9. Atmospheric CO_2, Environmental Stress, and the Evolution of C_4 Photosynthesis

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This paper is dedicated to the memory of Professor Vladimir Pyankov, an expert on C_4 dicots, who passed away unexpectedly in January of 2002.

9.1 Introduction

There are three modes of photosynthesis in terrestrial plants: C_3, C_4, and Crassulacean Acid Metabolism (CAM). C_3 photosynthesis is the oldest and most common of the three, having been present in the earliest land plants. It occurs in about 90% of all land plants and predominates in most life forms and taxonomic groups. CAM photosynthesis is the next most abundant in terms of species number and taxonomic distribution, with approximately 20,000 species (Winter and Smith 1996). C_4 photosynthesis is the least diverse of the three photosynthetic pathways, occurring in 17 families and some 7000 to 8000 species (Sage, Li, and Monson 1999). Despite the relatively small number of C_4 species, they have an ecological impact that far exceeds their numbers. C_4 grasses dominate warm-temperate to tropical grassland and savanna biomes, and tropical and subtropical marshes are often co-dominated by C_4 sedges (see Chapter 10) (Ueno and Takeda 1992; Knapp and Medina 1999;). C_4 plants are common in biomes that cover approximately 40% of Earth's land surface, and 20% to 25% of the global primary productivity occurs via the 3% of all plants species that use the C_4 pathway (Collatz, Berry, and Clark 1998; Sage, Wedin, and Li 1999).

While many consider the C_4 pathway as simply an interesting variety of photosynthesis, there are a number of reasons why C_4 plants deserve special attention. First, the biochemical technology of C_4 photosynthesis gives these species

an ability to exploit habitats that are too hostile for C_3 species. As a result, large expanses of the world's deserts and salt flats support a vegetation cover that might be far more sparse in the absence of C_4 photosynthesis. Second, unique features of the C_4 pathway promoted the evolutionary radiation of numerous animal groups following the expansion of C_4 dominated biomes (MacFadden 1997) (see also Chapter 12). Third, because of its unique physiology, C_4 photosynthesis responds differently to climate and atmospheric CO_2 change than do C_3 plants. C_4 plants perform well relative to C_3 species at CO_2 levels below the current atmospheric CO_2 level, particularly in warm to hot climates (Johnson, Polley, and Mayeux 1993; Sage 1995). Thus, whenever there are pronounced changes in atmosphere and climate, the distribution of C_4 relative to C_3 vegetation is altered (Cerling et al. 1997; Huang et al. 2001; Schefuss et al. 2003). Understanding the interactions among climate, atmospheric change, and C_4 vegetation is, therefore, important for interpreting how the current biota of the planet came into being, as well as predicting how the future biota will respond to anthropogenic global change.

In recent years there have been important advances in our understanding of past climates, atmospheric composition, and the systematic diversification of C_4 photosynthesis, such that it is now possible to propose robust models of C_4 evolution. In this review, I present a comprehensive summary of how variation in atmospheric CO_2 content and environmental stress may have affected the evolutionary diversification of C_4 photosynthesis. Considerable emphasis is placed on the distribution of C_4 photosynthesis in families of dicotyledonous plants. C_4 photosynthesis arose very recently and on multiple occasions in the dicots, and many of the immediate C_3 ancestors and intermediate forms have been identified (Ehleringer, Cerling, and Helliker 1997; Sage 2001). Analysis of the C_4 dicot lineages thus facilitates understanding of the conditions leading to the rise of the C_4 pathway in a manner that may not be possible in the older, more diverse C_4 grass and sedge lineages.

9.2 A Brief Review of C_4 Photosynthesis

C_4 photosynthesis is not a single metabolic pathway but instead consists of distinct biochemical reactions that concentrate CO_2 into an internal compartment where the enzyme Ribulose (1,5) bisphosphate carboxylase/oxygenase (Rubisco) is localized (Fig. 9.1). This internal compartment is commonly termed the bundle sheath; however, it is often derived from cell layers other than the true bundle sheath tissue and for this reason is often termed the photosynthetic carbon reduction (PCR) tissue (Dengler and Nelson 1999). In addition to localizing Rubisco into an internal compartment, all C_4 plants share the initial enzymatic step of phosphoenol pyruvate (PEP) carboxylation to form oxaloacetic acid, a four-carbon compound.

PEP carboxylase is located in the mesophyll tissue of the leaf in what is termed the photosynthetic carbon assimilation (PCA) compartment (Dengler and

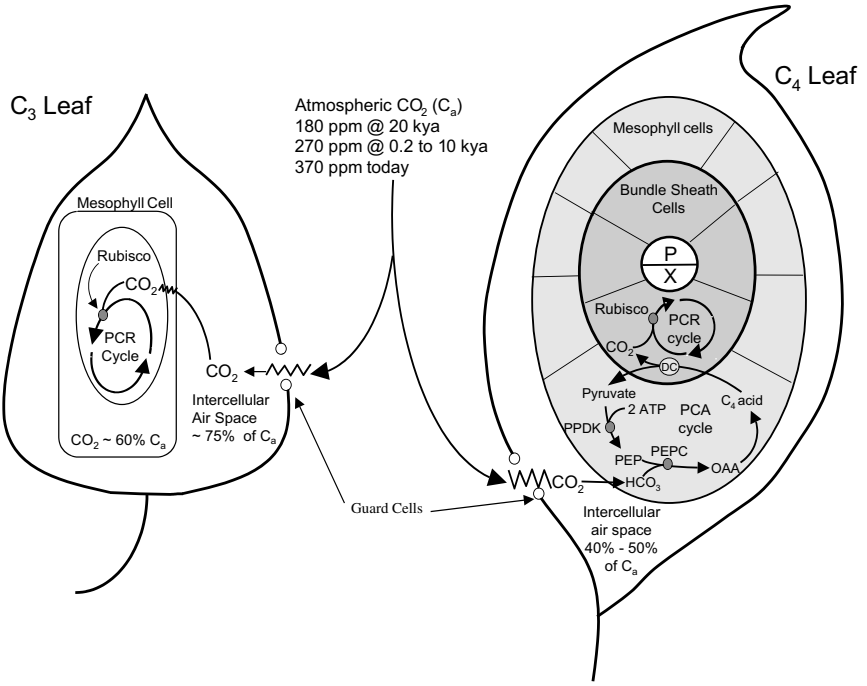

Figure 9.1. A schematic representation of photosynthesis in a C_3 and C_4 leaf. In the C_3 leaf, the outline of the biochemical path of the photosynthetic carbon reduction cycle (PCR) and photosynthetic oxidation cycle (PCO, or photorespiratory cycle) is shown for a single mesophyll cell, with the location of the Rubisco carboxylation and oxygenation steps highlighted. In the C_4 leaf, a cross section is shown for a single PCA-PCR unit comprised of adjacent mesophyll and bundle sheath cells, with a summary of key metabolic steps in the photosynthetic carbon assimilation (PCA) cycle. Atmospheric CO_2 levels (C_a) are shown for the late Pleistocene 20 thousand years ago (20 kya), in the Holocene before the industrial revolution (0.2–10 kya) and today. Other abbreviations: DC, the decarboxylase enzme; PEPC, phosphoenol pyruvate carboxylase; PPDK, pyruvate, phosphate-dikinase; p, phloem; x, xylem.

Nelson 1999). PCA cells usually surround PCR cells, creating a wreath-like arrangement termed Kranz anatomy (Metcalfe and Chalk 1979). The outer region of the PCA tissue is well exposed to the interecellular air species, while the PCR cells are positioned together along the inside walls of the PCA cells. This arrangement is important as it establishes a barrier that traps CO_2 in the PCR compartment.

