

# EVOLUTIONARY AND ECOLOGICAL ASPECTS OF PHOTOSYNTHETIC PATHWAY VARIATION

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## *Abstract*

C<sub>4</sub> and CAM photosynthesis are evolutionarily derived from C<sub>3</sub> photosynthesis. The morphological and biochemical modifications necessary to achieve either C<sub>4</sub> or CAM photosynthesis are thought to have independently arisen numerous times within different higher plant taxa. It is thought that C<sub>4</sub> photosynthesis evolved in response to the low atmospheric CO<sub>2</sub> concentrations that arose sometime after the end of the Cretaceous. Low CO<sub>2</sub> concentrations result in significant increases in photorespiration of C<sub>3</sub> plants, reducing productivity; both C<sub>3</sub>-C<sub>4</sub> intermediate and C<sub>4</sub> plants exhibit reduced photorespiration rates. In contrast, it may be argued that CAM arose either in response to selection of increased water-use efficiency or for increased carbon gain. Globally, all three pathways are widely distributed today, with a tendency toward ecological adaptation of C<sub>4</sub> plants into warm, monsoonal climates and CAM plants into water-limited habitats. In an anthropogenically altered CO<sub>2</sub> environment, C<sub>4</sub> plants may lose their competitive advantage over C<sub>3</sub> plants.

## INTRODUCTION<sup>1</sup>

Three photosynthetic pathways occur among higher plants. The most common and most primitive of these is the  $C_3$  pathway, or Calvin-Benson cycle, in which the initial carboxylation reaction results in phosphoglyceric acid, a three-carbon acid. The  $C_4$  pathway, or Hatch-Slack cycle, is a more evolutionarily recent photosynthetic pathway, in which the initial carboxylation reaction results in oxaloacetate, a four-carbon acid. The third photosynthetic pathway, crassulacean acid metabolism (CAM), is biochemically similar to the  $C_4$  pathway in that the initial carboxylation reaction results in a four-carbon acid, but this pathway differs from that in  $C_4$  plants in structural features as well as in the temporal activity of the initial carboxylation reaction. Climate has a profound effect on the performance of each pathway, and consequently variations in environmental parameters result in different ecological distributions of the three photosynthetic pathways. Here we explore the evolution of photosynthetic pathways in response to climatic variation and the ecological consequences of photosynthetic pathway variation on plant performance as well as to community- and ecosystem-level dynamics.

## PHOTOSYNTHETIC PATHWAYS

### *$C_3$ , $C_4$ , and CAM pathways*

Carbon dioxide fixation in  $C_3$  photosynthetic organisms is catalyzed by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Normally ribulose-1,5-bisphosphate (RuBP) is combined with atmospheric  $CO_2$  by this enzyme to produce two molecules of phosphoglycerate (PGA), a three-carbon molecule. The products of the photosynthetic light reactions (ATP, NADPH) are then used to further reduce PGA into a series of intermediate products in the photosynthetic carbon reduction cycle (PCR), synthesizing reduced sugars for further plant metabolism and producing RuBP to allow the PCR cycle to continue. Under present atmospheric conditions, Rubisco also catalyzes the oxygenation of RuBP, in which  $O_2$  combines with RuBP to produce one PGA and one phosphoglycolate (143). Further metabolism of phosphoglycolate results in the release of  $CO_2$ . The oxygenation of RuBP and eventual release of  $CO_2$  is termed photorespiration, a process that reduces the overall efficiency of net photosynthesis (143).

While an evolutionary trend exists among photosynthetic organisms for increased specificity of the Rubisco carboxylation reaction, the oxygenation reaction continues because of the susceptibility of RuBP-intermediates to react

<sup>1</sup>Abbreviations: CAM, crassulacean acid metabolism; BSC, bundle sheath cells; PCA, photosynthetic carbon assimilation; PCR, photosynthetic carbon reduction

with diatomic oxygen ( $O_2$ ). The oxygenase reaction appears not to have any useful function but is likely to be an inevitable consequence of the reaction mechanism under aerobic conditions. Historically, as oxygen levels in the atmosphere increased, photorespiration may have evolved as a mechanism to process phosphoglycolate and recycle as much fixed carbon as possible.  $CO_2$  and  $O_2$  are competitive substrates, but Rubisco has a much greater specificity for  $CO_2$  (2).  $CO_2$  concentrations are reduced as it diffuses through the stomata to photosynthetic cells. Under today's atmospheric conditions (0.035%  $CO_2$ , 21%  $O_2$ , and 78%  $N_2$ ), the  $CO_2$  concentration in the chloroplasts of  $C_3$  plants is approximately 1000 times lower than that of  $O_2$ . This low  $CO_2/O_2$  ratio allows a significant amount of photorespiration to occur, reducing the overall efficiency of net photosynthesis in  $C_3$  plants by approximately one third.

In  $C_4$  plants, a simple change in expression of the  $C_3$  cycle is used to overcome the reduced photosynthetic efficiency associated with photorespiration. Instead of allowing the  $C_3$  photosynthesis cycle to occur in all photosynthetic cells, the  $C_3$  cycle is limited to selected interior cells, typically the bundle sheath cells (BSC). A layer of mesophyll cells surrounds these bundle sheath cells. Within the mesophyll cells is phosphoenolpyruvate (PEP) carboxylase, an enzyme that catalyzes the initial photosynthetic reaction. This reaction involves phosphoenolpyruvate and atmospheric  $CO_2$  as substrates to produce oxaloacetate, a four-carbon acid; hence the name  $C_4$  photosynthesis. The  $C_4$  acid diffuses from the mesophyll through plasmodesmata to the bundle sheath cells, where the  $C_4$  acid is decarboxylated. Since PEP carboxylase has a higher affinity for its substrate and a greater maximum velocity than Rubisco, the  $CO_2$  concentration in the bundle sheath cells ends up being significantly higher than that in either the mesophyll cells or the surrounding atmosphere. The consequence is that PEP carboxylase effectively serves as a  $CO_2$  pump, concentrating  $CO_2$  within the bundle sheath cells. This results in  $CO_2$  concentrations within the chloroplasts that are approximately 0.25–0.30%, an order of magnitude higher than in  $C_3$  plants. As a consequence, the Rubisco reactions in  $C_4$  plants take place in an atmosphere with a high  $CO_2/O_2$  ratio, and photorespiration is effectively eliminated. Critical to the functioning of the  $C_4$  cycle is a distinct spatial separation of the activities of Rubisco and PEP carboxylase (Kranz anatomy)—such that with PEP carboxylase activity located between atmospheric  $CO_2$  and Rubisco, it provides a pump to raise the  $CO_2$  concentrations internally. Three biochemical enzymatic mechanisms are used in  $C_4$  plants to achieve  $C_4$  acid decarboxylation in the BSC - NADP-malic enzyme (NADP-me), NAD malic enzyme (NAD-me), and PEP carboxykinase (PEP-ck). The utilization of the alternative decarboxylases is expressed in conservative patterns across phylogenies (44, 69).

