's history, biological innovations have been developed to enhance photosynthetic efficacy across a wide range of environments. These enhancements, such as the C₄ photosynthetic pathway, do not alter the fundamental machinery of the Calvin-Benson cycle. Rather, they represent structural and biochemical modifications imposed around the Calvin-Benson cycle to improve its functioning.

The C₄ photosynthetic pathway represents a relatively minor physiological change from the basic C₃ template, but has distinct advantages over plants using solely the Calvin-Benson cycle. In general, the biochemistry and physiology of C₄ photosynthesis is understood and it is well known that the C₄ pathway acts as a CO₂ pump, concentrating CO₂ at the site of carbon fixation, increasing the efficiency of photosynthesis under low CO₂ conditions. However, important questions remain regarding the origin of C₄ plants and geographical expansion of the C₄ photosynthetic pathway.

PHOTOSYNTHETIC PATHWAYS

The C₃ Photosynthetic Pathway

C₃ photosynthesis, characterized by a series of biochemical reactions known as the Calvin-Benson cycle, is the most primitive photosynthetic pathway in terrestrial plants. This process fixes a diffusive flux of atmospheric CO₂ to ribulose 1,5-bisphosphate (RuBP) using the enzyme Rubisco to form an enzyme-bound intermediate, 2-carboxy-3-ketoarabinitol-1,5-bisphosphate (**Figure 1a**). This intermediate is unstable and is immediately hydrolyzed to form two molecules of 3-phosphoglycerate that are subsequently phosphorylated to 1,3-bisphosphoglycerate and then reduced to glyceraldehydes-3-phosphate (G3P) (the three-carbon sugar for which C₃ photosynthesis is named). For every three molecules of CO₂ assimilated by RuBP, six molecules of G3P are produced. Of these six G3P molecules, one molecule is converted to a simple sugar for storage and cell nutrition with the remaining five being recycled to regenerate RuBP.

Although Rubisco is a carboxylase and has a high specificity for CO₂, it can react with diatomic oxygen resulting in a short circuiting of carbon fixation and the release of CO₂ (**Figure 1a**). This process, known as photorespiration, is prevalent under specific environmental conditions, such as low CO₂/O₂ atmospheric conditions, and

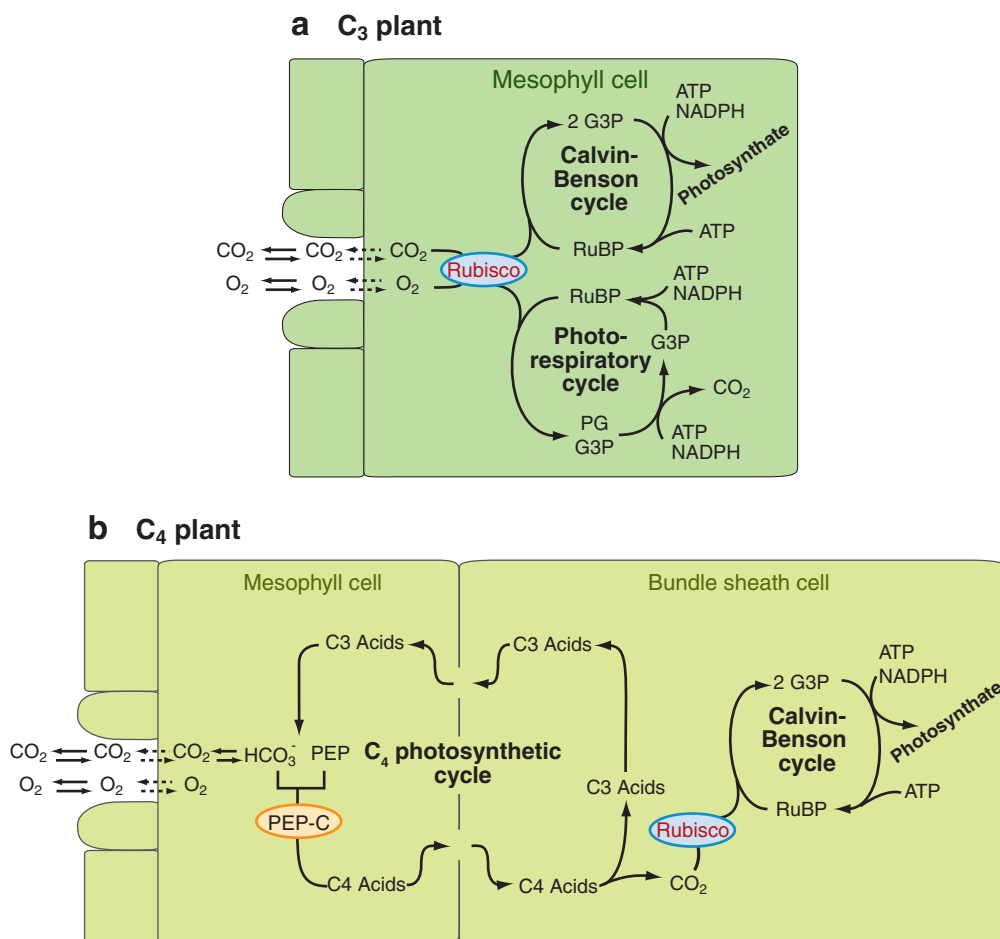


Figure 1

Schematic of the (a) C₃ and (b) C₄ plant physiology and biochemical pathways. Both C₃ and C₄ plants possess the Calvin-Benson cycle; however, the unique physiology of the C₄ plants provides a spatial separation between carbon assimilation and CO₂ fixation.

impedes the cycling of RuBP, reducing both its concentration necessary for carbon fixation and net photosynthesis. Under present atmospheric conditions, high rates of photorespiration in C₃ plants reduce the overall photosynthetic rate by 30%–40% (Percy & Ehleringer 1984, Ehleringer & Monson 1993, Ehleringer 2005). In addition, Rubisco oxygenase activity increases with temperature, whereas carboxylase appears to be temperature insensitive (Ehleringer 2005). Further, rates of photorespiration are intensified by high temperatures, aridity, and high-light intensity (Hatch 1987, Ehleringer & Monson 1993). Under these conditions, plants minimize their stomatal openings in an effort to reduce water loss from the leaf. This process leads to lower internal concentrations of CO₂ and enhanced rates of

CAM: Crassulacean acid metabolism

PEP: phosphoenolpyruvate

PEP-C: phosphoenolpyruvate-carboxylase

photorespiration. As a consequence, specific environmental stresses that enhance rates of photorespiration have forced several biological innovations on the basic C_3 model, namely Crassulacean acid metabolism (CAM) and C_4 photosynthesis.

The C_4 Photosynthetic Pathway

Terrestrial C_4 photosynthesis is not a single photosynthetic pathway, but rather a general CO_2 concentrating model with several distinct biochemical variations and anatomical modifications. Similar to C_3 plants, all terrestrial C_4 plants fix carbon through the Calvin-Benson cycle. However, C_4 plants possess a series of biochemical reactions prior to the Calvin-Benson cycle that convert aqueous bicarbonate (HCO_3^-) into a sequence of acids that are ultimately converted back to CO_2 and used to produce carbohydrates via the Calvin-Benson cycle (**Figure 1b**). This sequence of reactions is spatially divided into two specialized chloroplast-containing cell types: mesophyll and bundle-sheath cells, together called Kranz anatomy (**Figure 2b**). Within the mesophyll cells, HCO_3^- is fixed to phosphoenolpyruvate (PEP) by phosphoenolpyruvate-carboxylase (PEP-C) to yield a four-carbon acid, oxaloacetate (hence, the name C_4 photosynthesis) (Taiz & Zeiger 1998). Oxaloacetate is reduced to aspartate or malate depending on the C_4 photosynthetic pathway enzymatic subtype (Hatch & Osmond 1976). Within all C_4 subtypes, four-carbon acids are exported from mesophyll to bundle-sheath cells and decarboxylated, releasing the CO_2 that is used in the Calvin-Benson cycle. Importantly, PEP-C is not an oxygenase and has a greater affinity for HCO_3^- than Rubisco has for CO_2 , resulting in higher rates of carbon assimilation than the Calvin-Benson cycle alone (Hatch 1987).

