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Climate Change and the Evolution of C_4 Photosynthesis

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Plants assimilate carbon by one of three photosynthetic pathways, commonly called the C_3, C_4, and CAM pathways. The C_4 photosynthetic pathway, found only among the angiosperms, represents a modification of C_3 metabolism that is most effective at low concentrations of CO_2. Today, C_4 plants are most common in hot, open ecosystems, and it is commonly felt that they evolved under these conditions. However, high light and high temperature, by themselves, are not sufficient to favor the evolution of C_4 photosynthesis at atmospheric CO_2 levels significantly above the current ambient values. A review of evidence suggests that C_4 plants evolved in response to a reduction in atmospheric CO_2 levels that began during the Cretaceous and continued until the Miocene.

Plants possessing the C_3 photosynthetic pathway dominate most terrestrial ecosystems[1], and account for about 85% of all plant species. About 10% of the earth's flora possess CAM photosynthesis, and commonly grow in xeric sites, such as deserts and epiphytic habitats[2]. C_4 plants dominate warm to hot, open sites, but on a floristic basis comprise the lowest percentage of the terrestrial flora. Tropical and temperate grasslands, with abundant warm-season precipitation, are dominated by C_4 species.

C_4 plants have great economic significance, both as crops and weeds[3]. For this reason, C_4 plants have undergone much scrutiny since the discovery of the C_4 pathway in the mid-1960s. Although many aspects of C_4 metabolism are now well understood[4], questions remain about the initial evolution and subsequent expansion of C_4 plants. It is commonly thought that hot, arid conditions have favored their evolution[4]. However, while such environments have been common throughout the earth's history, the evolution of C_4 plants appears to be more recent (see below). The performance of C_4 plants relative to C_3 plants is highly dependent on levels of atmospheric CO_2: low CO_2 conditions favor C_4 species and high CO_2 levels favor C_3 species[5]. Geological evidence indicates that it has been only during the past 50 to 60 million years that CO_2 levels have declined to sufficiently low concentrations that C_4 photosynthesis has an advantage over C_3 photosynthesis[6]. Here we discuss the evidence that the primary selective factor influencing the evolution of C_4 photosynthesis was changes in the atmospheric CO_2 concentration, rather than aridity or high temperatures.

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Inefficiency of carboxylation in C_3 photosynthesis

Net carbon fixation in C_3 photosynthetic organisms is catalysed by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco)[7]. Rubisco normally catalyses the reaction between atmospheric CO_2 and RuBP to produce two three-carbon phosphoglycerate molecules (PGA), which are then further metabolized to the major end products of photosynthesis. However, Rubisco can also catalyse the oxygenation of RuBP to form one PGA and one phosphoglycolate, and further metabolism of phosphoglycolate results in the release of CO_2; these activities constitute photorespiration, a process that reduces the overall efficiency of net photosynthesis.

The oxygenase activity of Rubisco occurs, despite the physiological costs involved, because of particular aspects of the carboxylation reaction mechanism. During the carboxylation of RuBP, an intermediate is formed that is susceptible to reaction with oxygen[7]. Thus, the oxygenase activity of Rubisco may not have any useful function, but is simply an inevitable consequence of the reaction mechanism under aerobic conditions[7]. As oxygen in the atmosphere increased because of photosynthesis, the photorespiratory pathway evolved to process phosphoglycolate and recycle as much fixed carbon as possible. CO_2 and O_2 are competitive substrates, but Rubisco has a

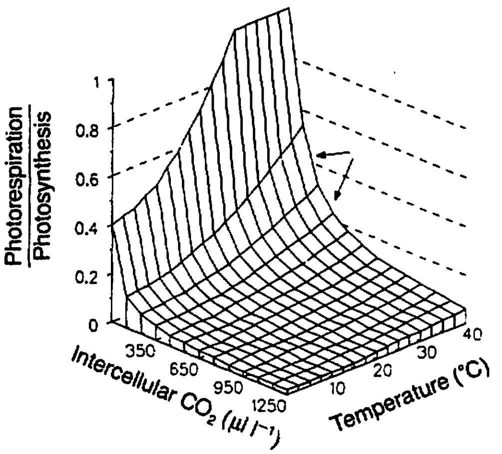

Fig. 1. Modelled CO_2 and temperature response of the ratio of photorespiration to the gross rate of photosynthesis in C_3 plants. Oxygen concentration was held constant at the current level of 21%. Arrows indicate the range of CO_2 concentrations typically occurring in leaves of C_3 plants under present atmospheric conditions. *Model derived from Ref. 11.*

much greater specificity for CO_2. Under current atmospheric conditions (350 $\mu l\ l^{-1}$ CO_2, 21% O_2, 78% N_2), however, the CO_2 concentration in the chloroplasts of C_3 leaves is approximately 1000 times less than that of O_2. This low CO_2:O_2 ratio allows a significant amount of photorespiration to occur, which reduces the efficiency of carboxylation during C_3 photosynthesis.