Four-carbon acids produced by PEP carboxylation diffuse from the PCA to the PCR compartment. Once in the PCR compartment, they are decarboxylated to release CO_2, which builds up to concentrations that saturate Rubisco and

inhibit RuBP oxygenation (see Fig. 9.1) (Hatch 1987; Kanai and Edwards 1999). RuBP oxygenation is a side-reaction catalyzed by Rubisco that produces phosphoglycolate, a two-carbon compound that is of no use to the plant and is toxic in elevated concentrations (Jordan and Ogren 1984; Sage 1999). Plants must metabolize phosphoglycolate back to PCR cycle intermediates to avoid toxic accumulation of the byproducts of RuBP oxygenation. In doing this, reducing power and (ATP) are consumed, while previously fixed CO_2 is released (Douce and Heldt 2000). Together, the uptake of O_2 by Rubisco and the release of CO_2 during metabolism of phosphoglycolate are termed photorespiration. Because of the energy cost, the loss of previously fixed CO_2, and the competition from O_2 for Rubisco active sites, photorespiration is highly inhibitory for photosynthesis under conditions promoting high oxygenase activity (Sharkey 1988; Sage 1999). In the current atmosphere, Rubisco oxygenase activity becomes significant as atmospheric CO_2 levels decline below 500 ppm, but only at warmer temperature (Jordan and Ogren 1984). Rising temperature stimulates oxygenase activity and hence photorespiration in C_3 plants at CO_2 levels of the current atmosphere and below, such that C_3 photosynthesis is significantly ($> 20\%$) inhibited by photorespiration above 25°C (see Chapter 10, Fig. 10.3). By accumulating CO_2 around Rubisco in the bundle sheath tissue, C_4 plants prevent significant levels of photorespiration at any temperature. The high CO_2 levels in the bundle sheath also allow Rubisco to operate near CO_2 saturation, so that its efficiency is maximized. In C_3 plants at warmer temperature, Rubisco operates at CO_2 levels well below saturation, and as a result its carboxylation potential is relatively low (Jordan and Ogren 1984; Sage 1999).

While all C_4 plants share certain features, such as PEP carboxylation, there are unique differences that demonstrate multiple evolutionary solutions to the challenge of concentrating CO_2 around Rubisco. For example, the decarboxylation of C_4 acids in the PCR compartment is catalyzed by one of three enzymes that vary between the different types of C_4 plants (Hatch 1987; Kanai and Edwards 1999). These are the NADP-malic enzyme (in NADP-ME type plants), the NAD-malic enzyme, and PEP carboxykinase (PCK). Differences also occur in the steps associated with metabolite transport between PCA and PCR cells, with the groups using the NADP-malic enzyme transporting C_4 acids as malate while the NAD-ME and PCK types transport C_4 acids as aspartate (Edwards and Walker 1983; Hatch 1987). Finally, while all C_4 plants segregate Rubisco and PEP carboxylation into physically distinct compartments, the source, location, and modifications of the PCA and PCR tissue vary substantially between different evolutionary lineages of C_4 photosynthesis. At least 8 different patterns of Kranz anatomy have been identified, based on the nature of the cellular arrangement, the ultrastructural patterns within photosynthetic cells, and the developmental lineage of the PCR tissue (Dengler and Nelson 1999). When all the differences in anatomy and decarboxylation types are accounted for, at least 16 different types of C_4 photosynthesis are apparent, and more appear likely as numerous groups of C_4 plants have yet to be studied.

Until recently, it was thought that Kranz anatomy was required for C_4 photo-

synthesis in order to trap CO_2 in the bundle sheath. This is no longer the case, because two species in the Suadeae tribe of the Chenopodiaceae have recently been shown to operate a complete C_4 pathway within single cells. One species, *Bienertia cycloptera*, localizes Rubisco into chloroplasts that reside in a cytoplasmic compartment in the center of barrel-shaped photosynthetic cells (Freitag and Stichler 2002; Voznesenskaya et al. 2002). PEP carboxylation occurs in cytoplasmic pockets located at the cell periphery and a large vacuole separates the central cytoplasmic region from the periphery. The second species is *Borszczowia aralocaspica*, a central-Asian shrub where photosynthetic cells form ranks of elongated columns surrounding the vascular tissue (Freitag and Stichler 2000; Voznesenskaya et al. 2001). Rubisco containing chloroplasts are located at the inner pole of the photosynthetic cells, while PEP carboxylation occurs at the outer end of the cell adjacent to the intercellular air spaces. As in *Bienertia*, *Borczscowia* has a large vacuole that separates the PCA and PCR regions of the cell and thus appears to serve as the diffusive barrier that limits CO_2 efflux.

9.3 The Taxonomic Distribution of C_4 Photosynthesis

C_4 photosynthesis in terrestrial plants is only found in the most advanced families of the angiosperms. It is absent in all of the more primitive plant groups (bryophytes, lycophytes, ferns, and gymnosperms), and in all the basal angiosperm orders, as shown in Fig. 9.2 (Kellogg 1999; Sage, Li, and Monson 1999).

C_4 photosynthesis occurs in 18 plant families in nine angiosperm orders, many of which are distantly related. Recent reports have identified C_4 photosynthetic metabolism in certain algae (for example, diatoms and the green algae *Egeria*; Reinfelder, Kraepiel, and Morel, 2000; Casati, Lara, and Andreo, 2000), but the issue of whether they constitute C_4 photosynthesis has not been fully resolved. Unlike terrestrial plants, C_4-like algae do not separate PCA and PCR functions into distinct compartments. Many C_3 plants also operate a C_4 cycle in vascular tissue of the stems (Hibberd and Quick 2002), but this is not considered to be C_4 photosynthesis because the C_4 metabolism is not linked to anatomical changes that facilitate CO_2 concentration around most of the Rubisco within the plant.

Most C_4 species are grasses (family Poaceae), with about 4600 of the estimated 10,000 grass species being C_4 (Sage, Li, and Monson 1999). Sedges (family Cyperaceae) are the next most abundant group with 1350 species, while the remaining 1200 or so C_4 species are from 15 families of dicots (Fig. 9.2 and Fig. 9.3).

In the dicots, 800 species are in the Chenopodiaceae and Amaranthaceae[1], 250 are in the Euphorbiaceae and 150 are scattered among 8 genera in the

1. Soltis et al. (2000) have merged the Chenopodiaceae into the Amaranthaceae based on molecular phylogeny data. Recent phylogenetic work with a larger number of species indicates separation of Chenopodiaceae and Amaranthaceae are justified (Kadereit et al. 2003). My treatment here recognizes the traditional split between the families.

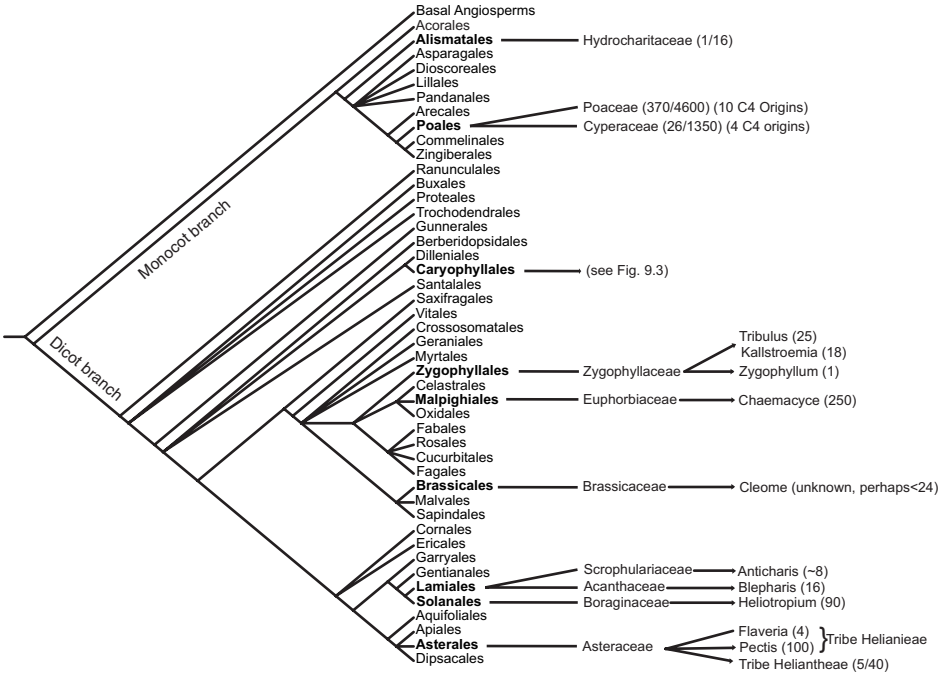

Figure 9.2. The distribution of C_4 photosynthesis in taxonomic orders of the angiosperms. Angiosperm orders containing C_4 species are shown in bold. Families containing C_4 species are shown for all orders except the Caryophyllales. Individual evolutionary lineages are shown for seven of the dicot families, with each lineage portrayed by the arrows connecting a family to the principle C_4 genera within the lineage. Genera/species numbers are shown in parentheses for grasses (Poaceae), sedges (Cyperaceae), the Hydrocharitaceae, and the tribe Heliantheae in the Astercacae. Estimated species numbers are shown for the genera in the other dicot lines. Based on the phylogeny of Stevens (2003). Estimates of genera and species numbers from Sage, Li, and Monson, 1999, as modified by recent carbon isotope surveys of photosynthetic pathway in Acanthaceae, Boraginaceae, Scrophulariaceae and Zygophyllaceae (Sage, unpublished).