CAM photosynthesis involves the same  $CO_2$ -concentrating mechanism as in  $C_4$  photosynthesis (PEP carboxylase). However, rather than a *spatial*

separation of the two carboxylation enzymes such as exists in  $C_4$  photosynthesis, there is a *temporal* separation of the two carboxylases in CAM, and both reactions occur within the same cell. In contrast to  $C_3$  and  $C_4$  plants, an inverted diurnal pattern of stomatal opening occurs in CAM plants. Stomata open during the evening, and atmospheric  $CO_2$  is fixed as a  $C_4$  acid (malate), which is then stored within the vacuole of the photosynthetic cell. During the following day, stomata remain closed, and malate is decarboxylated to release  $CO_2$ . The  $CO_2$  concentration within the cell remains high as  $CO_2$  cannot diffuse out through the closed stomata. As a result, Rubisco once again operates within a high  $CO_2/O_2$  environment, and photorespiration is eliminated. Thus, in CAM plants a temporal separation of the two carboxylase reactions occurs with Rubisco activity within the cell during the day and PEP carboxylase activity during the night.

The analysis of carbon isotopic ratio ( $\delta^{13}C$ ) of organic plant material is a powerful tool for distinguishing among photosynthetic pathways (55). Since the initial photosynthetic carboxylation reactions (PEP carboxylase in  $C_4$  and CAM plants or Rubisco in  $C_3$  plants) discriminate differentially against  $^{13}C$ , the isotopic composition of plant materials has proved to be a useful means of determining photosynthetic pathway differences among species and for addressing trophic-level interactions at the ecological level.  $C_4$  plants typically have a  $\delta^{13}C$  value of  $-13\text{‰}$  and  $C_3$  plants a value of  $-27\text{‰}$ . Further details of the biochemical and structural aspects of photosynthetic pathway variation appear in several recent reviews (55, 70, 92, 107, 143, 188).

### *Functional Consequences of Photosynthetic Pathway Differences*

**LIGHT-USE EFFICIENCY** The operation of the  $C_4$  cycle requires two additional ATP to reduce a  $CO_2$  molecule, with the additional ATP associated with the regeneration of phosphoenolpyruvate from pyruvate. Based on this, the quantum yield of photosynthesis or light-use efficiency (mol  $CO_2$  fixed per mol photon absorbed) should be lower in  $C_4$  plants than in  $C_3$  plants (46, 47, 96). However, in today's environment this is observed only at leaf temperatures below  $25\text{--}30^\circ C$ . This is because photorespiration in  $C_3$  plants increases with temperature, resulting in a continual decrease in light-use efficiency as leaf temperatures increase. Since photorespiration does not occur in either  $C_4$  or CAM plants, light-use efficiency remains constant as temperatures increase (46, 47, 83). Within natural canopies, leaf area development or overstory development is sufficiently high that light-use efficiency differences between  $C_3$ ,  $C_4$ , and CAM plants can play a role in determining overall rates of productivity.

Light-use efficiency of  $C_3$  plants will also be influenced by atmospheric



CO<sub>2</sub> levels (46, 47, 83), since photorespiration is directly related to the CO<sub>2</sub>/O<sub>2</sub> ratio. Over a range of 150–350  $\mu\text{L L}^{-1}$  CO<sub>2</sub> (= 0.015–0.035%), overall light-use efficiency will vary three-fold in C<sub>3</sub> plants. As is discussed further below, these atmospheric CO<sub>2</sub> concentrations are well within the known modern range of values (between glacial maxima 18,000 years ago and today's environment) and are likely to have had a significant effect on plant productivity and the competitive interactions between C<sub>3</sub> and C<sub>4</sub> plants.

**WATER-USE EFFICIENCY** The leaf epidermis imposes a diffusional barrier between the outside atmosphere and the chloroplast where photosynthesis is occurring. The stomata can be thought of as pores through this barrier, restricting both water vapor loss from the leaf and CO<sub>2</sub> diffusion into the leaf. One consequence of the CO<sub>2</sub>-concentrating mechanism in C<sub>4</sub> plants, however, is that the photosynthetic rate is largely independent of CO<sub>2</sub> concentration inside the leaf (117, 119). This implies that while changes in the degree of stomatal opening play a major role in affecting inward CO<sub>2</sub> diffusion, and thus photosynthetic rates in C<sub>3</sub> plants, they exert little influence on photosynthetic rates in C<sub>4</sub> plants over a broad range of stomatal opening. Yet in both C<sub>3</sub> and C<sub>4</sub> plants, rates of transpirational water loss are directly proportional to the degree of stomatal opening. The consequence is that water-use efficiency (the ratio of photosynthesis to transpirational water loss) is higher in C<sub>4</sub> plants than it is in C<sub>3</sub> plants. In effect, at equivalent rates of water loss, a C<sub>4</sub> leaf is expected to photosynthesize more than an adjacent C<sub>3</sub> leaf operating under the same set of environmental conditions.

Water-use efficiency, measured instantaneously at the leaf level or long-term under single-pot or field conditions, is always greater in C<sub>4</sub> plants than in C<sub>3</sub> plants. On an absolute scale, C<sub>4</sub> plants produce one gram of biomass for every 250–350 grams of water transpired, whereas in C<sub>3</sub> plants, this ratio is one gram of biomass produced for every 650–800 grams of water transpired (154). CAM plants exhibit even higher water-use efficiencies, because stomata open only at night, when the evaporative water loss gradients are less than during the day. Higher water-use efficiency may allow a decreased expenditure of water for a given amount of photosynthetic carbon gain. Under circumstances of limited soil moisture or where factors such as salinity may limit the capacity of a plant to extract water from the soil, an enhanced water-use efficiency may result in a competitive advantage.

**NITROGEN-USE EFFICIENCY** Rubisco represents a significant nitrogen investment, typically accounting for 25–30% of the total nitrogen in the leaf of C<sub>3</sub> plants (53). C<sub>4</sub> plants contain three to six times less Rubisco than C<sub>3</sub> plants (84, 139), and overall leaf nitrogen content is lower in C<sub>4</sub> plants (120–180 mmol N m<sup>-2</sup>) than in C<sub>3</sub> plants (200–260 mmol N m<sup>-2</sup>) (18, 137). Yet C<sub>4</sub>

plants exhibit equivalent or higher maximum photosynthetic rates than  $C_3$  plants (43, 117, 119). Thus,  $C_4$  plants are able to achieve higher nitrogen-use efficiencies (ratio of photosynthetic rate to nitrogen investment in the leaf) than are  $C_3$  plants (18, 19, 137, 138, 140, 186). Higher nitrogen-use efficiency may allow the plant the opportunity to allocate more nitrogen to the production of those structures associated with the increased capture of the resources that most limit overall plant growth. For instance, in soils that are nutrient deficient, allocating that nitrogen to increased root production would increase plant growth and presumably competitive ability.

## SELECTIVE PRESSURES AND EVOLUTION OF PHOTOSYNTHETIC PATHWAY VARIATION

### *CO<sub>2</sub>, O<sub>2</sub>, and Aridity as Selective Pressures for Photosynthetic Pathway Variation*

If the  $C_3$  cycle is the primitive or ancestral photosynthetic pathway and both the  $C_4$  cycle and CAM are evolutionarily more recent pathways, what selective factors are likely to have contributed to their evolution? To answer that question a logical starting point is to ask under what environmental conditions do plants with these derived pathways thrive today?  $C_4$  and CAM plants are most common in hot, arid climates (discussed in greater detail below)—environmental conditions that have existed since plants invaded land in the Upper Silurian. The extent of global arid zone development will be a function of the size of continental land masses, and GCM modeling studies indicate that arid regions have been common for at least the last 250 million years (7, 38). A second climatic factor associated with the distribution of many  $C_4$  and CAM plants is the summer monsoon. Conditions favoring the development of seasonal monsoonal air flows are likely to have always existed in subtropical and tropical land-sea interfaces. Yet Asian summer monsoons may have intensified only recently, following the extensive development of the Himalayas and Tibetan Plateau (33, 128).