The ratio of photorespiration to photosynthesis is not fixed, but varies with environmental conditions. It is dependent on three factors: CO_2 concentration, O_2 concentration and leaf temperature. Increasing temperature reduces the specificity of Rubisco for CO_2 and decreases the concentration of CO_2 relative to O_2 within the chloroplast[8–10]. Using established equations[11], we can model the ratio of photorespiration to photosynthesis as a function of CO_2 and temperature, while keeping O_2 constant at current levels (Fig. 1). At either low temperature or high CO_2 concentration, photorespiration is minor. However, under present atmospheric CO_2 concentrations, photorespiration is a major component at moderate temperatures and becomes even greater as temperature is further increased. On the other hand, elevating CO_2 from the current ambient level of 350 $\mu l\ l^{-1}$ significantly reduces the rate of photorespiration. For example, a doubling of ambient CO_2, such as is anticipated to occur within the next 50–100 years, will result in approximately a 50% reduction in the rate of photorespiration. Increasing current atmospheric CO_2 levels five-fold would nearly eliminate photorespiratory activity in C_3 plants.

Benefits associated with C_4 photosynthesis

There are two mechanisms that can be used to improve the carboxylation:oxygenation ratio of Rubisco: increases in the CO_2/O_2 specificity of the enzyme and/or increases in the ratio of CO_2 to O_2 present at the enzyme's active site. There is evidence that the relative specificity of Rubisco for CO_2 is greater in angiosperms than in more primitive plant groups, but there is little variation in Rubisco's characteristics among vascular C_3 plants[7]. The most successful mechanism for reducing photorespiration and thus increasing carboxylation efficiency is the C_4 photosynthetic pathway.

In C_4 plants, atmospheric carbon is initially fixed in a reaction catalysed by phosphoenol pyruvate carboxylase (PEP carboxylase)[12]. This reaction takes place in mesophyll cells of C_4 plants where Rubisco is absent (Fig. 2). The resulting four-carbon organic acids are transported internally from the mesophyll to bundle sheath cells where they are decarboxylated to release CO_2. Chloroplasts in the bundle sheath cells contain Rubisco and fix the CO_2 released by C_4-acid decarboxylation using normal C_3 photosynthetic metabolism (Fig. 2). Since PEP carboxylase has a higher affinity for its substrate and a higher maximal velocity than Rubisco, the C_4 pathway acts as a CO_2-concentrating mechanism, increasing the CO_2 concentration within the bundle sheath cells. In plants using C_4 photosynthesis, mesophyll CO_2 concentrations are approximately 100 $\mu l\ l^{-1}$, whereas bundle sheath CO_2 concentration may be 10- to 20-fold higher[1]. The carboxylation efficiency of Rubisco is improved, therefore, and photorespiration becomes negligible in C_4 plants. Secondary improvements in water-use and nitrogen-use efficiencies will also occur in C_4 plants, associated with advantages of the CO_2-concentrating mechanism[1,13].

The presence of the C_4 photosynthetic pathway leads to a markedly different response of net photosynthesis to changes in atmospheric CO_2 or O_2 concentration than that found in C_3 plants[5,14]. While at low CO_2 concentrations C_4 plants typically have higher photosynthetic rates than C_3 plants, C_4 photosynthesis becomes saturated at concentrations above the current atmospheric levels, whereas C_3 photosynthesis does not (Fig. 3a). Also, because of Rubisco oxygenation and subsequent photorespiration, the quantum yield or light-use