Asteraceae. With the exception of the Chenopodiaceae and Amaranthaceae, the C_4 dicot species are relatively minor in their respective families. For example, the Asteraceae has 1530 genera and 23,000 C_3 species, but only 140 C_4 species scattered among 8 genera (Bremer 1994; Sage, Li, and Monson 1999). Ten of the 15 dicot families containing C_4 plants have three genera or less and each of these has fewer than 80 species. Many of the dicot genera with C_4 species also contain C_3 species, including a few (*Salsola, Heliotropium, Alternanthera, Moricandia, Flaveria*) that have species intermediate for C_3 and C_4 photosynthesis (Frohlich 1978; Monson 1989; Sage, Li, and Monson 1999; Pyankov et al.

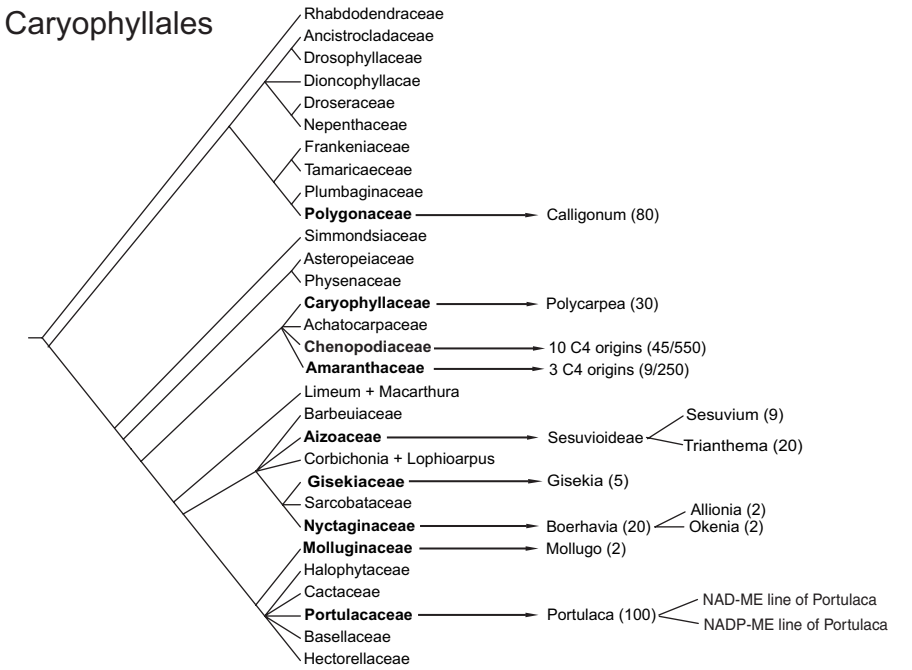

Figure 9.3. The distribution of C_4 photosynthesis in the Caryophyllales. Families with C_4 species are shown in bold, and species numbers corresponding to the individual genera are shown in parentheses. The genera beside the arrows are those where the C_4 pathway is postulated to have first arisen. Derived genera are shown for the smaller lineages. The systematic treatment follows Cuenoid et al. 2002. Species number estimates are from Sage, Li, and Monson, 1999, with modification based on carbon isotopic screens of herbarium materials (Sage, unpublished).

2001a,b). The presence of C_3/C_4 intermediates indicates that evolution from C_3 to C_4, or vice versa, is an ongoing process (Ehleringer and Monson 1993).

9.4 How Many Times Did C_4 Photosynthesis Evolve?

By comparing taxonomic affinity, biochemical traits, and anatomical features of the existing C_4 species, it is possible to identify distinct evolutionary origins of C_4 photosynthesis (Kellogg 1999; Pyankov et al. 2001a,b). The presence of C_4 photosynthesis in distantly related taxonomic families (see Fig. 9.2) clearly indicates distinct evolutionary lineages (Kellogg 1999). Within a given family, independent origin is indicated by differences in decarboxylation enzyme and Kranz anatomy type (Pyankov et al. 2001a,b). Recent phylogenetic analyses of

the C_4-inclusive orders now allow for improved estimates of the number of origins of C_4 photosynthesis. For example, in the grass family, over 10 distinct C_4 origins are evident (see Fig. 9.2) if it is assumed that reversion from C_4 to C_3 photosynthesis is uncommon (GPWG 2001; Giussani et al. 2001). In the sedge family, four distinct evolutionary origins are indicated by phylogenetic treatments (Soros and Bruhl 2000; Sage 2001). In the dicots, 31 distinct evolutionary lines can be postulated (see Fig. 9.2, 9.3). Many independent origins in the dicots are obvious as they are single evolutionary lines within distinct families: for example, in the Acanthaceae (*Blepharis*), Boraginaceae (*Heliotropium*, section *Orthostachys*), Brassicaceae (*Cleome*, section *Gynandropsis*), Euphorbiaceae (*Chaemacyce*) and Scrophulariaceae (*Anticharis*) (Kellogg 1999; Sage, Li, and Monson 1999). Four distinct origins are evident in the Asteraceae: two in the tribe Helianieae (two in *Flaveria* and one in *Pectis*) and one in the Heliantheae, subtribe Coreopsideae (Karis and Ryding 1994; Korpiva et al. 1996; Kellogg 1999). Two lines are evident in the Zygophyllaceae, with one origin in *Tribulus* or *Kallstroemia*, and a second in *Zygophyllum* (Sheahan and Chase 1996; Kellogg 1999). In *Zygophyllum*, only one species, *Zygophyllum simplex*, is C_4 (Sage, unpublished based on a carbon isotope survey of the Zygophyllaceae).

After the grass order Poales, the most prolific order in terms of C_4 evolution is the dicot order Caryophyllales, with nine families containing C_4 species (see Fig. 9.3). C_4 species appear to derive from single evolutionary origins in six families of the Caryophyllales. Four independent origins are estimated in the Amaranthaceae (Kadereit et al. 2003; Kellogg 1999; and Sage unpublished). In the Portulacaceae, two independent lines are postulated, one using the NAD-malic enzyme type of C_4 photosynthesis, and a second using the NADP-malic enzyme type (Guralnick and Jackson 2001). The Chenopodiaceae is the most prolific dicot family in terms of producing independent C_4 lineages. Ten distinct C_4 origins are estimated based on a combination of taxonomic, anatomical, and physiological treatments (Kellogg 1999; Pyankov et al. 2001a,b; Freitag and Stichler 2002; Kadereit et al. 2003). Two genera in the Chenopodiaceae seem particularly adept at evolving C_4 species. C_3 *Salsola* species are postulated to have given rise to two C_4 lineages, one using NADP-ME as the primary decarboxylating enzyme, and a second line using NAD-ME (Pyankov et al. 2001a,b). The genus *Suada* in the subtribe Suadeae holds the generic record for distinct C_4 evolutionary origins, with four transitions from C_3 to C_4, including the only two known cases of single-celled C_4 photosynthesis in terrestrial plants (Freitag and Stichler 2002; Voznesenskaya et al. 2001, 2002; Schütze et al. 2003).

When the number of probable C_4 lineages is tabulated, more than 40 distinct evolutionary origins of C_4 photosynthesis appear likely. Given this high number of independent origins, it is apparent that the evolution of C_4 photosynthesis is relatively easy in certain groups of plants, such as grasses, sedges, and chenopods. However, the absence of C_4 photosynthesis in most groups of plants, in-

cluding many families that share habitat or life form with C_4 plants, indicates there may be unique features in certain plant families that predisposed them to evolve C_4 photosynthesis on multiple occasions. The nature of these features is unknown, although it has been suggested that adaptations to aridity that affect bundle sheath size and vein spacing may be important (Ehleringer, Cerling, and Helliker 1997; Sage 2001). Enlarged bundle sheaths cells, reduced mesophyll cell number, and closer vein spacing may enhance leaf water status in environments with high evaporative demand (Sage 2002). These changes may, in turn, reduce the diffusive distance between mesophyll and bundle sheath cells, thus facilitating the exchange of metabolites between these tissues. Enhanced potential for metabolic exchange between mesophyll and bundle sheath cells is proposed to be an important early step in the evolution of C_4 photosynthesis (Monson 1999).

9.5 Where Did C_4 Photosynthesis Evolve?

The ability to resolve distinct C_4 evolutionary lineages in the taxonomic record allows for identification of probable centers of origin for C_4 photosynthesis, at least in the dicots. In the grasses, identifying centers of origin are more problematic because the origination events are older, the taxa more numerous, and the systematics more complicated than in the dicot lines where C_4 photosynthesis evolved. Usually, centers of diversity identify centers of origin. Using this approach, in combination with the location of the closest C_3 relatives and any C_3-C_4 intermediates, it is possible to postulate five general centers of origin for most of the C_4 dicot lineages (Fig. 9.4).