Atmospheric carbon dioxide levels in the Phanerozoic are thought to have been substantially lower than at the time photosynthesis first appeared in primitive photosynthetic bacteria. Based on geochemical carbon balance models (11, 31, 32), estimates are that the atmospheric  $CO_2$  concentration during the Triassic, Jurassic, and Cretaceous may have been four to eight times greater than today. These models indicate that atmospheric  $CO_2$  levels dropped after the Cretaceous from levels of 1400 to 2800  $\mu L L^{-1}$  to values below 1000  $\mu L L^{-1}$  in the Eocene, Miocene, and Pliocene. Over the past 160,000 years, direct measurements (6) of the atmospheric air trapped as bubbles in ice cores reveal that  $CO_2$  levels have remained low, fluctuating

between 180 and 280  $\mu\text{L L}^{-1}$  during glacial and interglacial periods, respectively.

Two factors have contributed to decreasing atmospheric  $\text{CO}_2$  levels over geological time: the burying of organic matter as coals, oils, and peats, and silicate-rock weathering. The silicate-rock weathering-calcite reactions for recently exposed rock can be summarized as:



Over geological time, this process has been a major factor in the reduction of atmospheric  $\text{CO}_2$  levels, resulting in the burial of carbon dioxide as carbonates in deep ocean sediments. Raymo & Ruddiman (132) suggested that increased weathering rates since the Cretaceous have significantly reduced atmospheric  $\text{CO}_2$  levels. Increased global weathering has been associated with the collision of the Indian subcontinent with Asia and the lifting of the Tibetan Plateau, which exposed new, unweathered silicate rocks. The calcite formed in these reactions is transported to the ocean floor, where, over time, it is subducted with the movement of the continental plates. Sediment flows associated with erosion of the Himalayas and Tibetan Plateau are high and account for the primary global input into the oceans. Thus, collision of the Indian subcontinent with Asia and the associated mountain building may have been of sufficient magnitude to induce a global decrease in atmospheric  $\text{CO}_2$  levels that could ultimately have been the selective force for a modification on the basic mechanism of photosynthesis in land plants (e.g. evolution of  $\text{C}_4$  photosynthesis).

At the same time, atmospheric oxygen levels appear to have fluctuated little (12). Therefore, the geological trend has been one of continual decreases in atmospheric  $\text{CO}_2$  levels as well as continual decreases in the  $\text{CO}_2/\text{O}_2$  ratio. Both of these factors have a negative impact on  $\text{C}_3$  photosynthesis—partly because of a decreased  $\text{CO}_2$  diffusion gradient and partly because of increased photorespiration rates. It has been hypothesized that some time after the Cretaceous, atmospheric  $\text{CO}_2$  levels and the  $\text{CO}_2/\text{O}_2$  ratio had decreased sufficiently to favor the evolution of  $\text{C}_4$  photosynthesis (48). Warm temperatures alone in the past may not have been sufficient to favor the evolution of  $\text{C}_4$ , but the reduction in  $\text{CO}_2$  may have been the critical factor.

### *Geological Evidence of Photosynthetic Pathway Variation*

The scant fossil record of  $\text{C}_4$  plants sheds little light on the origins of  $\text{C}_4$  photosynthesis. The oldest known fossil evidence of  $\text{C}_4$  plants has been dated to the late Miocene, an epoch between 5 and 7 million years ago (106, 162). The oldest soil carbonate data indicating the presence of the  $\text{C}_4$  pathway also dates to this age (33, 34, 128), suggesting that  $\text{C}_4$  photosynthesis appeared in different global regions at approximately the same time.



Recent studies have used soil carbonate carbon-isotope composition as a means to reconstruct historical changes in the expansive grassland biomes of Pakistan and East Africa (32, 128). A notable result from these studies is the striking shift in carbon-isotope signature during the Miocene, indicating rapid ecosystem-level increases in the proportion of  $C_4$  grasses (34). Reductions in atmospheric  $CO_2$  concentrations during the Miocene coupled with the warm climates of these subtropical latitudes would result in substantial increases in photorespiration and reductions in the carboxylation efficiency of  $C_3$  plants. In such an environment, plants that utilize the  $C_4$  pathway, with their inherently higher carboxylation efficiency, would exhibit obvious advantages compared to  $C_3$  plants (48). Climate changes such as the evolution of the Asian monsoons (128), in addition to those of atmospheric  $CO_2$  concentration, may have played a role in triggering the  $C_3$  to  $C_4$  shifts. Measurements of oxygen-isotope ratios in the same carbonate samples provided evidence of a Miocene climate shift toward increased summer rains, coincident with the  $C_3$  to  $C_4$  shift and the elevation of the Himalayas (128). Given the greater growth potential for  $C_4$  grasses relative to  $C_3$  grasses when grown at warm temperatures and low  $CO_2$  concentration, increased rain during the warm season could have triggered an increased representation of  $C_4$  species in these grassland communities. In a separate study, the oxygen-isotope signal from East African carbonates revealed a shift toward a warmer, drier climate approximately 0.1 million years before the apparent shift from  $C_3$ -dominated grasslands to  $C_4$ -dominated grasslands (32). In this case, the higher water-use efficiency expressed by  $C_4$  plants may have provided them with a competitive advantage in a warmer, drier climate, shifting the community balance toward the  $C_4$  extreme.

Fossil evidence of CAM tissue has not been discovered. The oldest material of definitive CAM origins comes from 40,000 year old pack rat middens in the southwestern United States (170). Based on arguments of facilitation—the apparent ease with which CAM has developed from other metabolic processes (e.g. pH balance)—as well as the expression of CAM in such a broad diversity of taxa, Griffiths (60) concluded that the evolution of CAM predated the  $C_4$  pathway. CAM may date back to the Cretaceous, but based on its presence in ancient groups such as isoetids and possibly cycads (e.g. *Welwitschia*), it could date to the Triassic (60).

### *Evolution of Structure/Function Associations in $C_4$ and CAM Plants*

Evolution of the distinctive  $C_4$  architecture, wherein PCR tissue lies at the innermost extreme of the leaf's carbon assimilation tissue, can be explained as the most efficient arrangement for directional carbon flow through the



sequential  $C_3$  and  $C_4$  cycles from the atmosphere to the vascular cells. The intimate association of PCR cells with the leaf's vascular tissue would ensure efficient transport of photosynthate to various nonassimilatory sinks of the plant, and the close proximity of the mesophyll cell layer (functionally described as the photosynthetic carbon assimilation layer, PCA) to the atmosphere ensures the effective assimilation of atmospheric  $CO_2$ . Additionally, due to the unique "wreathed" or radial architecture of most  $C_4$  leaves, the PCR cells lie at the inner convergence of numerous PCA cells. This pattern provides for a "funneling" of  $CO_2$  to the photosynthetic reduction cycle and facilitates the high  $CO_2$  concentrations that are achieved in the PCR cells. Such explanations are easily accepted because they fit so comfortably within the established structure/function design of  $C_4$  photosynthesis. However, recent studies with the so-called  $C_3$ - $C_4$  intermediate species suggest that in some cases the unique architecture of  $C_4$  leaves evolved in response to the photorespiratory constraints imposed on the  $C_3$  photosynthetic system, not the photosynthetic constraints imposed by the requisite coordination of carbon transport between the  $C_4$  and  $C_3$  cycles (92, 98).