The reactions occurring in the C_4 mesophyll cells can be viewed as a CO_2 pump, increasing CO_2 concentrations within the bundle sheath cell and keeping CO_2 concentrations high enough to saturate Rubisco with CO_2 , effectively eliminating

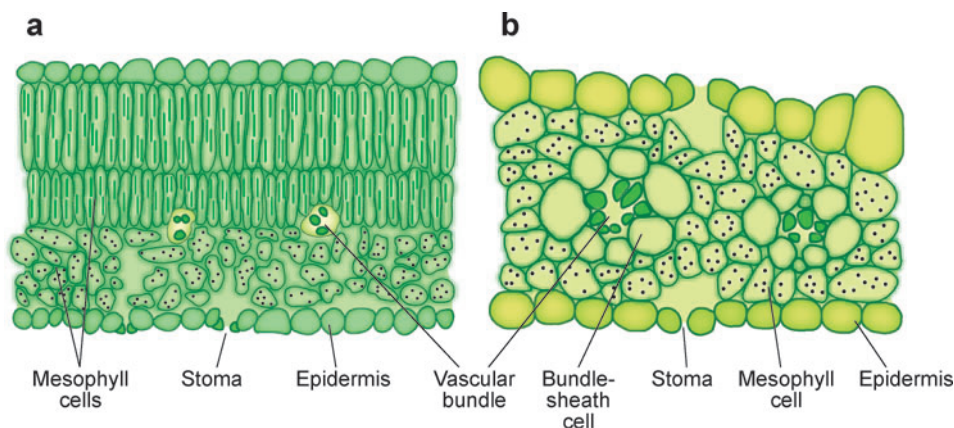


Figure 2

Idealized cross-sections of a (a) C_3 and (b) C_4 leaf, with the C_4 cross-section showing Kranz anatomy, the large bundle-sheath cells around the vascular strand in the C_4 plant.

photorespiration. Under modern atmospheric CO_2 levels, the high affinity of PEP-C for HCO_3^- facilitates enzyme saturation. As a consequence, C_4 plants can decrease their stomatal width and reduce transpiration, while fixing CO_2 at rates equal or greater than C_3 plants under similar conditions (Taiz & Zeiger 1998). As a result, C_4 plants' water-use efficiency (water loss per unit carbon assimilated) is twice that of C_3 plants at $\sim 25^\circ\text{C}$ (Hatch 1987). Moreover, higher rates of carbon assimilation can be maintained under elevated water-stressed conditions.

Given the physiological advantages of the C_4 pathway, it is not surprising to find that C_4 flora are better adapted to hot, high-light, and dry environments (Sage et al. 1999a), making up more than two-thirds of all grasses in tropical and subtropical regions, and more than 90% of tropical savanna floral ecosystems (Sage 2001). Only five species represent the C_4 photosynthetic pathway in C_3 -dominated region above 60°N (Sage et al. 1999a). Seasonal aridity with a wet growing season is also an important environmental constraint influencing C_4 floral distributions, but represents a secondary control (Sage 2001).

Higher Plant Carbon Isotopic Composition

Differences in the physiologies of C_3 and C_4 plants result in distinct stable carbon isotope signatures (Figure 3). The stable carbon isotopic composition of all higher plants is a function of the carbon isotopic composition of atmospheric CO_2 ($\delta^{13}\text{C}_{\text{CO}_2}$) and the ratio of partial pressure of atmospheric CO_2 (p_i) inside the leaf relative to atmospheric p_{CO_2} (p_a) (O'Leary 1981, Farquhar et al. 1989). Farquhar et al. (1989) empirically demonstrated that the $\delta^{13}\text{C}$ composition of bulk C_3 plant material can be expressed by the following equation:

$$\delta^{13}\text{C}_{\text{C}_3\text{plant}} = \delta^{13}\text{C}_{\text{CO}_2} - a - (b - a)\frac{p_i}{p_a}, \quad (1)$$

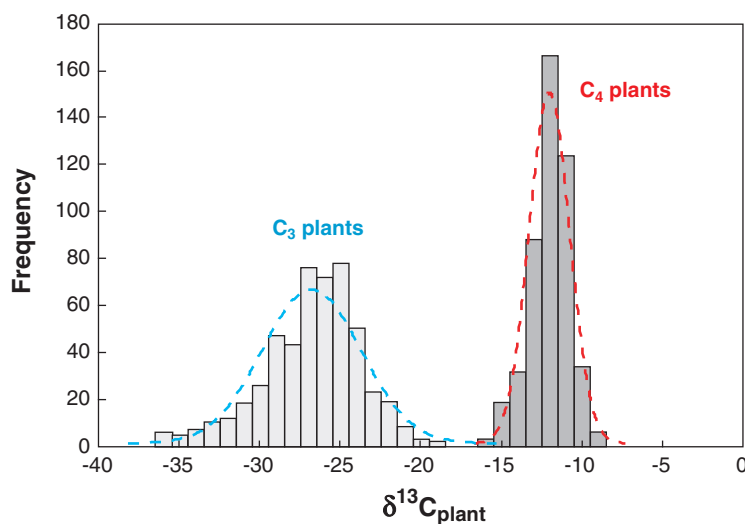


Figure 3

Histogram showing normal distribution of bulk C_3 and C_4 plant isotopic values. Data from Cerling & Harris (1999).

where a is the carbon isotopic fractionation that occurs during diffusion of CO_2 into the leaf (4.4‰) and b is the carbon isotopic fractionation expressed by carboxylation by Rubisco (27‰). Additionally, the $\delta^{13}\text{C}$ composition of bulk C_4 plant material can be expressed as

$$\delta^{13}\text{C}_{\text{C}_4\text{plant}} = \delta^{13}\text{C}_{\text{CO}_2} - a - (b_4 + b\phi - a)\frac{p_i}{p_a}, \quad (2)$$

where a and b are the same as in Equation 1, b_4 is the fractionation associated with carboxylation of PEP-C (−5.7‰), and ϕ is the proportion of carbon fixed by PEP-C that leaks out of the bundle sheath cell (Farquhar 1983).

C_3 plants have a fairly wide range of isotopic values, ranging from −20‰ to −35‰, whereas C_4 plants have a more narrow range, between −10‰ to −14‰ (**Figure 3**). Differences between the mean $\delta^{13}\text{C}$ composition of C_4 and C_3 plants can be accounted for by the affinity of PEP-C for ^{13}C , as well as the nature of carbon leakage (ϕ), which is proportional to the concentration of CO_2 within the bundle sheath cell. As a result, the term $(b_4 + b\phi)$ limits Rubisco's isotope discrimination in C_4 plants (Equation 2). Differences in the range of isotopic values among C_3 and C_4 plants are attributed to environment stresses (water availability, irradiance levels) and source effects (localized differences in the $\delta^{13}\text{C}$ value of CO_2 within a canopy) imposed on C_3 plants. In contrast, the majority of isotopic variation for C_4 plants is attributed to differences between C_4 enzymatic subtypes (Hattersley 1982). C_3 plants living under high-water stress tend to incorporate more ^{13}C as a result of lower stomatal conductance, followed by lower internal CO_2 concentration as the plant attempts to conserve water (Ehleringer 1993). When stomatal conductance is high, internal $p\text{CO}_2$ (p_i) is higher, allowing the carbon isotope fractionations that occur during carbon fixation (term b in Equations 1 and 2) to be more fully expressed (i.e., p_i/p_a approaches unity).

Anatomical and isotopic differences between C_3 and C_4 species have been used to document variation in plant types on local, regional, and larger scales. However, where modern studies use fresh plant material, ancient records require the preservation of these signals in the geologic record. As a consequence, paleorecords of C_3 and C_4 dynamics are inherently sparse.

PALEORECORDS

Fossil Records

The majority of the modern C_4 syndrome occurs in Poaceae (true grasses), both systematically (Sage et al. 1999b) and in biomass (Lloyd & Farquhar 1994), and thus suggest that the earliest C_4 plants were grasses as well. Anatomical differences between C_3 and C_4 grasses are striking in modern specimens (**Figure 2**). Kranz anatomy or the ring-like cell structure around the vascular bundle is a telltale sign of a C_4 plant, but not a necessity (Voznesenskay et al. 2001). Preservation of this structure, let alone grass leaves, is extraordinarily rare because grasses tend to grow in environments conducive to organic matter decomposition. To date, only two established C_4 grass macrofossils have been discovered. The earliest indisputable C_4 macrofossil recovered from late

middle Miocene sediments is ~12.5 million years old (MacGinitie 1962, Nambudiri et al. 1978). These fossil grasses are characterized by Kranz anatomy, radiating mesophyll tissue, and few cells between veins (3–5 cells); all diagnostic characteristics of C₄ species. Notably, these macrofossils have stable carbon isotope ratios similar to modern C₄ plants (**Figure 3**) (Nambudiri et al. 1978). Interestingly, paleoecologic reconstructions from this locality suggest a wooded-savanna in Mediterranean-type climate with cool seasonal precipitation, similar to much of modern southern California (Axelrod 1939). Generally, C₄ plants are rare under these conditions. However, Axelrod (1939) also reports flora suggestive of mesic environments with well-watered soil. It is possible that these early C₄ grasses were associated with stream or lake banks similar to modern C₄ grasses living in Mediterranean climates (Cerling 1999). Also, fluvial environments are more likely to preserve plant macrofossils than open grassland soils, potentially biasing this interpretation. However, if valid, the earliest unequivocal C₄ plants were not living on open plains, but rather on highly disturbed floodplains.