Centers of origin for the C_4 Asteraceae (*Pectis and Flaveria*), Boraginaceae, Euphorbiaceae, and Nyctaginaceae are proposed for the arid interior of Mexico, while arid South America is the center of origin of the C_4 species in the *Gomphrena Alteranthera* complex (Amaranthaceae), in *Alternanthera* (Amaranthaceae), and in the Helianteae tribe of the Asteraceae. The African center appears to be the home for many C_4 dicot lineages in Acanthaceae, Aizoaceae, Amaranthaceae (*Aerva*), Brassicaceae, Molluginaceae, Scrophulariaceae, and Zygophyllaceae (see Fig. 9.4). Central Asia is the likely home for all Chenopodicaceae, and *Calligonum* in the Polygonaceae. The two C_4 lines postulated for *Portulaca* (Portulcaceae) certainly originate in the Southern Hemisphere (Geesink 1969), but where exactly cannot be resolved until better phylogenies are produced.

In *Polycarpea* (Caryophyllaceae), C_3 and C_4 species co-occur in Africa and Arabia, indicating C_4 photosynthesis in this genus arose in here (Sage, unpublished). C_3-C_4 intermediates of *Heliotropium* are from Mexico, indicating an American center of origin seems likely (Frohlich 1978).

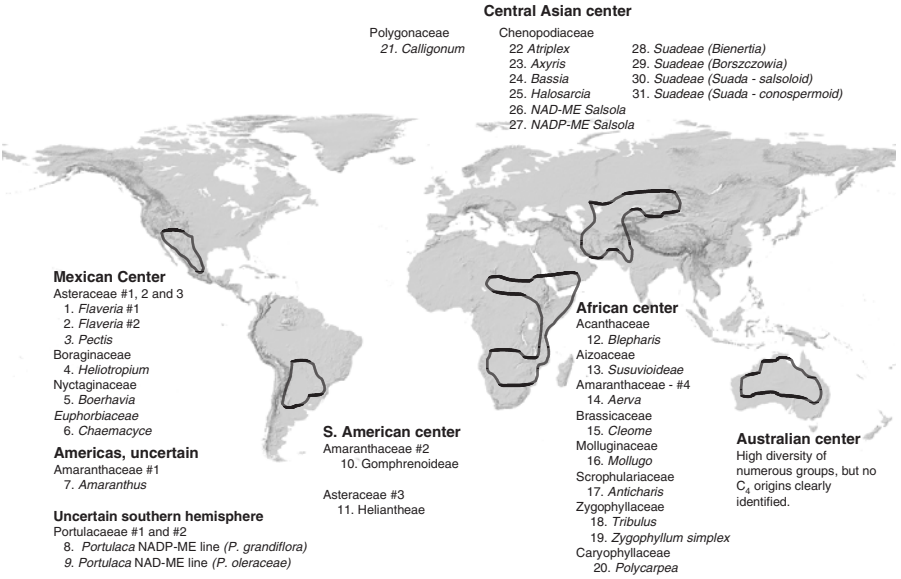

Figure 9.4. Postulated centers of origin for the C_4 dicot lineages. Centers of origin are based on (a) where the greatest diversity of C_4 taxa in a given lineage occur, (b) the distribution of any C_3-C_4 intermediates, and (c) the location of C_3 and C_4 species within a transitional genus. Key references for identifying distribution ranges include Volleson (2000, Acanthaceae); Hartmann (1993) for the Aizoaceae; Osmond, Björkman; and Anderson (1980), Kühn (1993), Pyankov et al. (2001a,b) and Townsend (1993) for the Amaranthaceae; Bremer (1994), Kadereit et al. (1996), and Powell (1978) for the Asteraceae; Johnston (1928), Frohlich (1978), and Hilger and Diane (2003) for the Boraginaceae; Bittrich (1993) for Caryophyllaceae; Webster, Brown, and Smith (1975) for the Euphorbiaceae; Endress and Bittrich (1993) for the Molluginaceae; Bittrich and Kühn (1993) for the Nyctaginaceae; Brandbyge (1993) for the Polygonaceae; Applequist and Wallace (2001) for the Portulaceae; plus Sage (unpublished, based on collection localities of over 1000 C_4 specimens housed in the herbaria of the Royal Botanical gardens at Kew, the Missouri Botanical Gardens, the New York Botanical Garden, and the Harvard University Herbarium).

9.6 How Old Is C_4 Photosynthesis?

9.6.1 The Evidence for Recent Evolution of C_4 Photosynthesis

Based on a number of criteria, the first C_4 ancestors of the existing C_4 flora most likely arose in recent geological time, with the earliest origin possibly occurring as far back as the mid-to-late Oligocene (23–30 Ma) (Kellogg 1999; Sage 2001). In rough terms, the greater the diversity in a C_4 lineage in terms of genera and species number, the older the lineage (Ehleringer, Cerling, and Helliker 1997). Using this criteria, the oldest C_4 lineage is in the grass family (370

genera, 4600 species), followed next by the sedge family (26 genera, 1350 species), and then the Chenopodiaceae (45 C_4 genera, 550 species), as shown in Fig. 9.5. Molluginaceae (one genus, less than 5 species), Scrophulariaceae (one genus, 6–10 species), and Brassicaceae (one genus with less than 20 species) would be very recent, possibly as late as the upper-Pleistocene (Ehleringer,

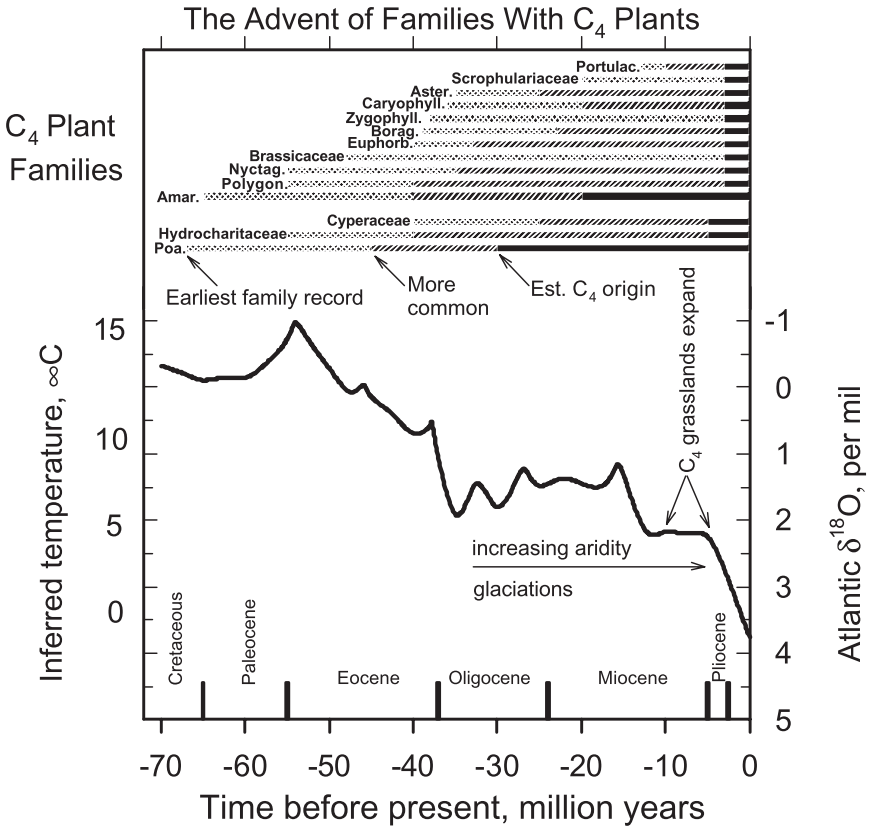

Figure 9.5. The occurrence of the families of plants containing C_4 photosynthesis in geologic time, with the corresponding inferred global temperature. Abbreviations correspond with the family names given in Fig. 9.2, 9.3. The solid portion of the histograms corresponds to estimated times for C_4 plant appearance in the respective families (after Kellogg, 1999, for grasses and Ehleringer, Cerling, and Helliker, 1997, and Kadereit et al. 2003 for dicots). Note: Amar refers to both the Amaranthaceae and Chenopodiaceae. Cyperaceae, Amaranthaceae, and Hydrocharitaceae are best guesses. The stippled middle portion of the histograms indicate times when family members become common in the fossil record, while the lightly stippled portions at the left end of the histogram indicate earliest reported origin (after Müller 1981 and Collinson, Boulter and Holmes, 1993) The inferred temperature is based on oxygen isotope ratios in Atlantic Ocean cores (Prothero 1994). From Sage (2001).