Photorespiratory  $CO_2$  loss in  $C_3$ - $C_4$  intermediate leaves is reduced, compared to  $C_3$  leaves, because of unique ultrastructural and biochemical partitioning patterns that appear to have been derived from the ancestral  $C_3$  system (94). Although the bundle-sheath cells of  $C_3$  plants may possess mitochondria and occasionally chloroplasts, those of  $C_3$ - $C_4$  intermediate species have greater numbers of these organelles with a centripetal organization (20). Often, the organelles are arranged such that mitochondria lie along the innermost tangential wall, interior to the chloroplasts (20). This provides a diffusive situation in which  $CO_2$  that is released from the mitochondria during the glycine decarboxylation of photorespiration is effectively reassimilated by chloroplasts before it escapes from the leaf. Brown & Hattersley (20) have demonstrated a correlation between the percentage of a leaf's photorespiratory organelles (mitochondria, peroxisomes, and chloroplasts) contained in the bundle sheath of  $C_3$ ,  $C_4$ , and  $C_3$ - $C_4$  species and the  $CO_2$  compensation point, an index of photorespiratory  $CO_2$  loss. Thus, an initial step in the evolution of  $C_4$  metabolism includes the increased provisioning of bundle sheath cells with photorespiratory organelles and their involvement in recycling photorespired  $CO_2$  before it diffuses from the leaf (94). In  $C_3$ - $C_4$  intermediate species of the genera *Moricandia*, *Panicum*, *Mollugo*, and *Flaveria*, the increased provisioning of bundle sheath cells with photorespiratory organelles is accompanied by the differential expression of glycine decarboxylase, the mitochondrial enzyme responsible for the release of photorespiratory  $CO_2$  (73). Activity of this enzyme is found only in the bundle-sheath cells (73, 103, 131). Glycine decarboxylase is composed of

four different subunits, with only one being isolated to the bundle-sheath cells and missing from the mesophyll cells (the P subunit, see 30). Because glycine decarboxylase is restricted to the bundle-sheath cells, all photorespired  $\text{CO}_2$  is released from the most interior part of the leaf, resulting in almost certain photosynthetic refixation before diffusing past the bundle-sheath and mesophyll chloroplasts.

The differential activity of glycine decarboxylase in bundle-sheath and mesophyll cells of  $\text{C}_3$ - $\text{C}_4$  intermediate species represents the first evolutionary chapter in an otherwise lengthy story of differential  $\text{C}_3$  and  $\text{C}_4$  cycle expression. What mechanism has evolved to depress the expression of the P-subunit of glycine decarboxylase in the mesophyll tissue, yet permit it in bundle-sheath cells? One possibility is the diffusible inhibitor of gene expression that has been invoked for the control of light-regulated genes involving phytochrome or one of the blue-light receptors (105). This inhibitor has been implicated as a possible control over the differential expression of Rubisco in  $\text{C}_4$  plants (107). Extending this model to  $\text{C}_3$ - $\text{C}_4$  intermediate species, a diffusible substance would be transported laterally from cells of the vascular system to mesophyll cells, where it would interact with a mesophyll-specific factor to repress transcription or translation of the P-subunit of glycine decarboxylase. Once a mechanism has evolved to control the differential expression of glycine decarboxylase, it could have developed further as a control over the repression of the Rubisco gene, and the promotion of the PEP carboxylase gene in the mesophyll cells of fully-expressed  $\text{C}_4$  plants. Langdale and coworkers have demonstrated that the differential expression of the principal  $\text{C}_4$  and  $\text{C}_3$  enzymes among PCA and PCR cells is due to regulation at the level of transcription (85, 107).

Several aspects of CAM appear to have evolved as minor modifications of processes in ancestral  $\text{C}_3$  species. For example, one of the key aspects of CAM is the temporal storage of organic acids within the cell vacuole. The synthesis and storage of organic acids is an integral part of cytoplasmic pH and osmotic balance in all terrestrial plants, irrespective of photosynthetic pathway (see 147, 129). Such processes have particular importance to nutrient assimilation and stomatal guard cell dynamics. Some past evolutionary scenarios for CAM have suggested modifications in the expression of guard cell metabolism as a principal event (37, 155). Griffiths (59), however, provides a compelling argument against restricting the evolutionary origins of CAM to one specific aspect of organic acid metabolism in  $\text{C}_3$  plants. Whatever the precise modifications, the ancestors to CAM clearly were already predisposed to the synthesis and storage of organic acids. Elaboration of these processes, as an evolutionary pattern, would have been required as nighttime  $\text{CO}_2$  assimilation gradually took on greater importance to the diurnal carbon budget of CAM plants.

### *Adaptation and Transitional Stages of C<sub>4</sub> and CAM Evolution*

Several studies have examined the gas-exchange traits of C<sub>3</sub>-C<sub>4</sub> intermediate species, with the aim of elucidating those environmental situations in which an advantage is expressed over C<sub>3</sub> species (13, 71, 92, 93, 95, 141). These studies have made it obvious that any advantages of C<sub>3</sub>-C<sub>4</sub> intermediate species are subtle in most environmental situations. Two situations in which they appear to exhibit some advantage are reduced intercellular CO<sub>2</sub> concentrations (71, 92) and elevated leaf temperatures (95, 141). By taking advantage of the modified bundle sheath layer, with its recycling of photorespired CO<sub>2</sub>, C<sub>3</sub>-C<sub>4</sub> intermediate species can realize a significant increase in CO<sub>2</sub> assimilation rate per unit leaf area, where photorespiration is increased, relative to photosynthesis (141). Additionally, because the CO<sub>2</sub> source for bundle-sheath assimilation is internal, the increased assimilation can be realized with higher water and nitrogen-use efficiencies, compared to C<sub>3</sub> species (141).

CAM is expressed in plants native to many ecological situations, e.g. in primitive aquatic plants (e.g. *Isoetes*), the chloroplast-containing, aerial roots of orchids, primitive, stomata-less bog plants (e.g. *Stylites*), tropical epiphytes (e.g. Bromeliads), and desert succulents (e.g. Cactaceae). The common denominator in all cases is that ambient CO<sub>2</sub> during the day is not readily available or is available only at extremely high costs (e.g. low daytime aquatic CO<sub>2</sub> concentrations and low diffusion coefficient for CO<sub>2</sub> in water, low tissue CO<sub>2</sub> concentrations due to lack of stomata, or high water costs as with desert succulents). An initial evolutionary step when exposed to such difficulties in obtaining ambient CO<sub>2</sub> appears to be nighttime assimilation of respired CO<sub>2</sub>, as a supplement to daytime CO<sub>2</sub> uptake (59, 92, 156, 167). Such recycling of respired CO<sub>2</sub> would enhance the plant's carbon balance by reducing nighttime respiratory losses. Additionally, during those periods when ambient CO<sub>2</sub> was acutely scarce, recycling of respired CO<sub>2</sub> would provide the plant with a means of ensuring adequate carbohydrate pools as substrate for maintenance respiration. The latter arguments focus on carbon balance as the principal advantage driving the evolution of CAM. Martin et al (89) have provided compelling evidence that CAM-cycling also leads to significant advantages in terms of water-use efficiency, and this has been acknowledged as a possible additional factor driving the evolution of CAM (60, 92).