The only other definitive C₄ grass fossil—a single C₄ grass leaf fragment with obvious Kranz anatomy—comes from the latest Miocene Ogallala Formation in northwestern Kansas (Thomasson et al. 1986). Further, epidermal structures, stomata shapes, and silica body forms are suggestive of Chloridoideae grasses (Thomasson et al. 1986), one of the two grass subfamilies that contain C₄ grasses (Sage 2001).

A third and final locality with questionable C₄ grass fossil evidence comes from the middle Miocene (~14 Ma) Fort Ternan Formation of Kenya (Retallack et al. 1990, Dugas & Retallack 1993). Although macroflora remains provide evidence for Africa's earliest wooded grasslands (Retallack et al. 1990, Dugas & Retallack 1993, Jacobs et al. 1999), tentative evidence for C₄ flora is based on cuticle morphology and the identification of species with modern C₄ affinity. However, the diagnostic C₄ Kranz anatomy was not recognized owing to poor internal preservation of the fossils.

Although grass fossils are exceedingly rare in the geologic record, grass pollen is very abundant given the plants adaptation to wind pollination (Jacobs et al. 1999). However, morphologically grass pollen is nearly identical, prohibiting taxonomic identification lower than the family level (Linder 1986), preventing its use as a proxy for past C₃ and C₄ plant dynamics.

Besides macrofossils, another potentially useful fossil grass component is internal silica deposits called phytoliths. Phytoliths or plant opal form in a variety of shapes and act as a defense mechanism against herbivory and increase structural integrity (Piperno 1988). The majority of extant grasses have specialized silica-accumulating cells, called idoblasts, which form characteristic phytolith shapes (Fredlund & Tieszen 1994). Paleophytolith assemblages have the potential to yield information on C₃ and C₄ grass communities (Fredlund & Tieszen 1994, 1997; Thorn 2004), as well as provide a repository of occluded plant organic matter that can be potentially used to evaluate stable carbon isotope compositions (Kelly et al. 1991, Lu et al. 2000, Smith & Anderson 2001, Smith & White 2004).

Morphological analysis of fossil phytolith assemblages have been used to reconstruct plant ecosystems during the Neogene (Strömberg 2004, Thorn 2004). However, interpretations are compromised because a single phytolith form can be

produced by several different plants. Further, issues of redundancy, multiplicity, and variations in phytolith production among C_3 and C_4 genera hinder paleoapplications. Most importantly, it is unclear if ancient C_4 grasses produced the same phytolith forms as today. All of these factors hinder the applicability of phytolith morphological and isotopic analysis for paleointerpretation of C_3 and C_4 grasses.

ISOTOPE PROXY RECORDS

Tooth Enamel

The isotope signatures preserved in mammal bioapatites, such as enamel, dentine, and bone, can be used to reconstruct the organism's diet, ecology, body temperature, and environmental parameters such as the hydrologic system (for a review see Koch 1998). The most reliable work to date derives from fossil tooth enamel, given its abundance in the fossil record, ease of identification, and resistance to isotopic diagenesis stemming from its low porosity and large crystalline structure (Wang & Cerling 1994, Kohn & Cerling 2002).

The $\delta^{13}C$ value of tooth enamel ($\delta^{13}C_{\text{enamel}}$) is directly linked to the stable carbon isotopic composition of a mammal's diet. Modern studies have shown that the tooth enamel of large herbivores is enriched in ^{13}C by $\sim 14\%$ compared to their diet (Cerling & Harris 1999). Further, large herbivores are selective eaters, a habit that amplifies the isotopic signature of their diets (Cerling et al. 1999). Accordingly, large herbivore tooth enamel has been extensively used to reconstruct the abundance of C_4 plants (Wang et al. 1994, Morgan et al. 1994, MacFadden et al. 1996, Cerling et al. 1997, Latorre et al. 1997, Clementz & Koch 2001, Passey et al. 2002, Wang et al. 2006). Importantly, studies generally focus on a few specific taxa rather than whole local faunas, potentially biasing ecosystem reconstructions toward either C_3 or C_4 plant characteristics. Therefore, the clear absence or presence of a C_4 isotope signature derived from tooth enamel records does not necessarily imply the lack or existence of C_4 plants, but rather the nature of herbivore feeding selectivity.

An interpretation of a definitive C_4 dietary component from $\delta^{13}C_{\text{enamel}}$ requires knowledge of the range of $\delta^{13}C_{\text{enamel}}$ values from both pure C_3 and C_4 feeders. Given an $\sim 14\%$ fractionation between diet and tooth enamel $\delta^{13}C_{\text{enamel}}$, a modern pure C_3 diet would result in average $\delta^{13}C_{\text{enamel}}$ values of $\sim -13\%$ and range from $\sim -20\%$ for closed canopy environments to $\sim -9\%$ for water-limited C_3 plant diets (Cerling & Harris 1999). In contrast, $\delta^{13}C_{\text{enamel}}$ values for a pure C_4 diet range between 0% to 4% . Given these constraints, the most conservative approach in the reconstruction of ancient plant communities is to assume that $\delta^{13}C_{\text{enamel}}$ values more positive than $\sim -9\%$ reflect the influence of C_4 plants in the organism's diet. This assumes that the carbon isotopic composition of atmospheric CO_2 —the primary control on the carbon isotopic composition of plants (Equation 1 and 2)—was similar to the modern value.

Cerling et al. (1997) compiled a survey of equid, proboscidean, and notoungulate $\delta^{13}C_{\text{enamel}}$ records from the Americas, East Africa, Pakistan, and Europe spanning the past 20 million years (**Figure 4**). A substantial positive shift in $\delta^{13}C_{\text{enamel}}$ occurs in

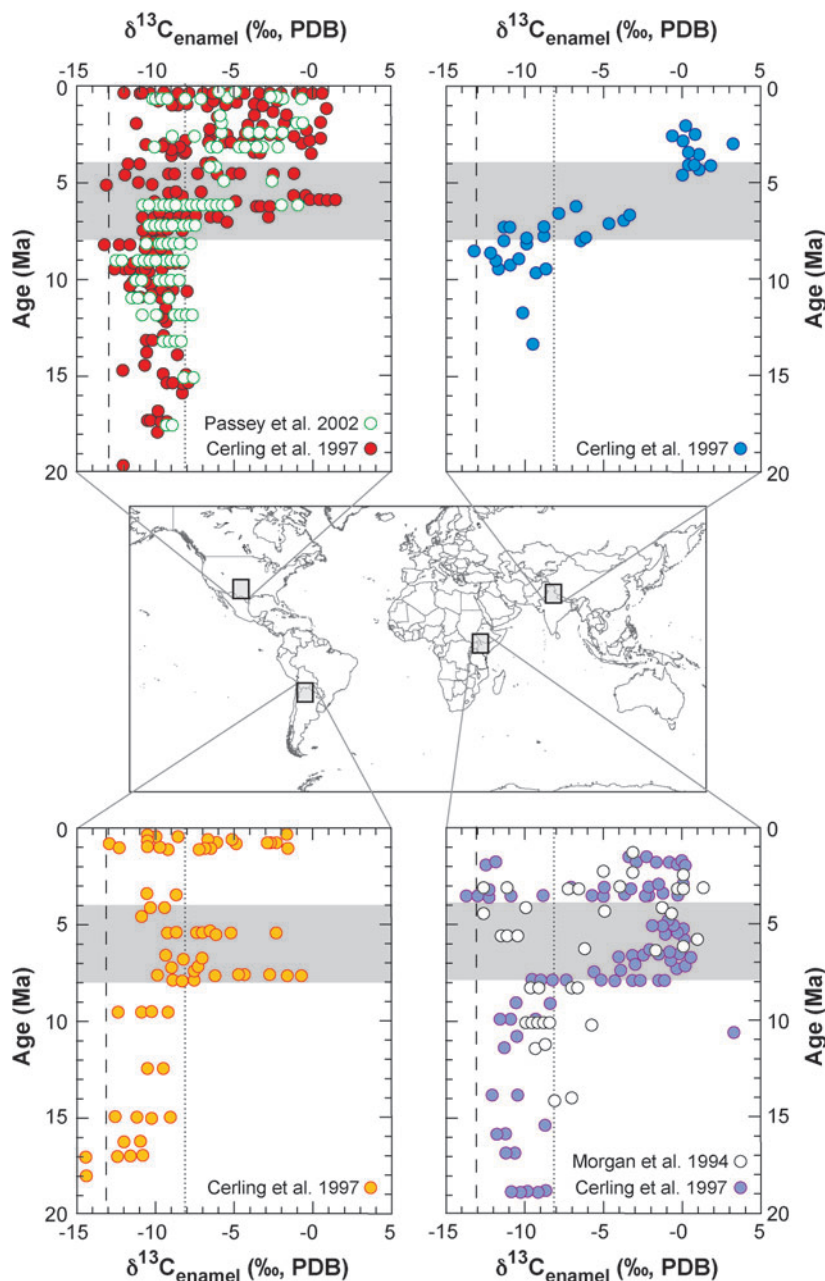


Figure 4

Carbon isotopic values of large herbivore tooth enamel from North America, South Central Asia, East Africa, and South America for the past 20 million years. Data from Morgan et al. (1994), Cerling et al. (1997), Passey et al. (2002). Conservative (-8‰) and average (-13‰) tooth enamel values for a pure C_3 diet are represented by the dotted and dashed lines, respectively. Isotopic values represent the end-member compositions of C_3 plants assuming both extreme and average water-stress conditions. Values more positive than the end-members represent some contribution of C_4 dietary influence. Estimates assume the isotopic composition of atmospheric CO_2 was similar to preindustrial values (-6.5‰). Map data from ERSI.