Cerling, and Helliker 1997). Most lines of C_4 dicots are suggested to originate in the Pleistocene, and it is possible that some of the less diverse grass and sedge lineages may date to the Pleistocene as well (see Fig. 9.5). For example, *Flaveria* is likely recent because there are only four C_4 species, and numerous C_3-C_4 intermediate species (Powell 1978; Kopriva, Chu, and Bauwe 1996). *Aerva* (Amaranthaceae) and *Halosarcia* (Chenopodiaceae) have multiple C_3 species, but these genera contain only one or two C_4 species, indicating a very recent transition to C_4 (Sage, unpublished data).

The earliest definitive evidence of C_4 photosynthesis in the geological record is represented by leaf samples that are 12 million years old in the Ricardo formation of California that have Kranz anatomy and C_4-like isotopic signatures (Nambudiri et al. 1978; Cerling 1999). Grass samples from Kenya that are 14.5 million years old have been suggested to be C_4, based on the presence of cuticles and other anatomical features that are similar to features that occur in modern C_4 grasses (Dugas and Retallack 1993). However, these samples have not been confirmed by isotopic screens or by Kranz anatomy (Cerling 1999). Isotopic signals from megafaunal remains and fossilized soils have been reported to indicate C_4 presence in East Africa as early as 15 million years ago (Kingston, Marino, and Hill 1994; Morgan, Kingston, and Marino 1994); however, C_4 species do not appear to dominate any ecosystems in East Africa prior to 10 million years ago (Cerling 1999). Beginning 10 million years ago, significant shifts in carbon isotope ratios from C_3 to C_4 values occur in many tropical sites, demonstrating ecosystems dominated by C_4 plants become widespread by the late Miocene (Cerling et al. 1997).

Evidence from molecular phylogenetic research with grasses indicates C_4 photosynthesis arose well before 12 to 15 million years ago. Using a molecular clock approach to analyze gene sequence differences between related grass genera that are both C_4 (hence the ancestor had to be C_4), Gaut and Doebley (1997) estimated distinct C_4 grass lineages were diverging by 25 million years ago. Kellogg and Russo (cited in GPWG 2001) estimated the C_4 grass *Danthoniopsis* diverged by 16 million years ago. Taking these observations into account, it is reasonable to propose that C_4 photosynthesis first arose in grasses by the mid Miocene, and possibly as early as the mid Oligocene. These species were relatively uncommon in most ecosystems until the late Miocene when they expanded across the globe, creating the first C_4 dominated biomes by 8 million years ago (Cerling 1999).

The Oligocene period ($\sim$34–23 Ma) is characterized by global environmental deterioration that left Earth colder, drier, and with an atmosphere depleted in CO_2 (see Fig. 9.5) (Prothero 1994; Zachos et al. 2001). Pagani, Freeman, and Arthur (1999); and Pearson and Palmer (2000) estimate that by 25 million years ago, high CO_2 levels ($>$500 ppm) of the Eocene and earlier had declined to below 300 ppm (see Chapter 3). This reduction correlates with the molecular clock data for C_4 divergence in major grass lineages.

In addition to the reduction in CO_2, the Oligocene was also a time when a number of other developments occurred that may have been essential for the

rise of C_4 photosynthesis. First, the taxa and functional groups in which C_4 photosynthesis would appear showed marked diversification during this epoch (see Fig. 9.5). Although grasses pre-date the Oligocene by some 30 million years, they become common in the fossil record during the Oligocene (Kellogg 2000). Sedges also diversify about this time, as do most of the major dicot families that would one day contain C_4 plants (Collinson, Boulter, and Holmes 1993). In addition to the radiation of the taxonomic groups, herbaceous life forms become common in the fossil record only after 40 million years ago, while the annual plant life form, and drought-adapted features, becomes common after the early Oligocene (Wolfe 1997). In the large majority of the postulated C_4 lineages, the C_4 pathway primarily occurs in herbaceous species. Many of these are annual, and drought-adapted features prevail. In sum, the Oligocene is a time when a number of conditions favorable for C_4 photosynthesis first occurred. The biotic source material (advanced herbaceous angiosperm families) capable of evolving the pathway appears, atmospheric preconditions are met, and climate deterioration created extensive habitat favorable to C_4 species.

9.6.2 Did C_4 Photosynthesis Pre-Date the Oligocene Period?

A number of studies have proposed more ancient origins of C_4 photosynthesis than the Oligocene. Low CO_2 and high O_2 atmospheres favoring C_4 photosynthesis are postulated to have occurred in the Carboniferous period 300 Ma ago (Wright and Vanstone 1991), but clear evidence for C_4 plants in the Carboniferous has not yet been identified (Cerling 1999; Chapter 6). During the Carboniferous, angiosperms did not exist, and if C_4 plants were present, they would have occurred in ferns and related species, or gymnosperms. No evidence has ever been presented for C_4 photosynthesis in nonflowering plants. Kuypers, Pancost, and Damsté (1999) also propose C_4 plants were present during a possible low CO_2 excursion during an oceanic anoxic event 91 million years ago. This work is based on a sudden ($<$60 kya) isotopic shift in putative leaf waxes from marine deposits and is interpreted to indicate an expansion of C_4 community onshore in Northwest Africa. Without other supporting data, this interpretation is difficult to accept, as it would require either a ready source of C_4 species, or rapid evolution of C_4 photosynthesis, in taxa whose modern relatives contain no C_4 species.

9.7 Factors Promoting the Origin of C_4 Photosynthesis

Although C_4 plants have now radiated into a diverse range of habitats, including swamps, alpine tundra, and wet grasslands (Jones 1986; Long 1999), the general consensus is that they evolved in hot, arid, and often saline habitats (Ehleringer and Monson 1993). The greatest diversity of C_4 grasses corresponds to hot, dry environments (Hattersley 1992; Schulze et al. 1996); as shown in Fig. 9.4, the centers of diversity of the C_4 dicot lineages are arid interiors of continents. The

main exception to these patterns may be the C_4 sedges, which tend to occur in wet habitats (Ueno and Takeda 1992). Ecological disturbance also may have played an important role, because many C_4 dicot species and C_3-C_4 intermediate species are common in recently disturbed habitats (Monson 1989).

High temperature is clearly important for the success of C_4 plants because C_4 photosynthesis has its greatest advantage over C_3 photosynthesis above 35°C (Pearcy, Tumosa, and Williams 1981; Osmond, Winter, and Ziegler 1982; Pearcy and Ehleringer 1984). For example, the temperature optimum for C_4 photosynthesis occurs at temperatures that are inhibitory for C_3 photosynthesis (Berry and Raison 1981; Sage and Pearcy 2000). At current CO_2 levels and below, the cause of the different temperature effects on C_3 versus C_4 photosynthesis is a high rate of photorespiration that occurs in C_3 plants above 30°C (see Chapter 10). By contrast, C_4 plants experience negligible inhibition from photorespiration at all temperatures (Kanai and Edwards 1999). Differences in photosynthetic performance also reflect ecological patterns. Studies comparing competitive abilities of C_3 and C_4 species in similar habitats demonstrate that C_4 plants dominate C_3 plants of similar growth form at elevated temperature (Pearcy, Tumosa, and Williams 1981; Sage and Pearcy 2000), and biogeographic surveys show C_4 grasses comprise over 90% of the biomass of grasslands at low latitude and altitude (Tiezen et al. 1997; Sage, Wedin, and Li 1999; Wan and Sage 2001).

The significance of aridity and salinity for C_4 evolution is threefold. First, the C_4 pathway provides clear advantages over the C_3 pathway in situations where the supply of freshwater is limited (Long 1999). C_4 plants have higher water use efficiency than do C_3 plants, a quality that gives them a greater capacity to produce biomass and to set seed on a limited amount of water (Osmond, Winter, and Ziegler 1982; Schulze and Hall 1982; Brown 1999). On saline soils, higher water use efficiency reduces the water flux through C_4 plants and, hence, the amount of salty water the plants encounter (Sage and Pearcy 2000). Second, drought and salinity reduce the density of plants, such that light levels remain high and surface heating of the ground maintains elevated temperatures near the plants. Third, drought and salinity stress reduce competitive interactions, so that transitional species with traits intermediate between C_3 and C_4 photosynthesis, and therefore potentially less efficient, may persist rather than being displaced by strong competitors.