## PHYLOGENY AND PHOTOSYNTHETIC PATHWAY DISTRIBUTION

### *Multiple Origins and Common Patterns*

Numerous past studies have superimposed the distribution of C<sub>4</sub> photosynthesis and CAM upon general phylogenetic schemes for the Angiospermae (e.g. 48, 81, 155). All studies come to the same conclusions: (i) both C<sub>4</sub> photo-



synthesis and CAM are derived conditions, based upon their being confined to more advanced families, and (ii) both  $C_4$  photosynthesis and CAM have evolved independently several times within the Angiospermae and may have evolved independently among several genera as well (54, 81, 147, 155). When one narrows consideration of phylogenetic relationships to specific families, additional evolutionary insight is possible. Below, we present six case studies of  $C_4$  and CAM evolutionary patterns to illustrate some of the diversity and similarity that has occurred within the plant kingdom as a whole.

### *Patterns Among Key Families*

**POACEAE** Most phylogenetic evidence suggests that the presence of  $C_4$  photosynthesis in the monocotyledonous Poaceae and Cyperaceae is due to independent evolutionary events and that within the Poaceae,  $C_4$  photosynthesis may have evolved independently at least three times (70). The Poaceae share closer affinities to six small,  $C_3$  southern hemisphere families (Restionaceae, Centrolepidaceae, Anarthriaceae, Ecdeiocoleaceae, Flagellariaceae, and Joinvillaeceae) than to the Cyperaceae; the closest sister groups to the Poaceae appear to be the Restionaceae and the Joinvillaeceae (both  $C_3$ ) (24). A phylogeny of the Poaceae now recognizes five subfamilies, two of which contain only  $C_3$  species (Bambusoideae and Pooideae) (177). The other three include the Chloroideae, which is  $C_4$  except for one species, *Eragrostis walteri* (51), the Panicoideae, which has both  $C_3$  and  $C_4$  species, and the Arundinoideae, which is predominantly  $C_3$  but possesses five  $C_4$  genera.

**CYPERACEAE** With respect to  $C_4$  Cyperaceae, two groups are generally recognized, the chlorocyperoid and fimbristylid types. Both groups exhibit the "classical" NADP-type of  $C_4$  ultrastructure with agranal, centrifugal chloroplasts (25). These groups have been given subfamilial status by Raynal (133), who presented evidence of two independent evolutions of  $C_4$  photosynthesis. However, Brown (22) proposed a phylogeny based on anatomical patterns, with the chlorocyperoid group evolving from the fimbristylid group. The fimbristylid group is characterized by three sheaths—an inner PCR sheath, a middle mestome sheath, and an outer parenchymatous sheath. The inner PCR sheath appears to be homologous with phloem and xylem parenchyma (22, 25). The chlorocyperoid group is characterized by only two sheaths. Brown (22) suggested that the chlorocyperoid group evolved from the more primitive fimbristylid group through elimination of the outermost parenchyma sheath. Thus, the evolutionary pattern for  $C_4$  in the Cyperaceae is confused by conflicting interpretations.

**CHENOPODIACEAE (ATRIPLEX)** The most widely accepted phylogeny of *Atriplex* is that of Hall & Clements (64), which is based on embryo position in the seed. Using this criterion, two subgenera have been distinguished, *Euatri-*

*plex* and *Obione*. Primitive members of each subgenus are  $C_3$ . In *Obione*, more advanced species are entirely  $C_4$ . In *Euatriplex*, advanced species include both  $C_3$  and  $C_4$  photosynthetic pathways. Thus, there is evidence for at least two independent evolutions of  $C_4$  photosynthesis in this genus.  $C_4$  *Atriplex* species exhibit the NAD-malic enzyme type of  $C_4$  decarboxylation. The origins of *Atriplex* probably lie in central Asia, where at least 70% of the species demonstrate  $C_3$  photosynthesis (116).  $C_4$  photosynthesis appears to have evolved early in this genus, at least with respect to its radiation to the other continents. This is based on the observation that 85–100% of the species in Australia, South America, North America, and Central America are  $C_4$  (116). In fact, the early evolution of  $C_4$  photosynthesis in this genus likely accelerated phylogenetic diversification. Rapid diversification was promoted by its ability to invade marginal habitats, especially those characterized as arid or saline (116). The presence of  $C_4$  photosynthesis would certainly have provided for more water-use efficient  $CO_2$  assimilation, and thus enhanced growth, in such habitats.

**POLYPODIACEAE** CAM has been confirmed in several species of the fern genus *Pyrrhosia* (184, 187). Plants of this genus are epiphytic and widely distributed within the Palaotropics (Africa, Asia, and Australia). Using recent cladistic analyses of this genus with knowledge of CAM distribution, Griffiths (59) concluded that CAM exists in three separate monophyletic, relatively advanced groups. It is not yet clear whether these groups obtained CAM through independent lineages, as the potential exists for considerable floristic mixing during past continental movements. A comparison of phylogeny with geographic distribution in this genus suggests an origin in Africa, with an early split when Africa separated from Gondwana and Australia separated from India. The primitive life-form in this genus appears to be poikilohydric and native to seasonally dry habitats. There is evidence that derived species include both the poikilohydric and succulent epiphytic life-forms, with CAM evolving within the succulent lineages (59). Kluge et al (80) have suggested that the apparent lack of drought tolerance in CAM species of *Pyrrhosia* reflects homology with  $C_3$  ancestors from rain forest habitats. If so, this suggests an early ecological dichotomy in this group, with the African/Central Asian groups diversifying within seasonally arid habitats, retaining the poikilohydric form, and the Australasian groups diversifying within more mesic rain forest habitats, taking on the succulent, CAM form. There is evidence that one species within the CAM group, *Pyrrhosia rupestris*, exhibits  $C_3$  photosynthesis (184). Griffiths (59) raised the possibility of this species representing an evolutionary reversal from CAM back to  $C_3$ .

**BROMELIACEAE** Within this family of neotropical origins, all three subfamilies contain CAM species (148). It appears as though CAM has evolved

independently at least twice in this family, and possibly more than once within the single genus *Tillandsia* (58, 148).  $C_3$  and CAM species occur in both terrestrial and epiphytic groups of this family (148). Within the subfamily Bromelioideae, there is evidence of some loss of CAM and reversal to the  $C_3$  state (148).

The Bromeliaceae provide unique opportunities to examine the relationship of CAM evolution to appearance of the epiphytic growth habit (90, 91, 148). Considering epiphytic species from all angiosperm families, over 13,000 species may exist with CAM (59)! With such estimates, CAM is obviously much more frequent within the epiphytic growth form than is the arid, terrestrial growth form typically associated with CAM (59, 110, 184).