samples from Africa, Pakistan, and subtropical South and North America, indicative of a change from C_3 to C_4 -dominated diets between 8–4 Ma. Prior to 8 Ma, $\delta^{13}C_{\text{enamel}}$ values average $\sim -10.6\text{‰}$ and range between -7.5 and -14.5‰ (Cerling et al. 1997) and are interpreted to represent a pure C_3 plant diet. Further, given that large herbivores can travel great distances, $\delta^{13}C_{\text{enamel}}$ records provide a regional-scale isotope signal of diet. Uncertainty in this interpretation stems from the assumed end-member isotopic value for C_3 plants. For example, a C_3 - $\delta^{13}C_{\text{enamel}}$ value of -8‰ requires that the average $\delta^{13}C$ value of C_3 plants was -22‰ , a very ^{13}C -enriched estimate given that modern C_3 plants with these $\delta^{13}C$ values (**Figure 3**) primarily occur in desert environments under extreme water-stress conditions (Ehleringer 1993, Williams & Ehleringer 1996).

Another source of uncertainty is in the $\delta^{13}C$ value of ancient atmospheric carbon dioxide ($\delta^{13}C_{\text{CO}_2}$). Miocene $\delta^{13}C_{\text{CO}_2}$ values were not necessarily similar to preindustrial compositions given evidence that $\delta^{13}C_{\text{CO}_2}$ has substantially changed over geologic time (Gröcke 2002). Reconstructions of ancient $\delta^{13}C_{\text{CO}_2}$ have been approached using $\delta^{13}C$ records of both fossil charcoal and foraminifera. Stable carbon isotopic records from fossil charcoal (pyrofusinite) have been used as a proxy for C_3 plant material given that C_3 plants produce secondary xylem (wood) and most C_4 plants do not. In this case, charcoal $\delta^{13}C$ is related to $\delta^{13}C_{\text{CO}_2}$ algebraically by Equation 1, where modern C_3 p_i/p_a values range between 0.7 and 0.5 (Evans et al. 1986, Polley et al. 1993). Records of $\delta^{13}C_{\text{CO}_2}$ derived from planktonic foraminiferal calcite ($\delta^{13}C_{\text{cc}}$) assume that the surface ocean and atmosphere are in isotopic equilibrium. The relationship between $\delta^{13}C_{\text{cc}}$ calcite and $\delta^{13}C_{\text{CO}_2}$ can be expressed by the equation

$$\delta^{13}C_{\text{CO}_2} = \{(\delta^{13}C_{\text{cc}} + 10^3)/[(\epsilon_{\text{cc-CO}_2}/10^3) + 1]\} - 10^3, \quad (3)$$

where $\epsilon_{\text{cc-CO}_2}$ represents the temperature-dependent fractionation factor between foraminiferal calcite and $\text{CO}_{2(\text{gas})}$ (Mook et al. 1974, Romanek et al. 1992) and is expressed as

$$\epsilon_{\text{cc-CO}_2} = 11.98 - 0.12 \times T(^{\circ}\text{C}). \quad (4)$$

To date, no charcoal-based $\delta^{13}C_{\text{CO}_2}$ records have been established for the Cenozoic. However, foraminifera-derived records suggest that $\delta^{13}C_{\text{CO}_2}$ varied between -4.9‰ and -6.9‰ over the past 20 million years compared with a preindustrial $\delta^{13}C_{\text{CO}_2}$ of -6.5‰ (Passey et al. 2002). When changes in $\delta^{13}C_{\text{CO}_2}$ are taken into account, a conservative pure C_3 diet $\delta^{13}C_{\text{enamel}}$ end-member through the Miocene is between -7.5‰ and -9.5‰ , whereas a more average C_3 diet $\delta^{13}C_{\text{enamel}}$ end-member varies between -10.5‰ and -12.5‰ (Passey et al. 2002). The latter case is reasonable, given that hyperbrowsers, such as giraffes, which have a predominately C_3 -based diet, have $\delta^{13}C_{\text{enamel}}$ values between -11‰ and -13.5‰ during the Miocene (Morgan et al. 1994, Feranec & MacFadden 2006).

In summary, if $\delta^{13}C_{\text{CO}_2}$ was $\sim 4\text{‰}$ more positive during the Miocene, then a C_3 plant end-member value of $\sim -22\text{‰}$ would likely represent an average value, which suggests that C_4 plants were absent prior to 8 Ma. However, if $\delta^{13}C_{\text{CO}_2}$ proves to be similar to preindustrial values, a less conservative C_3 -plant end-member is warranted. For example, if a C_3 composition of -24‰ ($\delta^{13}C_{\text{enamel}} = -10\text{‰}$) is assumed, late

Miocene $\delta^{13}\text{C}_{\text{enamel}}$ records would yield a considerable dietary C_4 component up to 27% prior to 8 Ma.

The latter interpretation deserves consideration given isotopic evidence that supports the appearance of C_4 plants earlier than 8 Ma. For example, equid, bovid, and rhinocerosid $\delta^{13}\text{C}_{\text{enamel}}$ records from the Baringo Basin of East Africa have values as high as $\sim -6\text{‰}$, between 15.3 to 12 Ma (**Figure 4**) (Morgan et al. 1994). Equid and rhinocerosid $\delta^{13}\text{C}_{\text{enamel}}$ from the well-dated Ashfall Fossil Bed in Northern Nebraska range from -7 to -4‰ at 11.8 Ma, clearly indicative of C_4 plant consumption (Clementz & Koch 2001; M. Clementz, personal communication, 2006). Although equid $\delta^{13}\text{C}_{\text{enamel}}$ records from Nebraska and Texas (coupled with paleo- $\delta^{13}\text{C}_{\text{CO}_2}$ corrections for the past 20 million years) provide evidence for a shift to C_4 -dominated diets by ~ 6 Ma (Passey et al. 2002), all but two of these data prior to 6 Ma are more ^{13}C -enriched than an average pure C_3 $\delta^{13}\text{C}_{\text{enamel}}$ end-member value, estimated at ~ -7.5 to -12.5‰ (**Figure 4**), and thus support some C_4 input during this time.

Collectively, these $\delta^{13}\text{C}_{\text{enamel}}$ records represent growing evidence that either large herbivores were restricted to highly water-stressed environments or that they consumed C_4 plant materials as part of their normal diets prior to 9 Ma (Passey et al. 2002). While these interpretations are potentially biased by feeding selectivity, $\delta^{13}\text{C}_{\text{enamel}}$ from large herbivores provide regional-scale information with relatively high spatial and temporal resolution.

Paleosols and Soil Carbonates

Pedogenic carbonate nodules form as a result of surface dissolution of Ca-bearing minerals and downward transport of Ca^{2+} and HCO_3^- through the soil profile, followed by dehydration, saturation, and CaCO_3 precipitation (van Breemen & Buurman 2002). Typically, soil carbonates readily form in soils with grasses and/or shrubs under less than 75 cm of rain per year (Cerling 1984), and rarely form in soils with > 100 cm of precipitation, characteristic of tropical and subtropical environments (Cerling 1984).