9.7.1 The Role of Low Atmospheric CO_2

While important, the action of heat, drought, and salinity stress is probably not strong enough to select for the evolution of C_4 photosynthesis if atmospheric CO_2 levels are high. At elevated CO_2, photorespiration is low, Rubisco operates at high efficiency and photosynthesis in C_3 plants is typically greater than in ecologically similar C_4 plants (Berry and Raison 1981; Ehleringer et al. 1991). Without the added energy and nitrogen costs associated with the C_4 pump, C_3 plants at high CO_2 can also exhibit greater light, water, and nitrogen use efficiency than do C_4 plants, and the impact of drought, salinity, and heat stresses

are reduced (Ehleringer, Cerling, and Helliker 1997; Hsiao and Jackson, 1999; Luo, Canadell, and Mooney 1999; Munns, Cramer, and Ball 1999; Sage and Cowling 1999). Furthermore, limitations associated with carbon supply become minor in high CO_2, and the control over productivity shifts from carbon supply to the availability of mineral nutrients, such as nitrogen and phosphorous (Agren, Shaver, and Rastetter 1999; Hungate 1999). Because of this, adaptations that improve the carbon economy of the plants, such as C_4 photosynthesis, have limited value in high CO_2 conditions.

At CO_2 levels less than those of today, C_4 plants show marked superiority over C_3 plants in terms of photosynthetic performance, growth, competitive ability, and reproductive output, particularly in warmer (>25°C) environments (Johnson, Polley, and Mayeux 1993; Dippery et al. 1995; Tissue et al 1995; Sage 1995). Furthermore, as CO_2 declines, the degree of inhibition associated with a given level of drought or heat stress substantially increases in C_3 species, potentially to the point where the survival of the plant is threatened (Sage and Cowling 1999). For example, in a variety of C_3 crops, biomass production at 200 ppm CO_2 and elevated temperature (35°C/29°C day/night) is reduced by 80% to 90% relative to plants at moderate temperature and current CO_2 levels (see Table 9.1). This level of yield reduction indicates plants exposed to warmer conditions in low CO_2 environments would have difficulty completing their lifecycle during a growing season.

A useful way to evaluate the ability of plants to survive at low CO_2 is to model the minimum CO_2 requirement for photosynthesis, vegetative growth, and reproduction (Fig. 9.6). If CO_2 supply is insufficient to support growth and reproduction, then the life cycle cannot be completed and plants will eventually disappear from a habitat. The minimum amount of CO_2 that is required to sup-

Table 9.1. Vegetative biomass (in grams) of three C_3 crop species grown in a 2 × 2 factorial experiment combining two growth CO_2 levels (380 and 200 ppm) and two growth temperature regimes (25°/19°C day/night and 35°/29°C day/night). The percent of the control value at 380 ppm and 25°/19° is shown in parentheses. The bean data is from Cowling and Sage (1998) and wheat and tobacco data from Sage and Cowling (1999). Means ± SE. DAP = days after planting.

		Growth Condition		
Species	380 ppm 25°/19°C	380 ppm 35°/29°	200 ppm 25°/19°	200 ppm 35°/29°
Phaseolus vulgaris Black turtle bean at 23 DAP Whole plant dry weight, N=15	4.29 ± 0.15 (100%)	2.74 ± 0.14 (64%)	2.70 ± 0.12 (63%)	0.82 ± 0.08 (19%)
Nicotiana tabacum Tobacco at 21 DAP Shoot dry weight, N=6	1.20 ± 0.06 (100%)	0.84 ± 0.06 (70%)	0.51 ± 0.06 (43%)	0.09 ± 0.01 (8%)
Triticum dicoccum Emmer wheat at 41 DAP Shoot fresh weight, N=3	62 ± 2 (100%)	14 ± 1 (23%)	33 ± 3 (53%)	5 ± 1 (8%)

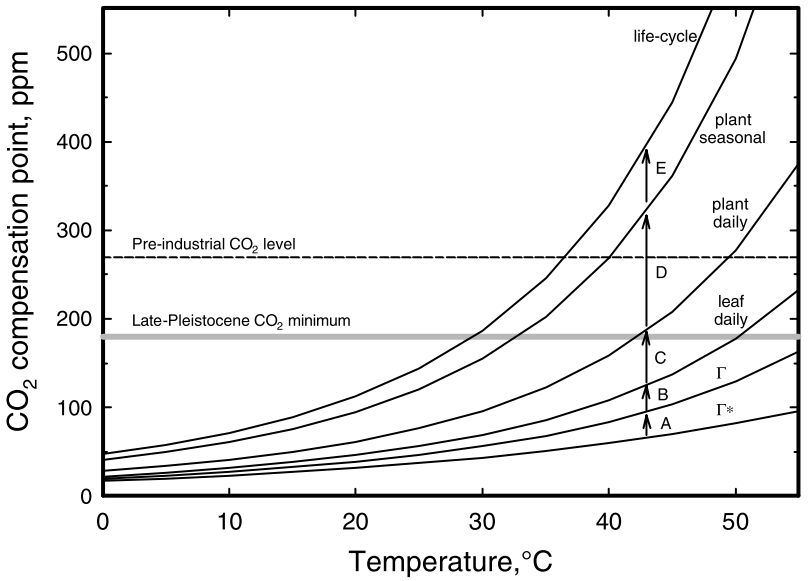

Figure 9.6. Hypothetical CO_2 compensation points for various processes in plants, as a function of temperature. Γ* is the CO_2 compensation point in the absence of mitochondrial respiration. Γ is the CO_2 compensation point for net photosynthesis, which incorporates the mitochondrial respiration rate in leaves in the light (indicated by arrow A). Leaf daily incorporates nighttime respiration of leaves (arrow B). Plant daily incorporates the respiration of nonphotosynthetic tissues over 24 hours (arrow C). Plant seasonal incorporates respiration and carbon construction costs associated with growth over the growing season (arrow D), and life cycle incorporates respiration and carbon costs for reproduction. Γ* and Γ responses modeled using Equations 2.37 and 2.32, and parameters in Table 2.3 from von Caemmerer (2000). These responses are experimentally supported but do not account for acclimation effects. To incorporate respiration and construction costs at higher levels than Γ, the R_d term in Equation 2.37 of van Caemmerer 2000 was increased to account for the additional respiratory carbon consumed during the added interval. Thus, for example, to account for nighttime respiration in leaves, R_d was doubled under the assumption that 50% of the leaf respiration occurs at night. For plant daily, R_d was doubled again on the assumption that 50% of the respiring plant biomass is nonphotosynthetic. Seasonal construction costs were assumed to be double maintenance costs, and R_d was increased another 25% to account for reproductive costs. Respiration costs at the whole plant level were loosely based on Lambers (1985) and are very approximate.

port this process is described in terms of the CO_2 compensation point. CO_2 compensation points vary with the frame of reference, rising as the scale increases from the biochemical to the whole plant level, and as the time frame increases from flux rates expressed per second to CO_2 exchange integrated over an entire life span. All versions of the CO_2 compensation point in C_3 plants are ultimately dependent on the ratio of oxygenation to carboxylation of RuBP, which is a property of the Rubisco enzyme (von Caemmerer 2000).

The CO_2 compensation point of gross photosynthesis in C_3 plants (Γ^*) occurs at the CO_2 level where RuBP carboxylation equals the rate of photorespiration. The CO_2 compensation point for net photosynthesis (Γ) occurs at the CO_2 level where the rate of carboxylation equals the sum of the photorespiration and mitochondrial respiration (van Caemmerer 2000). Because photorespiration and mitochondrial respiration increase with rising temperature at a faster rate than the rate of carboxylation, CO_2 compensation points also increase with temperature (Brooks and Farquhar 1985; von Caemmerer 2000).

For a plant to survive, carbon acquisition has to be greater than carbon loss through photorespiration and maintenance respiration, as well as having the carbon requirements to build new tissue. Respiratory losses are substantial, generally consuming 30% to 80% of the photosynthate gained by plants in the current atmosphere (Lambers 1985; van der Werf, Welschen, and Lambers 1992). For a leaf to have a positive photosynthesis rate over the course of a day, the daily gross photosynthesis rate has to exceed the sum of photorespiration and of mitochondrial respiration during both the light and dark periods of a diurnal cycle. The inclusion of the nighttime respiration costs yields the leaf-daily CO_2 compensation point, which is greater than the CO_2 compensation point for the instantaneous net photosynthesis (see Fig. 9.6). Similarly, CO_2 compensation points rise when respiration costs of nonphotosynthetic tissues are accounted for over a day (see the plant daily response) and an entire growing season (see the plant seasonal response).