**CRASSULACEAE** Teeri (155, 156) has provided evidence through  $\delta^{13}C$  analyses, combined with cytogenetic and morphologic features, that CAM has evolved twice in trans-Mexican Crassulacean species. North American Crassulaceae tend to exhibit only CAM-cycling ( $C_3$  uptake of atmospheric  $CO_2$  with the capacity to recycle respired  $CO_2$  at night). Thus, in this family, fully expressed CAM has evolved only in the more southerly taxa, presumably because of the increased frequency of radiation into habitats of extremely high aridity. All of the species so far examined in this family have at least some capacity for nighttime accumulation of organic acids (whether it be through CAM-cycling or fully expressed CAM), except for two, *Crassula erecta* and *Sedum ternatum*. Teeri (156) offered the suggestion that the latter species represents the product of evolutionary reversal, from CAM or CAM-cycling to fully expressed  $C_3$ . In support of this, some of the genera in this family (e.g. *Dudleya* and *Villadia*,) have been classified, on the basis of  $\delta^{13}C$ , as representing intermediate steps in the evolution of fully expressed CAM from CAM-cycling (156). In a survey of Semipervivoideae (Crassulaceae) from Teneriffe (Canary Islands), Tenhunen et al (161) provided evidence from a single subfamily of adaptive radiation from ancestral,  $C_3$  succulents that occur in mesic, cloud forest environments that they produce a variety of CAM taxa occupying relatively arid habitats.

## ECOLOGICAL ASPECTS OF PHOTOSYNTHETIC PATHWAY VARIATION

### *Variation in Relation to Abiotic Factors*

**$C_3$  AND  $C_4$  DISTRIBUTIONS ALONG GEOGRAPHIC AND ENVIRONMENTAL GRADIENTS** One of the more fruitful approaches to understanding the ecological significance of photosynthetic pathway variation has come through correlative analyses of pathway abundance and specific environmental factors. In pioneering work, Teeri & Stowe (158) found that the percent  $C_4$  grass representation in local floras was most highly correlated with the average July



minimum temperature. Since this work, numerous studies taking a similar approach have verified some aspect of growing season temperature as the strongest correlate with  $C_4$  representation, especially when considered over fairly broad geographic scales (17, 30, 35, 52, 66, 68, 87, 114, 136, 153, 173, 175, 178). Each of these distribution patterns is mechanistically consistent with previous evidence that increased photorespiration and decreased light-use efficiency at elevated temperatures play a significant role in influencing plant carbon balance. Modeling the consequences of light-use efficiency differences on predicted productivity of  $C_3$  and  $C_4$  canopies, Ehleringer (45) predicted distribution patterns that were qualitatively similar to the above observations.

$C_4$  dicots do not follow the climate relationships that have been reported for  $C_4$  monocots. Stowe & Teeri (152) found that the representation of  $C_4$  dicot species in local floras of North America was more highly correlated with indices of aridity than indices solely describing temperature. However, even  $C_3$  species in those families that contained  $C_4$  dicots exhibited significant correlations with aridity, suggesting a phylogenetic component, independent of photosynthetic pathway type. Assuming that  $C_3$  photosynthesis was ancestral in these families, these results suggest a pattern of  $C_4$  evolution in those North American dicot taxa predisposed to growth in arid habitats. Okuda & Furukawa (115) found that Japanese  $C_4$  dicots did not exhibit numerical decreases as latitude increases, in contrast to the trend for  $C_4$  monocots. In  $C_4$  dicots, the advantages of  $C_4$  photosynthesis appear to have facilitated diversification and expansion into warm areas of progressively greater aridity. In  $C_4$  monocots, the advantages of  $C_4$  photosynthesis appear to have facilitated diversification and expansion into warm areas with relatively high amounts of summer precipitation.

At more local scales, summer precipitation takes on increased importance, reflecting the influence of moisture on  $C_4$  distribution within a relatively homogenous temperature regime (42, 69). Generally,  $C_4$  species tend to predominate in areas of lower precipitation or soil moisture (35, 174). Several studies have emphasized that the evolution of a  $C_4$ - versus  $C_3$ -dominated flora depends on the interaction between temperature and precipitation—i.e. whether it is warm or cool during the season of highest moisture (68, 174, 175). In the arid regions of the western United States (144) and South Africa (179),  $C_3$  species predominate during the winter rainy season whereas  $C_4$  plants predominate during the summer season. Along topographic moisture gradients,  $C_3$  and  $C_4$  species tend to sort themselves along patterns similar to those observed for broad geographic regions— $C_4$  species often occur at the drier extreme of the gradient, and  $C_3$  species at the cooler, wetter extreme (3, 11, 99, 166, 178, 189).

Well-defined crossovers in the relative dominance of  $C_3$  versus  $C_4$  species

in a local flora have been described along elevational gradients at equatorial latitudes (35, 136, 166). Along the ascent of Mount Kenya, Tieszen et al (165) observed a switch from a  $C_4$ -dominated flora to a  $C_3$ -dominated flora at altitudes above 2300 m. This elevation was characterized by a mean annual minimum temperature of 8°C (the mean minimum temperature at this elevation is relatively constant year-round). Along elevational gradients in Hawaii and Costa Rica, the crossover in  $C_3/C_4$  grass representation occurred at different elevations than in Kenya, but at similar mean minimum temperature ranges (9–11°C) (35, 136). In a survey of  $C_3/C_4$  distributions, Long (88) concluded that  $C_4$  species do not occur in areas with mean summer temperature minima below 8°C, although it should be noted that genera such as *Echinochloa* (125) and *Muhlenbergia* (142) commonly occur at this lower temperature limit. Even so, there is a marked convergence in the low temperature limits of  $C_4$  distribution among widely different floras. These temperature-related distributional limits for  $C_4$  plants are probably due to the evolutionary origins of  $C_4$  photosynthesis in floras of warm-temperature latitudes, rather than to any inherent weaknesses in the design of the  $C_4$  system (88).

In temperate latitudes of North America,  $C_3$  and  $C_4$  plants may occupy the same habitat, with ecological divergence occurring across seasonal and topographic gradients in temperature and moisture (17, 79, 97, 100, 104, 180, 181). In arid land regions of the southwestern deserts with bimodal precipitation, seasonal differences in the activities of  $C_3$  and  $C_4$  plants are pronounced, with  $C_3$  grasses and herbs dominating the winter season and  $C_4$  the summer season (104, 144). In the mixed-grass prairie of South Dakota, significant increases in the  $\delta^{13}C$  of bulk aboveground biomass occur during midsummer compared to spring and fall (5, 113). These observations reflect the increased abundance of  $C_4$  biomass, relative to  $C_3$  biomass, during the warmest part of the growing season and demonstrate a distinct difference in the seasonality of  $C_3$  and  $C_4$  grasses in this ecosystem. A similar divergence in the timing of seasonal growth might be expected for the short-grass prairie of North America, but recent measurements have indicated that although phenologies differ for  $C_3$  and  $C_4$  grasses in this ecosystem, there is no difference in the timing of maximum seasonal growth (99).