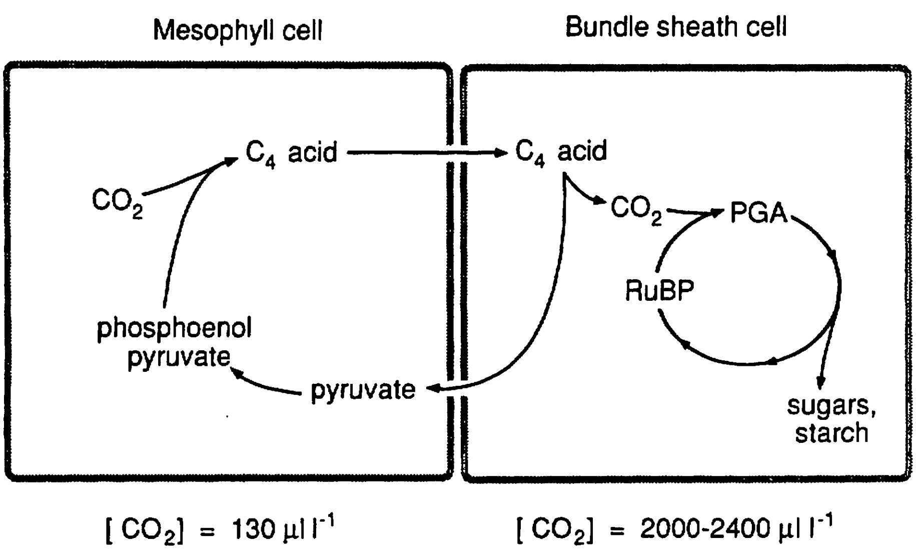

Fig. 2. Diagrammatic representation of C_4 photosynthesis. Atmospheric carbon is initially fixed inside leaf mesophyll cells in a reaction catalysed by phosphoenol pyruvate (PEP) carboxylase. The resulting C_4 acid is decarboxylated inside the bundle sheath cell, providing a source of CO_2 for ribulose-1,5-bisphosphate carboxylase (Rubisco) and the normal C_3 photosynthetic cycle. C_4 photosynthesis acts as a CO_2-concentrating mechanism. The CO_2 concentration inside the bundle sheath, where Rubisco functions, is 10- to 20-fold higher than the CO_2 concentration in the leaf mesophyll cells. PGA, phosphoglycerate; RuBP, ribulose bisphosphate.

efficiency of C_3 plants is strongly dependent on CO_2 (Fig. 3b). In C_4 plants, the light-use efficiency is essentially independent of atmospheric CO_2, but does not attain the maximum levels of C_3 plants because of the extra ATP costs of the CO_2-concentrating mechanism[15]. Under changing atmospheric CO_2 levels, though, it is clear that low CO_2 levels should favor C_4 photosynthesis whereas high CO_2 levels should favor C_3 photosynthesis. Studies of growth and competition between C_3 and C_4 plants under elevated CO_2 conditions have borne out this prediction[16–18].

Diversification of C_4 taxa in the plant kingdom

The C_4 photosynthetic pathway represents a modification of the C_3 photosynthetic cycle, and is, therefore, considered to be the derived, more evolutionarily advanced pathway. There are no enzymes or anatomical structures present in C_4 plants that are not already present in C_3 plants[19]. Thus, the evolution of the C_4 pathway from plants originally possessing the C_3 pathway appears to have involved relatively minor changes in enzyme characteristics, enzyme distribution and structural modification such as the enlargement of the bundle sheath cells. That these changes might have arisen easily and rapidly is suggested by the high degree of heritability of key aspects of C_4 metabolism in C_3–C_4 crosses[20–22], and by the large number of species that are anatomically and/or biochemically intermediate between C_3 and C_4 metabolism, especially in those genera such as *Moricandia* and *Parthenium* that have not yet developed C_4 taxa[23]. In addition, there is at least one species, *Eleocharis vivipara*, capable of expressing either C_3 or C_4 photosynthesis in different tissues depending on environmental conditions[24].

Because C_4 photosynthesis occurs in diverse, distantly related angiosperms with no common C_4 ancestors, it is likely that C_4 photosynthesis evolved independently many times[4,19] (Fig. 4). C_4 photosynthesis is present in at least 18 families, and may have evolved independently in all of them, since each family contains both C_3 and C_4 genera[25,26]. Even within genera, C_4 photosynthesis may have evolved independently several times. For example, in the grass genus *Panicum*, three species exhibit enough biochemical variation in C_4 photosynthesis to indicate separate origins[19]. It is likely that C_4 photosynthesis developed in many different regions simultaneously, perhaps in response to some common change in the global environment.