The rise in the CO_2 compensation point for the plant over a growing season may be particularly pronounced because this response includes the construction costs to produce a mature vegetative plant. For a C_3 plant to complete its life cycle, the minimum carbon costs required to produce fruits and seeds must also be accounted for, and this further increases CO_2 compensation points. From an evolutionary perspective, the life cycle CO_2 compensation point is the most significant because it reflects the amount of CO_2 required for a population to avoid extinction.

In Fig. 9.6, the temperature response of the CO_2 compensation points for gross photosynthesis and net photosynthesis are experimentally well described (e.g., Brooks and Farquhar 1985). CO_2 compensation points at higher orders of complexity, such as the whole plant, have not been studied, and the curves presented are educated guesses. Despite this limitation, the hypothetical responses shown in Fig. 9.6 indicate that the rise in the CO_2 compensation point with increasing scale and temperature could be substantial enough to exceed prevailing atmospheric CO_2 levels during the Pleistocene, and possibly even the Holocene. As indicated in Fig. 9.6, the hypothesized rise in the CO_2 compensation point for completion of a life cycle may have exceeded the CO_2 level in the atmosphere of the late Pleistocene below 40°C. Above 40°C, the CO_2 compensation point for the life cycle is suggested to exceed the preindustrial CO_2 level of 270 ppm. If these responses are valid, they demonstrate that C_3 plants could reproduce neither in the late Pleistocene environments warmer than 30°C, nor in the Holocene environments above 40°C. The responses in Fig. 9.6 also indicate that vegetative C_3 plants could not grow above 40°C in late Pleistocene times, be-

cause the CO_2 compensation point for a positive daily carbon balance of whole plants is greater than atmospheric CO_2 levels of the time. Because few environments are consistently above 40°C, the importance of this may seem minor. However, seedlings experience temperatures that are well above air temperature in the boundary layer of the soil, and thus CO_2 requirements for establishment of C_3 seedlings could have been above what the prevailing climate conditions might indicate (Schulze et al. 1996; Sage and Sage 2002).

The situation would be even more perilous for C_3 plants if stomatal closure occurred to save water or to reduce salt loads, because this would effectively reduce the CO_2 availability to the plant, making it more likely that minimum CO_2 requirements were not met. As a result, it seems probable that many areas of the planet that support C_3 vegetation cover may have been unable to do so in low CO_2 environments of the past. These areas may thus have been wide open for colonization by genotypes expressing carbon-conservation traits that improved the carbon balance of the plant. Some of these carbon conservation mechanisms may have been the first steps in the evolution of C_4 photosynthesis.

9.7.2 Photorespiration: The Metabolic Bridge to C_4 Photosynthesis

In the origin of C_4 photosynthesis, there was no "final plan" for evolution to work toward. Instead, C_4 photosynthesis arose in a series of incremental steps from C_3 ancestors, and each step had to be adaptive in its own right (Monson and Rawsthorne 2000). Because each stage was likely necessary for subsequent stages to occur, the first stage may have been particularly important, as this would determine whether a population could even begin the C_4 evolutionary process (Sage 2002).

The first steps in C_4 evolution are thought to involve the localization of the enzyme glycine decarboxylase into the bundle sheath tissue (Monson 1999; Monson and Rawsthorne 2000). Glycine decarboxylase releases CO_2 in photorespiration, and its localization to the bundle sheath would force the photorespired CO_2 to diffuse through the mesophyll tissue, where it could be refixed by Rubisco. In addition, when photorespiration is high, any Rubisco in the bundle sheath could experience elevated CO_2 levels, allowing it to operate with greater efficiency. In this manner, the localization of glycine decarboxylase to the bundle sheath allows plants to operate a weak CO_2 concentrating mechanism that can partially offset the deleterious effects of high rates of photorespiration (Fig. 9.7). However, this will only be relevant when photorespiration is high, which is only in atmospheres of depleted CO_2.

The efficiency of a photorespiratory CO_2 pump can be increased in environments where photorespiration is elevated by enhancing Rubisco expression in the bundle sheath (Monson and Rawsthorne 2000). The next stage in the evolution toward C_4 photosynthesis may involve the enhancement of the CO_2 scavenging capacity by elevating PEP carboxylase activities in the mesophyll, and decarboxylase activity in the bundle sheath tissue (Monson 1999). Because C_3

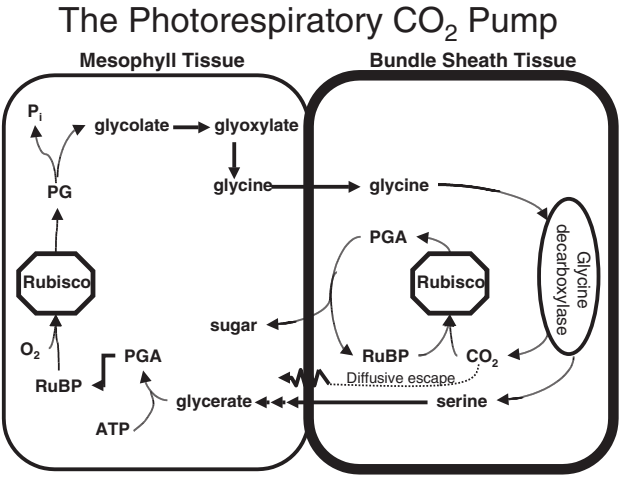

Figure 9.7. The photorespiratory CO_2 pump. In C_3-C_4 intermediate species, the first step on the evolutionary sequence to C_4 photosynthesis is proposed to be a shift in glycine decarboxylase expression from the mesophyll to the bundle sheath compartment (Monson 1999). Phosphoglycolate formed from RuBP oxygenation is converted to glycine in the mesophyll and then is transported to the bundle sheath where glycine decarboxylase metabolizes it to serine and CO_2. CO_2 levels in the bundle sheath become elevated during high rates of photorespiration and in doing so increase the efficiency of Rubisco use in the bundle sheath compartment. Derived from Sage 2001.

plants already have significant activities of decarboxylating enzymes in bundle sheath tissues (possibly to metabolize organic acids used in long-distance transport), this step may simply require the enhancement of existing patterns of gene expression (Hibberd and Quick 2002).

As the photorespiratory CO_2 pump develops, bundle sheath cells may enlarge to accommodate more Rubisco, while ratios of mesophyll to bundle sheath cells would further decline to reduce diffusion distances and to allow for close coordination of the various phases of the photorespiratory pump (Monson 1989). At some point, a recognizable PCA cycle would emerge as the primary role of PEP carboxylase shifts from refixation of photorespiratory CO_2 to assimilating CO_2 diffusing in from the atmosphere. In the final stages in C_4 evolution, Rubisco would be restricted to the bundle sheath cells, PEP carboxylase activity would be enhanced to meet the Rubisco capacity for CO_2 fixation, and enzyme regulation and cell ratios would be optimized to allow close coordination of the PCA and PCR cycles (Monson 1989).

In summary, the key initial step in the evolution of C_4 photosynthesis is thought to be the development of the photorespiratory CO_2 pump, which then makes the subsequent evolutionary stages possible. In this regard, photorespiration acts as the evolutionary bridge leading from C_3 photosynthesis to the intermediate stages where the PCA cycle can begin to develop (Sage 2001). To

generate enough photorespiratory metabolites to allow a photorespiratory CO_2 pump to arise, low CO_2 is required because at high CO_2, Rubisco oxygenase activity is suppressed at all temperatures. Hence, for the evolutionary bridge to form, low CO_2 conditions must prevail.

9.8 Ecological Scenarios for C_4 Evolution

Once the preconditions for C_4 photosynthesis were met, how might the C_4 pathway have evolved in natural ecosystems? One scenario for C_4 evolution is indicated by the occurrence of the two single-celled C_4 species, *Bienertia cycloptera* and *Borszczowia aralocaspica* (Freitag and Stichler 2000, 2002). Both species occur on extremely saline soils in depressions, the shores of saline lakes, and along marine coastlines of central Asia; notably, they are found beyond the leading edge of the halophytic C_3 vegetation (Freitag and Stichler 2000; 2002). This distribution indicates C_4 photosynthesis may be the key trait enabling the single-celled C_4 species to exist on soils too saline for most C_3 competitors. If this is the case, the adaptive radiation of single-celled C_4 photosynthesis could have occurred on soils where the salinity is too high for C_3 competitors to occur. The process might have begun when individuals expressing the initial stages of C_3-C_4 intermediacy were able to survive on marginal soils just beyond the edge of the ancestral C_3 vegetation. Offspring of these colonists that had greater levels of intermediacy may then have been able to colonize soils of even greater salinity. In turn, some of their offspring may have exhibited a greater expression of C_3-C_4 intermediacy and, as a result, may have colonized soils further out on the salinity gradient. Eventually, the salinity gradient may have selected for the right combination of traits, in the right sequence, to allow for a fully developed C_4 pathway to evolve.