**VARIATION IN  $C_4$  SUBTYPES IN RELATION TO ECOLOGICAL PATTERNS** Brown (22) and Hattersley (66) predicted correlations between  $C_4$  subtype and climate. Recent correlative analyses have revealed that  $C_4$  subtypes exhibit systematic distribution patterns over broad ecological gradients (52, 69, 126, 174, 179). In the  $C_4$ -dominated grasslands of the South African/Namibian region, NAD-me subtype species predominate at the more arid extremes (representing 67% of the  $C_4$  flora in the most arid districts), whereas NADP-me species exhibit a striking sensitivity to increasing aridity (decreas-

ing in proportional representation from 42% of the  $C_4$  species in the wettest districts, to 12% in the driest districts). The relationship of PEP-ck species to climate was less distinct, but the species tended to be most abundant at the median of the range. Hattersley (69) has provided unequivocal evidence that NAD-me grass species in Australia are negatively correlated in abundance with median annual rainfall, whereas both NADP-me and PEP-ck species exhibit significant, positive correlations with precipitation.

It is possible that the correlations noted above are the result of historical, phylogenetic linkages between  $C_4$  subtype and some unrelated factor of drought tolerance, because there is no simple mechanistic linkage between  $C_4$  subtype and drought tolerance. Past studies have demonstrated definitive differences among the subtypes with respect to  $CO_2$  leakage from the PCR tissue (47, 67), which is likely to affect growth when water is available, but not necessarily performance when exposed to drought. The leakage of  $CO_2$  from PCR tissue is almost certain to influence  $CO_2$  partial pressures in the intercellular spaces surrounding PCA tissue, and this may, in turn, influence stomatal conductance and transpiration rate. Whatever the mechanism underlying these recently uncovered correlations, there is certain to be future interest in their implications for phytogeographical studies of global grasslands and applied aspects of grassland management (see 69).

**CAM AND ARIDITY** Over broad geographical scales, the distribution of CAM is clearly most highly influenced by aridity (61, 101, 102, 149, 159, 179, 182, 184). The CAM-aridity distribution falls into two primary groupings: succulents from the arid and semi-arid regions, and epiphytes from tropical and subtropical regions. With limited exceptions (151, 168), CAM plants are succulents, and the development of CAM is a function of leaf thickness (151, 184). Species of aridland Cactaceae and Crassulaceae in North America are most abundant in regions with high indices of aridity (159); along gradients of increasing aridity in North America, CAM increases in frequency (50, 49, 101). The distributional limits of many CAM species are determined by tolerance to low-temperature extremes (e.g. 111), though once again this is probably due to their evolutionary origins in warm climates and not to any weakness of CAM per se. In CAM plants from cold environments, the period of maximum productivity occurs during the summer, not the winter as in most desert CAM succulents (108, 109, 110).

**WATER-USE EFFICIENCY AND PLANT DISTRIBUTION** While the increased water-use efficiency of  $C_4$  plants over  $C_3$  plants has been considered to be of adaptive value, it is difficult to distinguish the effects of high temperature from water use on plant performance. High leaf temperature results in an increased evaporative gradient for both  $C_3$  and  $C_4$  plants, but when combined



with the increased photorespiration rate of  $C_3$  plants, higher leaf temperature results in a greater reduction in water-use efficiency of the  $C_3$  plants. The advantages to  $C_4$  plants conferred by a lack of photorespiration are directly convertible to a water-use efficiency advantage, but is there evidence that water conservation is the important factor? Since temporal activities of  $C_3$  and  $C_4$  plants are often seasonally displaced, equivalent, direct comparisons among similar life forms are limited. Robichaux & Pearcy (135) could not detect any advantage of increased water-use efficiency in a comparison of  $C_3$  and  $C_4$  shrub species along a moisture gradient in Hawaii. Knapp (82) suggested that the higher water-use efficiency of  $C_4$  plants and the greater capacity to conserve water while responding to light-level fluctuations places  $C_4$  plants at a competitive advantage under grassland situations, especially when drought may be an intermittent factor. Saline environments also impose a stress on plants, resulting in partial stomatal closure, and one in which water-use efficiency may be important for long-term survival. As soil salinities increase in tidal marshes or inland sinks,  $C_3$  plants increase water-use efficiency, but their values are not as high as those of the  $C_4$  plants that replace them on more saline sites (23, 63, 76, 56). In cold deserts, productivity of  $C_3$  and  $C_4$  shrubs may not differ significantly, but  $C_4$  plants achieve equivalent productivity on more saline soils losing less transpirational water (23). Over longer time scales, water-use efficiency of  $C_3$  plants is sensitive to decreased global  $CO_2$  levels and is much greater today than it was during glacial periods when  $CO_2$  concentrations were lower (123, 124), implying that, even with a reduced water-use efficiency,  $C_3$  plants were at a competitive disadvantage under glacial-period atmospheric  $CO_2$  levels.

Changes in photosynthetic pathway are a means of increasing water-use efficiency and extending activity into drought periods. Common among the leaf succulents of the arid regions of North America and Africa are plants that reversibly switch between  $C_3$  and CAM in response to water stress (65, 77, 157, 169, 183). The additional carbon gained through CAM activities increases reproductive output under conditions of drought and salinity stress (185). An interesting variation on this theme are African succulents that have drought-deciduous  $C_3$  leaves and evergreen CAM stems (86, 176). Shifts between  $C_3$  and  $C_4$  photosynthesis within a plant are not known, with the exception of the unique aquatic plant *Eleocharis vivipara* (172).

### *Competitive Interactions Among $C_3$ and $C_4$ Plants*

As discussed above, comparative carbon balance studies of  $C_3$  and  $C_4$  species within an ecosystem often show a seasonal displacement of activities, which tends to reduce competitive interactions. However, for plants growing in saline or disturbed environments, the growing periods coincide, and there are opportunities for comparing competitive abilities among  $C_3$  and  $C_4$  plants.

Differences in the temperature dependence of photosynthesis (14, 120) and greater nitrogen-use efficiency (18, 36, 43, 137) provide a competitive edge to  $C_4$  plants, but exceptions to this pattern may exist (42, 57). There appears to be a competitive advantage to  $C_4$  photosynthesis under limited water stress (42, 118), unless that stress is so extreme that canopy closure does not occur (120). While cool, shade situations should favor  $C_3$  plants (45), differences in the capacity to use intermittent sunflecks in gap situations could place  $C_4$  plants at a competitive advantage if sunfleck frequency is sufficiently high (134, 150).

Gurevitch (62) observed that the  $C_3$  grass *Stipa neomexicana* occupied dry ridgetop sites in a semi-arid grassland of southern Arizona, whereas the lower, wetter sites were dominated by  $C_4$  grasses. This reversal of the typical pattern, in which  $C_4$  species typically dominate drier portions of the topography, is due to the exceptional dryness of these ridgetop sites and the extreme drought tolerance of *S. neomexicana*. Physiological features other than photosynthetic type probably represent the principal determinant of extreme drought tolerance. While the  $C_4$  pathway, in and of itself, does not confer a drought tolerance, it does confer an advantage in terms of rapid growth during the hot summers of this Arizona site, when rainfall reaches its monthly maximum. Thus, at the lowland sites,  $C_4$  grasses are better able to compete for the midsummer moisture, restricting the  $C_3$  species to those extreme sites that lack  $C_4$  competitors. This pattern makes clear, once again, that the temperature during the rainy season is an important determinant of whether a local flora is  $C_3$ - or  $C_4$ -dominated.  $C_3$  and  $C_4$  species will sort along temporal and spatial environmental gradients according to their relative competitive advantages. At the environmental extremes, however, success may depend more upon the evolved traits of stress tolerance than on the greater resource-use efficiencies that typify  $C_4$  plants.