Conditions favoring the evolution of C_4 photosynthesis

Primitive photosynthetic organisms evolved in an atmosphere in which the concentration of CO_2 was high and the concentration of O_2 was minimal. In this environment, the oxygenase activity of Rubisco would have been insignificant[27]. As long as CO_2 concentration remained high, oxygenase activity and photorespiration would have been minor components (Fig. 1), even as atmospheric O_2 concentration increased

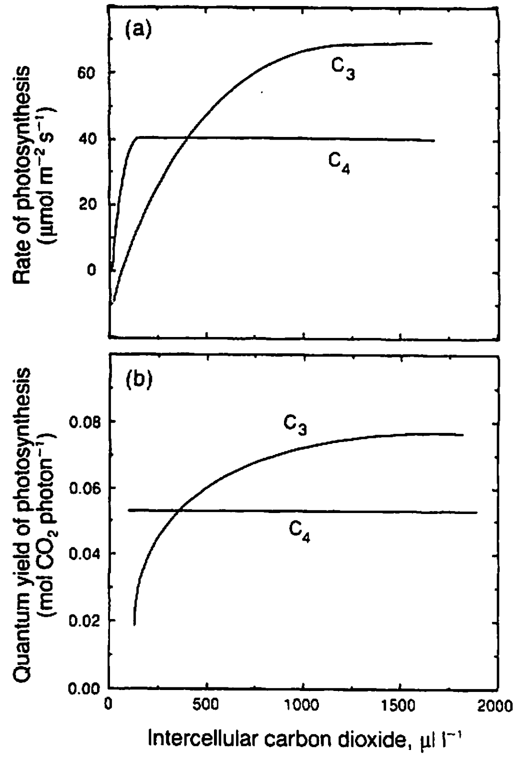

Fig. 3. Effect of CO_2 concentration on the absolute rate of photosynthesis (a) and the quantum yield of photosynthesis or light-use efficiency (b) in C_3 and C_4 plants. *Redrawn from Refs 5 and 15.*