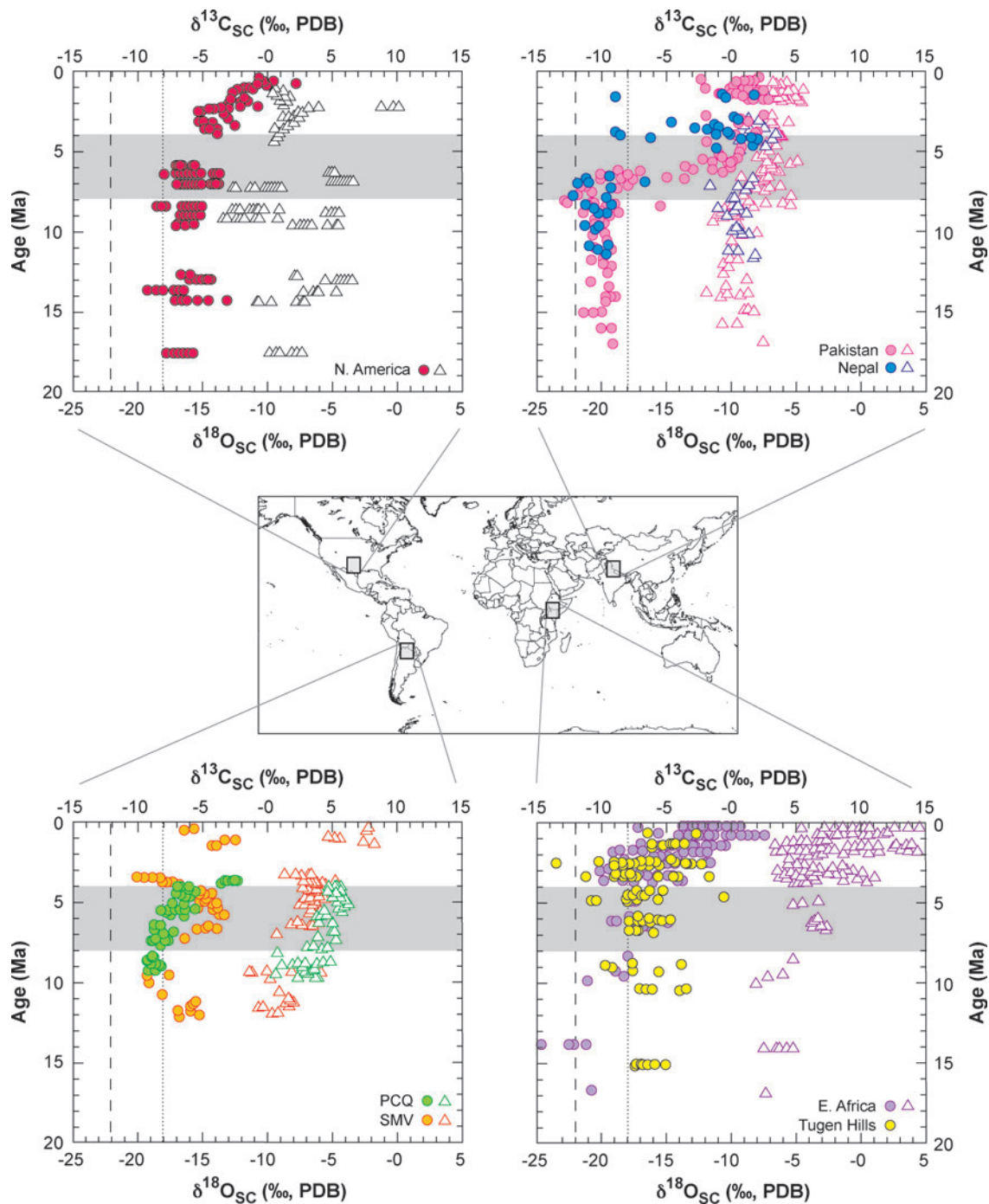
Field experiments have shown that soil carbonates are in isotopic equilibrium with soil CO_2 (Cerling et al. 1989, Quade et al. 1989a), whereas CO_2 fluxes are a function of diffusion. As such, standard diffusion mathematical models describe the concentration and isotopic distribution of soil CO_2 (Kirkham & Powers 1972, Cerling 1984). CO_2 fluxes include atmospheric CO_2 and soil-respired CO_2 (Cerling 1991a), where the latter derives from root-respiration and/or microbial decomposition of organic matter (Cerling 1991a). Consequently, the isotopic character of soil CO_2 is related to both the $\delta^{13}\text{C}$ of the soil-respired CO_2 (-13‰ to -30‰) and atmospheric CO_2 (-6‰ to -8‰) in moderate to high respiration rate soils (Cerling 1984, 1991a). Differences in the diffusion coefficients for $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ (Craig 1953) result in soil CO_2 that is ^{13}C -enriched by $\sim 4.4\text{‰}$ compared with soil-respired CO_2 (Cerling 1991a). Finally, equilibrium fractionation between CO_2 and CaCO_3 is -9.8‰ to -12.4‰ , depending on temperature (between 0° and 35°C) (Deines et al. 1974). As a consequence, soil carbonates are $\sim 14\text{‰}$ to 17‰ more ^{13}C -enriched relative to soil-respired CO_2 .

In soils with moderate to high respiration rates (10^{-6} to 10^{-3} mole cm^{-2} year $^{-1}$), the primary control on the isotopic composition of soil CO_2 ($\delta^{13}\text{C}_{\text{SoilCO}_2}$) is the isotopic composition of soil-respired CO_2 ($\delta^{13}\text{C}_{\text{SR-CO}_2}$), where $\delta^{13}\text{C}_{\text{SR-CO}_2}$ is determined by the proportion of C_3 and C_4 plant biomass present (Cerling 1984, 1992a). At lower respiration rates ($<10^{-6}$ mole cm^{-2} year $^{-1}$), $\delta^{13}\text{C}_{\text{SoilCO}_2}$ becomes more enriched owing to a greater influence of atmospheric CO_2 in $\delta^{13}\text{C}_{\text{SoilCO}_2}$ (Cerling 1984). Accordingly, only soils with adequately high respiration rates can be used as a proxy for $\delta^{13}\text{C}_{\text{SR-CO}_2}$. In modern systems, the $\delta^{13}\text{C}$ of soil carbonates associated with C_3 ecosystems range between -14‰ to -8‰ , with the most ^{13}C -enriched values associated with highly water-stressed C_3 plants under arid conditions (Quade et al. 1989a). Thus, a pure C_3 end-member value of -8‰ is extremely conservative but can be used as an end-member value above which indicates C_4 input.

Numerous soil carbonate $\delta^{13}\text{C}$ records ($\delta^{13}\text{C}_{\text{SC}}$) have been used to evaluate the Tertiary history of C_4 photosynthesis (Quade et al. 1989b, 1994; Cerling & Quade 1993; Cerling 1992b; Cerling et al. 1993; Kingston et al. 1994; Latorre et al. 1997; Fox & Koch 2003, 2004). In general, interpretations of $\delta^{13}\text{C}_{\text{SC}}$ data assume soil respiration rates where high and $\delta^{13}\text{C}_{\text{CO}_2}$ values were similar to preindustrial values. Perhaps the most well-known records derive from the Potwar Plateau of Pakistan, which include the Siwalik Group (Quade et al. 1989b, Quade & Cerling 1995). Paleosol carbonates from the Siwalik paleosols record an abrupt positive $\delta^{13}\text{C}$ shift (**Figure 5**) by 7.7 Ma, indicative of a transition from C_3 to C_4 grasses (Quade et al. 1989b, Quade & Cerling 1995). Associated with this $\delta^{13}\text{C}$ shift is a gradual positive trend in $\delta^{18}\text{O}$ values, implying long-term changes in the hydrologic cycle (**Figure 5**) (Quade et al. 1989b, Quade & Cerling 1995). In particular, the $\delta^{18}\text{O}$ shift has been interpreted to reflect monsoonal intensification during the late Miocene that propelled the expansion of C_4 grasses in the region (Quade et al. 1989b). If valid, then monsoonal intensification began ~ 2 million years earlier than the expansion of C_4 grasses, and continued into the Pliocene. Further, if monsoonal intensification was the primary driver for C_4 plant expansion, its expression was regionally heterogeneous. For example, Nepalese Siwalik $\delta^{13}\text{C}_{\text{SC}}$ and $\delta^{18}\text{O}_{\text{SC}}$ records from ~ 500 km to the east of the Pakistan record show similar trends in magnitude and abruptness of isotope shifts (**Figure 5**) (Quade et al. 1995). However, southern Nepal records indicate C_4 plants

Figure 5

Carbon (*circles*) and oxygen (*triangles*) isotopic values of paleosol soil carbonates from North America, Pakistan, Nepal, East Africa, and South America for the past 20 million years. Data compiled from Quade et al. (1989b, 1995), Cerling (1992b); Kingston et al. (1994); Quade & Cerling (1995); Latorre et al. (1997); Kleinert & Strecker (2001); Fox & Koch (2003, 2004). Conservative (-8‰) and average (-12‰) soil carbonate values for a pure C_3 ecosystem represented by the dotted and dashed lines, respectively. Isotopic values represent the end-member compositions of C_3 plants assuming both extreme water stress and average conditions. Values more positive than the end-members represent some contribution of C_4 biomass influence. In addition, estimates assume the isotopic composition of atmospheric CO_2 was similar to preindustrial values (-6.5‰) and soil respiration rates were high. Map data from ERSI.