Given the sensitivity of light-use efficiency and photorespiration to low atmospheric  $CO_2$  levels,  $C_3$  plants should become better competitors in elevated  $CO_2$  environments (26, 190). Yet allocation patterns and competition for other resources make the competitive outcome of  $C_3$ - $C_4$  interactions difficult to predict (8, 10). Although reduced photorespiration favors  $C_3$  plants under elevated conditions, growth of  $C_3$  plants quickly becomes nitrogen-limited (39, 112), and the greater nitrogen-use efficiency of  $C_4$  plants may place them at a competitive advantage (9).

### *Plant-Animal Interactions*

DIFFERENTIAL HERBIVORY AMONG  $C_3/C_4$  PHOTOSYNTHETIC PATHWAY TYPES  
Carbon isotope ratios ( $\delta^{13}C$ ) have been used to establish feeding preferences of insects and large ungulates in various ecosystems (1, 15, 40, 163, 166).

Since "you are what you eat, isotopically," the proportion of  $C_3/C_4$  plants in an animal's diet can be estimated by analysis of the  $\delta^{13}C$  values of its tissues (hair, teeth, muscle, bone, etc.) (163, 164). These data reveal that most grazing species exhibit significant preference for one photosynthetic pathway or the other.

Caswell et al (29) hypothesized that there were greater herbivory rates on  $C_3$  than on  $C_4$  plants, because  $C_4$  plants were less nutritious. The basic tenets are that the bulk of leaf protein in  $C_4$  plants is protected within the thick cell walls of the BSC, and leaves of  $C_4$  plants contain less protein than those of  $C_3$  plants. In support of their hypothesis, a number of studies have shown feeding preferences for  $C_3$  over  $C_4$  plants (29, 72), a tendency for most of protein in leaves of  $C_4$  plants to be located within BSC (84), and evidence that bundle sheath cells tend to pass undigested through an insect gut (27, 28). However, others have observed the opposite pattern ( $C_4$  plants were preferentially taken before  $C_3$  plants—16, 74, 145) or no clear preference pattern (4, 121, 122). A more balanced approach might consider the possibility of co-evolutionary relationships between plants and their herbivores, which result in feeding preferences for plants with one photosynthetic pathway or the other.

Several distinct patterns emerge from studies comparing  $C_3$  versus  $C_4$  feeding preferences. First, the hierarchy of feeding preferences tends to be  $C_3 \rightarrow$  NADP-me  $C_4 \rightarrow$  NAD-me  $C_4$  or the opposite (74, 75, 127, 171). Second, there is a tendency for NAD-me  $C_4$  plants not to contain toxic secondary compounds, whereas NADP-me and  $C_3$  plants often contain secondary metabolites known to deter herbivores. Third, there are anatomical differences among  $C_4$  plants: BSC of NAD-me  $C_4$  are short and cubical, whereas those of NADP-me  $C_4$  and  $C_3$  are long and rectangular (21). Since the BSC of all  $C_4$  plants have thicker cell walls than do adjacent mesophyll cells, the bundle sheath cells of NAD-me  $C_4$  plants have essentially "protected" the leaf protein within a BSC cell that is more difficult to crush than have NADP-me  $C_4$  plants. This is not the case for NADP-me  $C_4$  plants, in which there is both a greater photosynthetic protein content within mesophyll cells (associated with producing malate as the transported  $C_4$  acid), and also bundle sheath cells that have a higher surface to volume ratio and should be therefore less difficult to crush. For the NADP-me  $C_4$  plants (as well as for  $C_3$  plants), much of the leaf protein is "exposed," contained within thin-walled mesophyll cells, and not within the bundle sheath cells; toxic secondary compounds may be necessary to reduce the likelihood of herbivory. Given this scenario, it is reasonable to hypothesize that insects capable of detoxifying secondary compounds may exhibit a preference for  $C_3$  and NADP-me  $C_4$  plants, whereas those insects lacking this capability should exhibit a preference for NAD-me  $C_4$  plants.



## SOME CONCLUSIONS CONCERNING C<sub>4</sub> AND CAM EVOLUTIONARY PATTERNS

The high frequency with which C<sub>4</sub> photosynthesis has evolved must reflect the existence of developmental and biochemical gene families with a propensity for frequent mutation. The architecture of certain genetic regulatory systems may predispose these systems to evolutionary change, providing for the rapid evolution of novel morphological and biochemical patterns (41). Though these patterns may, at first, be nonadaptive, in the case of C<sub>4</sub> evolution, they may become subject to selection shortly after their appearance. This hypothesis is based on the fact that the initial stages of C<sub>4</sub> evolution appear to entail the coordinated development of bundle-sheath cell ultrastructure and differential expression of the enzyme glycine decarboxylase between the bundle sheath cells and the mesophyll cells. The metabolic advantages of this evolutionary sequence are clear in terms of reducing photorespiratory CO<sub>2</sub> loss and improving net CO<sub>2</sub> assimilation rate in an environment of low atmospheric CO<sub>2</sub>. Strong selection in favor of such traits would probably occur with a combination of internal factors creating a high carbohydrate sink strength and external factors creating a low carbohydrate source strength. This combination might occur most frequently for plants with a genetic constitution favoring rapid growth rates (high carbohydrate sink strength) in warm environments with reduced CO<sub>2</sub> availability, where photorespiration is expressed at a high level (low carbohydrate source strength)—in other words, in those conditions in which plant growth is likely to be carbon limited.

The frequency of independent evolution of CAM suggests that it too has developed from gene families with a propensity for change. Like C<sub>4</sub> photosynthesis, the initial evolutionary changes leading to CAM involve both biochemical and ultrastructural changes. The biochemical changes involve increases in the expression of PEP carboxylase activity as a means of recycling respired CO<sub>2</sub> into malic acid. The ultrastructural changes involve elaboration of vacuolar storage capacity as a means of accommodating increases in nighttime synthesis of malic acid. The advantages of these evolutionary changes, however, are still unclear. On the one hand, it could be argued that the evolution of CAM occurred as a means of extending the period of carbon assimilation in an environment where daytime CO<sub>2</sub> availability is low. Such a case could be made for both aquatic environments (where daytime CO<sub>2</sub> availability is limited by intense competition from C<sub>3</sub> phytoplankton) (78) and arid, terrestrial environments (where daytime CO<sub>2</sub> availability is limited by low stomatal conductances). With such an argument, the evolution of CAM would best be classified as relieving carbon limitations to growth. Alternatively, the argument could be made that CAM facilitates a respiratory carbohydrate supply during particularly stressful periods for the maintenance

of cellular structure and function, allowing for rapid recovery upon the relaxation of stress. With the latter argument, the evolution of CAM would best be classified as enhancing stress tolerance and recovery.

In an anthropogenically altered environment, the environmental situation favoring the continued evolution of  $C_4$  photosynthesis (low atmospheric  $CO_2$ ) is being rapidly eliminated. As atmospheric  $CO_2$  levels continue to rise, the selective value of  $C_4$  photosynthesis is therefore diminished. At some elevated global  $CO_2$  concentration in the not-too-distant future, it is likely that photorespiration in  $C_3$  plants likely will be effectively eliminated and light-use efficiencies will be increased to the point that  $C_4$  plants are no longer at a competitive advantage over  $C_3$  plants in any ecological situation.

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