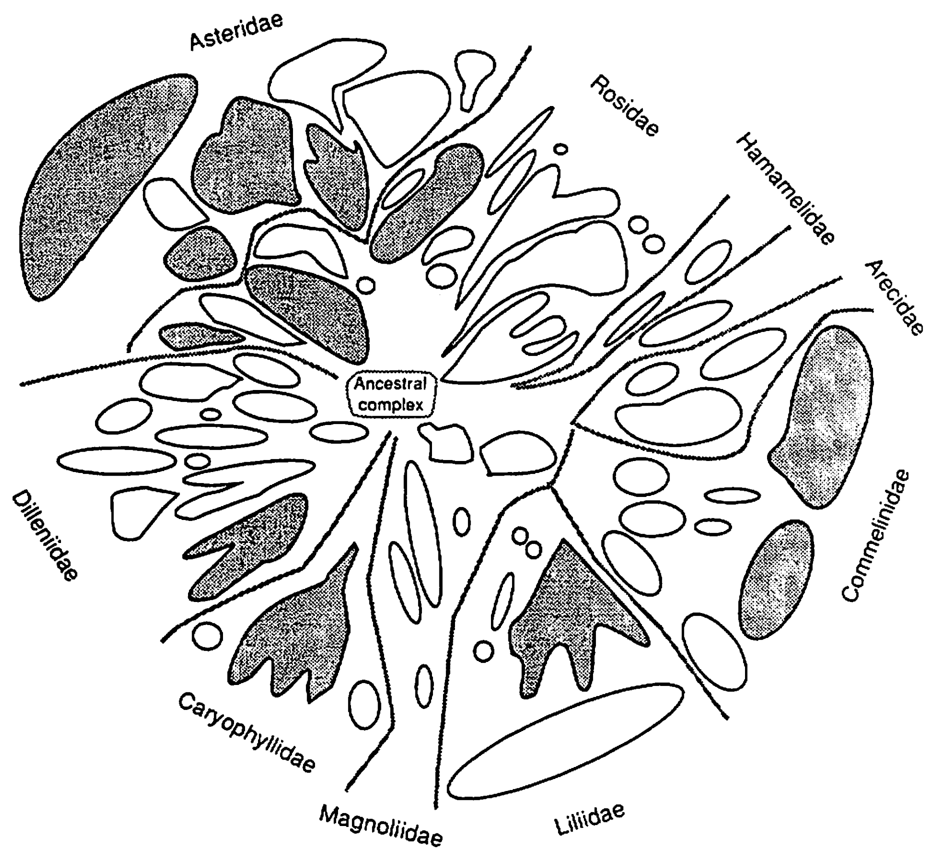

Fig. 4. Distribution of C_4 plants among the plant kingdom. The diagram is arranged so that primitive, ancestral plant groups are located near the center, with the more advanced, derived plant groups radiating out from the center. Plant groups containing C_4 species are shaded. *Adapted from Refs 42 and 43.*

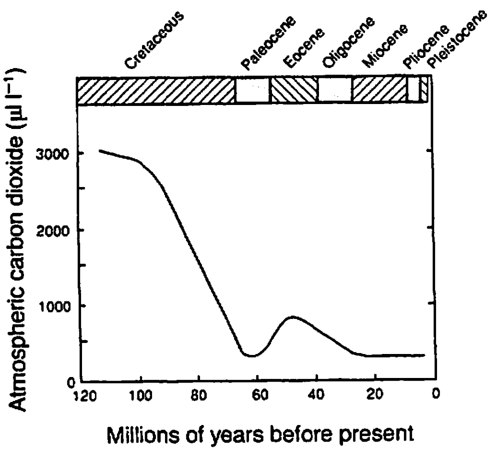

Fig. 5. Modelled change in atmospheric CO_2 concentration during the past 100 million years. *Based on data from Ref. 6.*

during the Paleozoic. A reduction in global CO_2 concentration to present-day levels, however, would result in a significant loss in carboxylation efficiency. The major selective force for the evolution of the C_4 pathway may have been an atmosphere with greatly reduced CO_2 levels.

It is well established that atmospheric CO_2 concentration has undergone dramatic changes throughout geological time[28–30,44,45]. When were CO_2 levels high enough to favor C_3 photosynthesis over C_4 photosynthesis? Estimates of atmospheric CO_2 concentrations during the Pleistocene and prior epochs are not precise, but there is general agreement that atmospheric CO_2 levels were at times significantly higher than they are today[28–30]. Claims that gases trapped inside amber could provide information about the CO_2 concentration in ancient atmospheres have proven unfounded[31]. Instead, we must rely on geological carbon-cycle models to estimate long-term changes in global, atmospheric CO_2 concentration.

Lasaga *et al.* estimate that atmospheric CO_2 levels were over 3000 µl l^{-1} in the mid Cretaceous, about 100 million years ago[6] (Fig. 5). Other estimates for this period[28,30] suggest that CO_2 levels were near 1500–2100 µl l^{-1}. Atmospheric CO_2 levels declined abruptly at the end of the Cretaceous, and during the Paleocene approached current values (Fig. 5). Atmospheric CO_2 levels have remained relatively low over the past 50 million years, being near current atmospheric levels in the Paleocene and again in the Miocene, and about double current levels during the Oligocene and Eocene (Fig. 5). Atmospheric O_2 levels are thought to have remained relatively constant since the Cretaceous.

The rapid drop in CO_2 concentration during the period of major expansion of the angiosperms would have provided strong selection pressure for increased carboxylation efficiency in C_3 plants both in terms of reduced photorespiration and increased light-use efficiency. On the basis of the data in Fig. 3 and the model in Fig. 5, C_4 photosynthesis may have first appeared in the Paleocene, but may have remained suppressed during the periods of elevated CO_2 levels that are thought to have occurred in the Eocene and Oligocene. By the Miocene, however, atmospheric conditions would have again favored C_4 photosynthesis, and as shown below, there is strong evidence that C_4 species flourished by the late Miocene. Temperature increase alone probably did not play a major role in promoting C_4 evolution, primarily because at elevated CO_2 concentrations temperature does not affect the carboxylation to oxygenation ratio to the extent that C_4 photosynthesis would be favored (Fig. 1). In addition, mean global temperatures have actually declined since the mid-Cretaceous[6,32].