PCQ: Puerta de Corral
Quemado, Argentina

SMV: Santa Maria Valley,
Argentina

expanded nearly a million years later (~ 7.0 Ma) than those in Pakistan (Quade et al. 1995). Whether or not monsoonal intensification was the primary driver, other data in addition to $\delta^{18}\text{O}_{\text{SC}}$ records, including paleobotanical and palynological evidence, support regional aridification in the Himalayan foothills, implicating changes in the hydrologic cycle as a mechanism of ecological change (Quade et al. 1995).

Cerling (1992b) expanded the Neogene record with a spatially diverse study that included six Kenyan and two Tanzanian hominid localities (**Figure 5**). $\delta^{13}\text{C}_{\text{SC}}$ records from East Africa become more ^{13}C -enriched from the middle Miocene to the present, consistent with an increase in the proportion of C_4 biomass. Data older than 10 Ma suggest that the local ecosystem was populated by C_3 vegetation, whereas soils younger than 10 Ma were predominately composed (up to 70%) of C_4 plants, with the highest percentages in the Late Pleistocene. As with the Siwalik paleosols, East African $\delta^{18}\text{O}_{\text{SC}}$ records document a positive shift in $\delta^{13}\text{C}_{\text{SC}}$, suggesting that drier climates became increasingly prevalent (**Figure 5**).

There are other East African sites, such as the Tugen Hills site in Kenya [just ~ 50 km from several localities in the Cerling (1992b) survey], that record no change in plant ecology during the late Miocene (**Figure 5**). The Tugen Hills record is unique in that it represents a continuous sampling from one locality (Kingston et al. 1994). Tugen Hills paleosol $\delta^{13}\text{C}_{\text{SC}}$ values represent a 15.3-million-year record of a mixed C_3 and C_4 mosaic ecosystem, with C_4 plants composing up to 30% of the East African paleoplant community, assuming preindustrial values for $\delta^{13}\text{C}_{\text{CO}_2}$. This, along with other East African paleosol and tooth enamel data (Cerling 1992b, Morgan et al. 1994, Cerling et al. 1997), indicates that the history of C_4 plants in East Africa was spatially heterogeneous, but C_4 plants were likely an important component of the East African plant community prior to the global expansion at ~ 7 Ma.

The spatial variability of C_4 distributions during the Miocene appears to have been a general characteristic beyond Africa and Asia. South and North American isotope records both indicate important C_4 contributions prior to the late Miocene C_4 expansion. Data from Puerta de Corral Quemado, Argentina (PCQ), that date from 9.1 to 3.5 Ma indicate that the presence of C_4 plants increased in association with drier climates (Latorre et al. 1997). Further, the C_4 transition near ~ 7 Ma is muted ($<1\%$ shift in $\delta^{13}\text{C}_{\text{SC}}$) compared with Asia, and it continued into the Pliocene with increased C_4 contributions between 3.7 and 3.9 Ma (**Figure 5**) (Latorre et al. 1997). Nonetheless, the PCQ record crosses the -8% C_4 -endmember threshold near 7 Ma, suggesting global linkages in the expansion of C_4 flora at this time.

As with Africa and Asia, it appears that local and regional climates influenced the appearance and distribution of C_4 flora in the Americas. For example, paleosol $\delta^{13}\text{C}_{\text{SC}}$ and $\delta^{18}\text{O}_{\text{SC}}$ records from Santa Maria Valley, Argentina (SMV), located within 100 km from the PCQ site, indicate that the abundance of C_4 plants was much higher relative to PCQ before 7 Ma (**Figure 5**) (Kleinert & Strecker 2001). SMV $\delta^{13}\text{C}_{\text{SC}}$ values prior to 10.5 Ma indicate that C_4 plants represented $\sim 20\%$ or more of the plant population (calculated with modern C_3 and C_4 soil carbonate end-member estimates). The influence of C_4 flora at SMV varied with time, with declining percentages between 10.5 and 9.5 Ma and increases between 7.0 and 5.5 Ma, similar to the PCQ and East African records (Cerling 1992b, Latorre et al. 1997, Kleinert & Strecker 2001). By

the early Pliocene, SMV returned to a C₃-dominated ecosystem (Kleinert & Strecker 2001), while the data from PCQ supports an increased C₄ presence (Latorre et al. 1997).

$\delta^{13}\text{C}_{\text{SC}}$ records from numerous localities in the North American Great Plains clearly show that C₄ plants made up a sizable component of the plant community throughout much of the Miocene (**Figure 5**) (Fox & Koch 2003, 2004). Assuming a preindustrial $\delta^{13}\text{C}_{\text{CO}_2}$ value, $\delta^{13}\text{C}_{\text{SC}}$ records from 28 sediment sections suggest more than 20% C₄ plant biomass (Fox & Koch 2003, 2004).

In summary, well-distributed $\delta^{13}\text{C}_{\text{SC}}$ records from four continents indicate that the expansion of C₄ plants during the Miocene was not globally synchronous. African and North and South American $\delta^{13}\text{C}_{\text{SC}}$ records clearly indicate C₄ plants made up sizable constituents of the terrestrial ecosystem prior to 7 Ma.

Paleosol carbonate $\delta^{13}\text{C}_{\text{SC}}$ records are inherently site-specific compared with tooth enamel $\delta^{13}\text{C}_{\text{enamel}}$ records that integrate a much greater spatial area across which an animal travels. However, $\delta^{13}\text{C}_{\text{SC}}$ records are not intrinsically biased toward a particular plant type, which occurs in enamel records owing to feeding selectivity, and thus provide a more integrated isotope signal of the actual plant community. As with any isotope proxy, diagenesis is a major concern and paleosol carbonates are susceptible to diagenetic alteration and recrystallization during burial (Koch 1998). In addition, special care must be taken to sample only pedogenetic carbonate material that was formed in situ in the soil horizon. Notably, the results of several $\delta^{13}\text{C}_{\text{SC}}$ records are potentially compromised by isotopic alteration (Kingston et al. 1994, Kleinert & Strecker 2001). Nonetheless, taken together, the $\delta^{13}\text{C}_{\text{SC}}$ records are consistent with $\delta^{13}\text{C}_{\text{enamel}}$ records and point toward a complex rise of C₄ plant-dominated communities on different continents with an important transition from C₃- to C₄-dominated ecosystems between 8–4 Ma.

Compound-Specific Isotopes Analysis

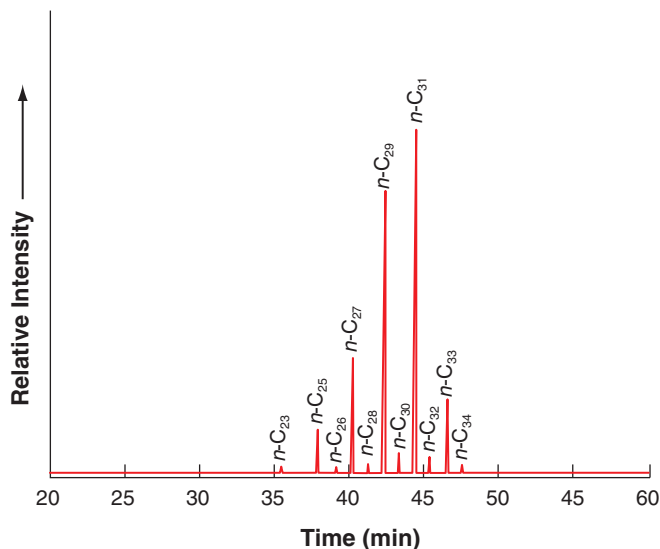
Important advancements are being made in paleoecology with the recent advent of compound-specific carbon isotope analysis (CSIA). This approach targets refractory compounds or biomarkers from higher plants that have widespread distributions in terrestrial, lacustrine, and marine sediments. In this regard, leaf wax lipids are ubiquitously distributed in the sediment record and are largely resistant to diagenetic alteration. In contrast to site-specific tooth enamel or soil carbonate records, *n*-alkanes transported to marine basins represent a regionally integrated signal of the terrestrial ecosystem. Thus, $\delta^{13}\text{C}_{\text{alkane}}$ records have the potential of revealing a much larger regional history of C₄ plant dynamics.