C_3–C_4 intermediate plants, such as *Flaveria ramosissima* and *F. brownii*, are believed to represent stages in the evolution of C_4 photosynthesis[23,33]. Apparent photorespiration rates of C_3–C_4 intermediates are reduced, but these plants have no other metabolic advantages over C_3 plants in terms of water-, light- or nitrogen-use efficiencies[23,34]. In most cases, reduced photorespiration rates in the C_3–C_4 intermediates result from the C_4 decarboxylase enzyme being confined to the cells of a rudimentary bundle sheath[35]. CO_2 that is released during photorespiration is then recycled within the bundle sheath cells, which reduces the photorespiration rate of C_3–C_4 intermediate plants. The initial step, therefore, during the evolution of C_4 photosynthesis from C_3 plants was a slightly improved carboxylation efficiency in C_3–C_4 intermediate plants[23,33]. This observation is consistent with the idea that C_4 photosynthesis evolved in response to a reduction in global CO_2 concentration because of the resulting improved carboxylation efficiency of the C_4 pathway.

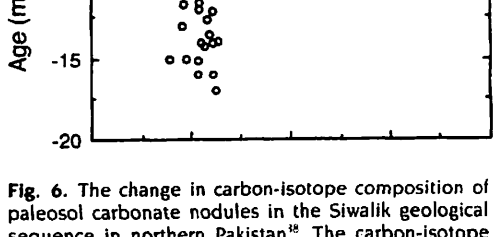

Fig. 6. The change in carbon-isotope composition of paleosol carbonate nodules in the Siwalik geological sequence in northern Pakistan[38]. The carbon-isotope composition of carbonate nodules is enriched approximately 14–16‰ above that of the carbon-isotope composition of plants growing in the soil at the time that carbonate is formed[39]. The negative $\delta^{13}C$ values before 7.5 million years ago indicate a C_3-dominated vegetation. The positive $\delta^{13}C$ values after 7.5 million years ago indicate a switch to a C_4-dominated flora.

Evidence for the appearance of C_4 plants

Geological evidence for the first appearance of C_4 plants is scanty, but what exists comes from two sources: (1) plant fossils with well-developed bundle sheath anatomy, and (2) the carbon-isotopic composition of fossil soil carbonate layers. Brown and Smith[36] *suggested* that plants with C_4 photosynthesis could have arisen as early as the Cretaceous, but fossil evidence is lacking. The oldest identifiable fossils with pronounced bundle sheath layers date from the late Miocene (approximately seven million years ago)[37].

The first clear indication of a shift in dominance from C_3 to C_4 plants comes from fossil carbonate layers in Pakistan[38]. Soil carbonate layers take on a carbon-isotopic composition related to that of the plants living on the site during carbonate formation[39]. Since C_3 and C_4 plants differ in carbon-isotopic composition[40], it is possible to document shifts in the abundance of C_3 and C_4 species within the flora from changes in the carbon-isotope ratios of fossilized carbonate layers. In Pakistan, an abrupt shift in the carbon-isotope composition of soil carbonate layers indicated a change from a C_3-dominated vegetation to a C_4-dominated flora during the Miocene, approximately 7.5 million years ago (Fig. 6). Similar shifts in the carbon-isotope composition of soil carbonate layers also appear during this same time period in locations in Africa and Nepal (J. Quade, pers. commun.). These dates are also consistent with pollen evidence, which indicates that the world's major grasslands developed during the Miocene[41]. The C_3 to C_4 vegetational change was associated with a local climatic shift as indicated by the oxygen-isotope composition of these paleosol carbonates. Quade *et al.*[38] have suggested that the changes in oxygen-isotope ratios are indicative of increased warm-season precipitation, perhaps associated with development of the Asian monsoon system in Pakistan.

Conclusions

While it is not currently possible to date the first appearance of C_4 photosynthesis precisely, it can be stated that C_4 photosynthesis has no identifiable advantage in an environment in which atmospheric CO_2 levels are significantly above current ambient levels of 350 $\mu l\ l^{-1}$, and that C_4 photosynthesis most likely evolved sometime after CO_2 levels first declined to near current levels in the Paleocene. Second, a rise in CO_2 levels during the Eocene and Oligocene may have restricted the occurrence of C_4 plants, but by the Miocene – when CO_2 levels were again reduced – C_4 plants flourished and dominated many grassland habitats. Further reductions in CO_2 levels during the Pleistocene would have further stimulated the spread of C_4 plants. It is now questionable whether the proliferation of C_4 plants will continue in an anthropogenically altered atmosphere.

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