Normal alkanes (*n*-alkanes) are straight-chain, saturated hydrocarbons that are common components in terrestrial plant leaf waxes. On average, *n*-alkanes make up the majority of the total lipid component of epicuticular waxes (Eglinton & Hamilton 1967). High-molecular-weight *n*-alkanes with chain lengths of 25 to 35 carbons (*n*-C₂₅–*n*-C₃₅) and odd-over-even carbon-number distributions (**Figure 6**) are widely ascribed to epicuticular waxes from terrestrial vascular plants. The odd-over-even nature of terrestrial plant *n*-alkanes is a function of the palmitic acid

CSIA: compound-specific isotope analysis

Figure 6

Gas chromatograph trace of higher plant-derived *n*-alkanes extracted from a modern leaf. Compound distributions are similar to *n*-alkanes extracted from sediments, where the odd-over-even predominance is used to identify unaltered higher plant material.



elongation-decarboxylation mechanism that preferentially produces odd carbon-chain length *n*-alkane molecules for incorporation into epicuticular leaf waxes (Monson & Hayes 1982).

$\delta^{13}\text{C}$ values of organic compounds from higher plants, such as *n*-alkanes, are a function of the $\delta^{13}\text{C}$ of carbon dioxide, isotope fractionations associated with plant physiology, and biosynthesis (Hayes 1993):

$$\delta^{13}\text{C}_{\text{lipid}} = \delta^{13}\text{C}_{\text{plant}} - \varepsilon_{\text{l-p}}, \quad (5)$$

where the apparent fractionation between *n*-alkanes and bulk organic matter ($\varepsilon_{\text{l-p}}$) is between 5‰–7‰ and 8‰–10‰ for C_3 and C_4 flora, respectively (Collister et al. 1994, Chikaraishi & Naraoka 2001). Therefore, *n*-alkane $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{alkane}}$) values from modern plants should average $\sim -36\text{‰}$ and -24‰ for C_3 and C_4 flora, respectively. Indeed, analyses of *n*-alkanes from modern leaf waxes indicate average values of -36‰ and -20‰ for C_3 and C_4 species, respectively (Collister et al. 1994, Chikaraishi & Naraoka 2001). From the available data, the variation in $\varepsilon_{\text{l-p}}$ is likely species and/or environmentally dependent (Lockheart et al. 1997). However, as with other plant $\delta^{13}\text{C}$ records, these environmental factors primarily affect C_3 flora. These influences, as well as the history of $\delta^{13}\text{C}_{\text{CO}_2}$, must be considered to refine paleoecologic interpretations.

Freeman & Colarusso (2001) provide clear evidence that $\delta^{13}\text{C}_{\text{alkane}}$ from the Late Miocene Siwalik paleosols and Bengal Fan sediments is similar in magnitude to the carbon isotopic shift recorded in Pakistani soil carbonate and bioapatite $\delta^{13}\text{C}$ records (Figure 7). However, $\delta^{13}\text{C}_{\text{alkane}}$ values from Siwalik sediments shift from a C_3 -dominated to C_4 -dominated ecosystem as early as 9 Ma—2 million years prior to the change recorded in Pakistani soil carbonates and fossil teeth records—and do not reach the most ^{13}C -enriched values until the Pliocene. Early to middle Miocene $\delta^{13}\text{C}_{\text{alkane}}$ records from the Indian and Atlantic Oceans have values similar to those

from the Bengal Fan during the latest Miocene/Pliocene (**Figure 7**) (Pagani et al. 2000, Freeman & Colarusso 2001). Assuming that Miocene $\delta^{13}\text{C}_{\text{CO}_2}$ was similar to preindustrial values, with average C_3 and C_4 isotopic end-members of -32‰ and -20‰ , respectively, we estimate that approximately 30% of terrestrial organic matter delivered to the deep ocean was derived from C_4 plants. If the most conservative known C_3 *n*-alkane end-member (-27‰ ; N. Pedentchouk, personal communication, 2006), indicative of extremely water-stressed conditions, is applied, only then can any C_4 plant influence be ruled out.

Again, the results from these limited studies suggest that C_4 flora either represented a substantial component of plant ecosystems prior to the late Miocene C_4 expansion or that C_3 plants were subjected to highly water-stressed conditions.

EVOLUTIONARY ORIGINS

Patterns in both C_4 plant form and distribution suggest an evolutionary selection for a photosynthetic system adapted to overcome increased photorespiration from a C_3 ancestor (Ehleringer et al. 1997). This reasoning promotes the supposition that the evolutionary patterns in C_4 plants are primarily a reflection of changes in the partial pressure of CO_2 ($p\text{CO}_2$) where critically low $p\text{CO}_2$ levels selected for CO_2 -concentrating mechanisms to overcome photorespiratory stresses.

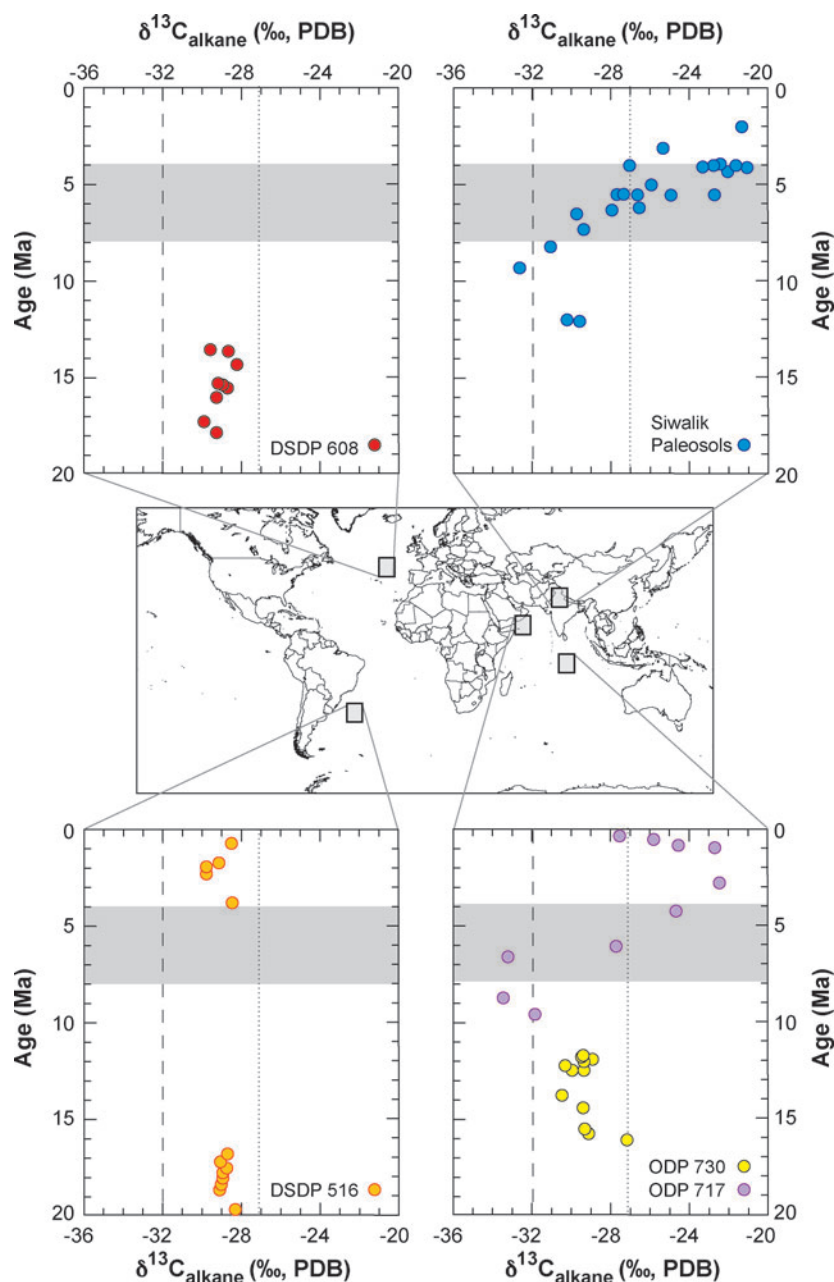
The possibility that the C_4 pathway evolved as a response to low CO_2/O_2 conditions is a predominant supposition in the field. Because photorespiration does not occur in C_4 plants, comparisons between C_3 and C_4 plant photosynthetic efficiency can be made by comparing quantum yield (i.e., light-use efficiency), which is primarily a function of temperature.

For a C_3 plant, quantum yield increases with increased atmospheric CO_2/O_2 ; however even at high CO_2/O_2 ratios, as temperature increases, quantum yield declines (Ehleringer et al. 1991). In contrast, the quantum yield for a C_4 plant is unaffected by changing temperature or CO_2/O_2 ratios. So at any given atmospheric CO_2 concentration, temperature controls the cross-over point when C_4 plants are favored over C_3 plants (Ehleringer et al. 1991).

The quantum yield model predicts that a drop in CO_2 concentration below a critical threshold would have provided strong selective pressure to reduce photorespiration in C_3 plants (Ehleringer et al. 1991) and, at any given CO_2 level, predicts that C_4 plants are more likely to occur in habitats with a high growing season temperature (Ehleringer et al. 1997). Indeed, numerous modern studies have shown C_4 plant distributions are strongly correlated to a warm growing season (Teeri & Stowe 1976, Ehleringer 1978). In addition to a warm growing season, C_4 taxa favor warm-season precipitation (Teeri & Stowe 1976). Changes in both seasonality and CO_2 have been implied as forcing mechanisms to account for C_4 plant distributions during the Last Glacial Maximum (LGM) (Huang et al. 1999; 2001). However, global vegetation model simulations of the LGM demonstrate that climate alone (temperature precipitation and modern CO_2 levels) cannot account for the plant biome changes observed in paleorecords (Harrison & Prentice 2003). When lower $p\text{CO}_2$ (200 ppmV) is included in model simulations, large changes in plant ecosystems are predicted with

Figure 7

Average $\delta^{13}\text{C}$ values of C_{27} , C_{29} , and C_{31} n -alkanes from DSDP sites 516 and 608, ODP sites 717 and 730, and Siwalik paleosols spanning the past 20 million years. Data gathered from Pagani et al. (2000) and Freeman & Colarusso (2001). Conservative (-27‰) and average (-32‰) values for a pure C_3 ecosystem represented by the dotted and dashed lines, respectively. Isotopic end-members assume the isotopic composition of atmospheric CO_2 was similar to preindustrial values (-6.5‰). Map data from ERSI.



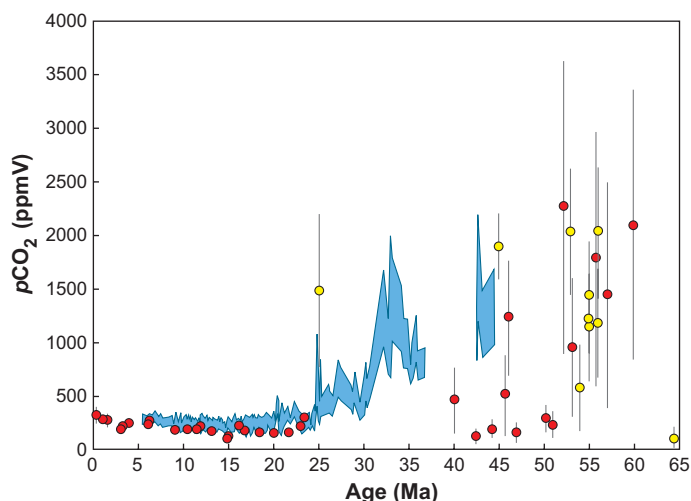


Figure 8

A compilation of paleoatmospheric CO₂ proxy records spanning the Cenozoic (Eckart et al. 1999, Pagani et al. 1999, Pearson & Palmer 2000, Royer et al. 2004). Red circles with error estimates are from boron isotope-based CO₂ proxy data (Pearson & Palmer 2000), yellow circles with error bars are pCO₂ estimates based on pedogenic carbonate records (Eckart et al. 1999, Royer et al. 2004), and the blue field represents the upper and lower pCO₂ estimates from alkenone-pCO₂ proxy records (Pagani et al. 1999, 2005; Henderiks & M. Pagani, unpublished data). Neogene-age soil carbonate-derived CO₂ estimates were excluded because error estimates (>250 ppmV) are much larger relative to the other proxy methods.

an expansion of grasslands and deserts at the expense of tropical forests and savannas (Harrison & Prentice 2003). These findings agree with predictions of quantum yield model where C₃ forests give way to C₄ grasslands during the LGM under a CO₂ concentration of ~200 ppmV (Ehleringer 2005).

Results from paleo-CO₂ reconstructions support higher pCO₂ levels prior to ~30 Ma (Cerling 1991b, Eckart et al. 1999, Pearson & Palmer 2000, Royer et al. 2004, Pagani et al. 2005), with a precipitous drop in pCO₂ during the Oligocene that approached modern levels by the earliest Miocene (**Figure 8**) (Pagani et al. 2005). Carbon dioxide levels during the Miocene remained low and relatively stable with concentrations similar to the present (Pagani et al. 1999, Pearson & Palmer 2000, Pagani et al. 2005). Under high CO₂/O₂ conditions during the Eocene and early Oligocene, photorespiratory stresses would have been at a minimum and favored the C₃ pathway. However, low atmospheric CO₂ conditions during the late Oligocene would have favored the C₄ pathway owing to a substantial increase in photorespiratory stresses specific to the C₃ pathway. Interestingly, molecular clock studies place the origin of the C₄ pathway between 25–33 Ma, during the time that CO₂ fell to near-modern concentrations (Gaut & Doebley 1997, Pagani et al. 2005).

Support for a middle Oligocene to early Miocene appearance of the C₄ pathway is growing from recent results from tooth enamel (Morgan et al. 1994, Passey et al. 2002), soil carbonate (Kleinert & Strecker 2001, Fox & Koch 2003, 2004), and

n-alkane analyses (Pagani et al. 2000). If an early origin for C₄ photosynthesis is valid, then the late Miocene C₄ grass expansion likely represents changes in ecological conditions on a near-global scale. Initially, Pagani et al. 1999 argued for an increase in aridity under low CO₂ conditions as a mechanism forcing C₄ plant expansion. However, a variety of environmental disturbances can influence plant distributions. In general, increased habitat disturbance (e.g., fires, herbivory, canopy clearing) favors fast-growing, colonizing plant communities, which under warm-season precipitation regimes and modern CO₂ conditions selects strongly for C₄ grasses (D'Antonio & Vitousek 1992, Cerling & Harris 1999, Heckathorn et al. 1999, Sage 2001, Bond et al. 2005).

An emerging hypothesis argues that the late Miocene C₄ grass expansion represents an ecological response to disturbance rather than a change in specific atmospheric conditions (Pagani et al. 1999; Keeley & Rundel 2003, 2005; Osbourne & Beerling 2006). In support of this supposition, a limited number of deep-sea records indicate that the flux of black carbon, indicative of fire disturbance, increased into the North Pacific Ocean during the late Miocene (Herring 1985). However, increased black carbon flux to the oceans can also be interpreted as an increase in black carbon delivery owing to greater wind intensity. Increased wind intensity during the late Miocene can be inferred by an increase in dust deposition in deep-sea records (Rea 1994, Rea et al. 1998), but could also reflect an increase of continental aridity (consistent with $\delta^{18}\text{O}_{\text{SC}}$ records from Asia, Africa, and the Americas). Although multiple interpretations are possible, wind intensity, aridity, and fire occurrence are not mutually exclusive. If average wind strength increased, so would fire intensity (Stocks & Kauffman 1997), especially under drier conditions, resulting in increased clearing of large tree stands and the inhibition of recolonization (Puyravaud et al. 1995, Dowciak et al. 2005).

In summary, it is possible that the late Miocene C₄ expansion represents an ecological response to increased fire disturbance propagated by changes in regional precipitation, as well as an increase in photorespiratory stresses associated with lower humidity and water stress during the late Miocene.

SYNTHESIS

Under modern CO₂ levels, the abundance of C₄ in a particular environment is positively correlated to high growing season temperatures and warm-season precipitation. This is predicted based on differences in the quantum yield between C₃ and C₄ plants. In addition, environmental disturbance also selects for C₄ plants. These climatic and nonclimatic ecological factors, however, appear secondary to lower CO₂ levels as the most important driver of C₄ viability when compared to C₃ plants.

Paleo-CO₂ proxies indicate that prior to the Oligocene, *p*CO₂ levels were several times higher than modern levels. Under these conditions, the C₃ photosynthetic pathway would have been favored, as predicted by quantum yield models. Indeed, no unequivocal evidence has been found for C₄ plants or C₄-dominated environments prior to the Oligocene. However, by the Oligocene/Miocene boundary, ~24 million years ago, *p*CO₂ appears to have fallen to near-modern levels. Under such conditions,

a plant possessing a CO₂-concentrating mechanism would have an adaptive advantage over the C₃ photosynthetic pathway, and limited molecular clock studies indicate that the C₄ photosynthetic pathway diverged during this time. To date, no physical or isotopic evidence supporting the origin of C₄ plants has been found during the latest Oligocene/earliest Miocene. However, by the mid-Miocene, multiple isotope C₄ plant proxy records indicate minor to substantial contributions from C₄ plants.

During the late Miocene, a well-documented C₄ expansion is recorded in a variety of proxy records. Initially, the late Miocene expansion appeared globally synchronous; however, more detailed studies suggest that its expansion was regionally heterogeneous. C₄ plant proxy records point toward a complex rise of C₄ plant-dominated communities with spatial and temporal differences during the late Miocene. These variations were likely driven by local or regional environmental factors, including disturbance and changes in precipitation.

UNRESOLVED ISSUE

Cenozoic records of the stable carbon isotopic composition of paleoatmospheric CO₂ are needed to better estimate both C₃ and C₄ plant δ¹³C end-members.

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