A second scenario describes how nonhalophytic C_4 species may have evolved along heat and aridity gradients, with selection at the seedling stage playing a major role (Fig. 9.8). In the hot deserts of the world, the establishment of seedlings is the stage of the life cycle when mortality is greatest. A particularly stressful feature of the establishment phase is that seedlings experience elevated temperature in the boundary layer of the soil. Insulation provided by the boundary layer retains heat from incoming solar radiation, causing temperatures near the soil surface to warm above air temperature (Oke 1987).

In the hot deserts of the world, daytime air temperatures commonly exceed 40°C (Walter, Harnickell, and Mueller-Dombois 1975), so boundary layer temperatures should be well into the 45°–55°C range. In low CO_2 environments, the high temperature of the boundary layer probably causes the growth CO_2 compensation point of the C_3 plants to approach the prevailing atmospheric CO_2 level, hindering the establishment of C_3 seedlings. Seedlings that localize glycine decarboxylase to the bundle sheath may experience enough improvement in carbon acquisition to be able to colonize marginally lethal microsites. In turn, their offspring with greater levels of C_3-C_4 intermediacy might be selected for

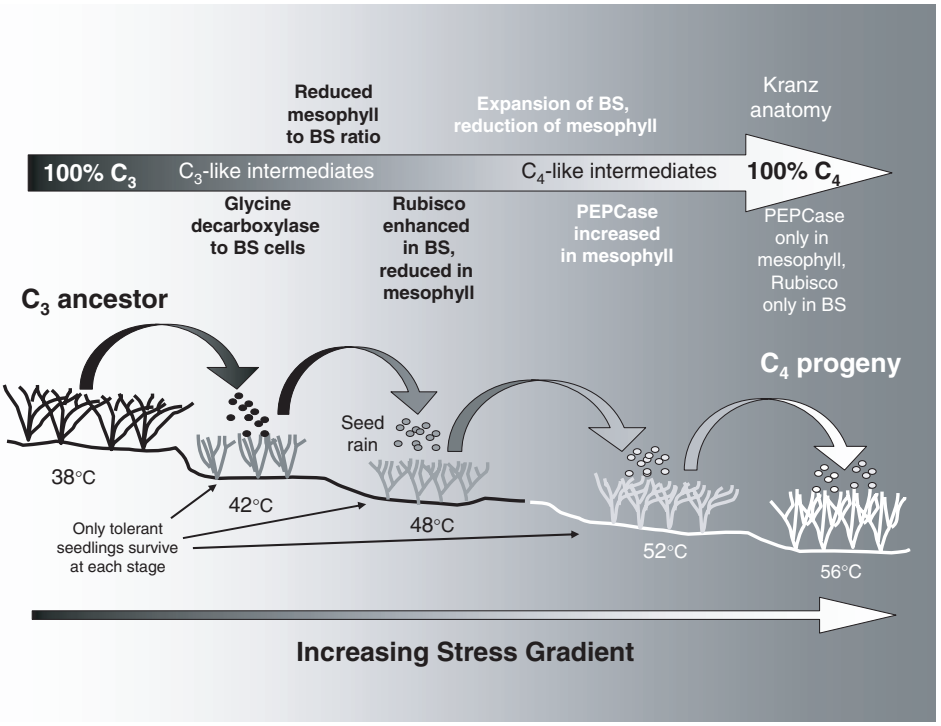

Figure 9.8. A conceptual model of how a stress gradient may select for varying stages of C_3-C_4 intermediacy and C_4 photosynthesis. Along the top of the figure are key steps in the evolutionary sequence of a complete C_4 pathway, which is based on recognized intermediate types in *Flaveria* species (Monson 1999). The temperatures at each stage represent possible temperatures in the boundary layer of desert soils along a microtopographic gradient. Where depressions form along the gradent, air movement slows, boundary layers thicken, and surface temperatures rise (Oke 1987). In the model, seed from C_3 progenitors will fall onto sites where the heat stress is slightly greater than can be tolerated by C_3 plants in a CO_2-depleted atmosphere. Most seedlings die, but seedlings with a weak carbon conservation mechanism, such as glycine decarboxylase localization to bundle sheath cells, will survive and establish. Seed from these individuals will rain onto sites of greater stress, and only offspring with a greater level of C_3-C_4 intermediacy will survive. In turn, only those offspring with an even greater degree of intermediacy will establish on the next most severe site, and so on until fully evolved C_4 progeny appear on sites with the most extreme stress.

progressively hotter sites, until eventually a full C_4 pathway evolved (see Fig. 9.8). Drought and salinity would act in concert with boundary layer heating, because other options for heat amelioration, such as opening stomates to transpirationally cool leaves, would not be possible where water is limited. As with salinity, in drought and high temperature scenarios, the inability of C_3 species to meet their carbon needs creates a gradient of open niches where experiments in carbon conservation and C_4 evolution could occur without interference from C_3 competitors. Once the C_4 pathway successfully evolved, the prototype C_4 plants would have a superior photosynthetic system that would allow them to spread back across the stress gradient and out of the C_4 so-called nursery. The novel C_4 species would then be able to radiate into C_3 dominated biomes and eventually adapt the wide range of habitats where C_4 photosynthesis is now successful, including swamps, wet grasslands, and river floodplains.

9.9 Summary

Significant advances in systematics, paleoecology, and climatology in recent years are now providing new perspectives that facilitate the generation of robust hypotheses of C_4 evolution. Fossil and isotopic evidence show when C_4 plants became common in the geological record, while phylogenetic evidence indicates when C_4 lineages began to diverge. The many independent origins of C_4 photosynthesis demonstrate the evolution of the pathway must be relatively easy in certain taxa; however, the restriction of C_4 photosynthesis to advanced angiosperm families indicates the evolution of these families may have been an important precondition that constrained the timing of C_4 evolution. The appearance of herbaceous and annual plant life forms in the Oligocene may also be significant, because C_4 photosynthesis is very successful in these groups, particularly in arid ecosystems (Sage 2001).

A plausible scenario indicated by the observations described here is that the superior reproductive system of the angiosperms allowed for the radiation of herbaceous species in hot, dry, and saline habitats. These plants in turn evolved drought adaptations that predisposed them to evolve C_4 photosynthesis once appropriate environmental conditions appeared. The full set of conditions promoting the success of C_4 photosynthesis may have first appeared during the Oligocene, when the global climate became drier, the tropics remained hot, and the atmospheric CO_2 levels declined below the high levels of the early Tertiary. In more recent geological time, climates deteriorated further, most notably during the late Miocene and early Pleistocene, allowing for widespread expansion of deserts and semi-arid landscapes. Although a further reduction in atmospheric CO_2 in the late Miocene has been debated (Cerling et al. 1997; Pagani, Freeman, and Arthur, 1999), it is clear from ice core records that record low CO_2 levels were present by late Pleistocene times (Petit et al. 1999; Zachos et al. 2001).

In summary, a picture emerges in which the Oligocene was a time when a number of critical factors coalesced to facilitate the origin of the first C_4 plants,

and the phylogenetic evidence indicates major groups of C_4 grasses were diverging 20 to 30 million years ago. While there has been much emphasis on the first origin of C_4 plants, the current flora is the product of many separate origins, and many if not most of these occurred more recently, possibly following late Miocene and early Pleistocene climate and atmospheric change. A late Miocene pulse corresponds to when C_4 grasslands expanded across continents at low latitude, while the Pleistocene is associated with the radiation of C_4 photosynthesis in most dicot groups.

The relationship between CO_2 reduction and the late Miocene expansion of C_4 grasslands is uncertain; however, it is known that very low CO_2 levels were present by the late Pleistocene. These levels were so low that it is probable hot regions of the earth could not support C_3 plants because CO_2 requirements to complete a life cycle were greater than the prevailing atmospheric value. Under these conditions, widespread C_4 evolution could be expected in the dry tropics, because the heat, low humidity, limited water availability, and in many areas elevated salinity would have selected for traits that enhanced the carbon balance of the plant. The rate of photorespiration is high under these conditions. This would have promoted the rise of C_4 photosynthesis by limiting productive potentials in C_3 plants as well as by providing an opportunity to concentrate CO_2 by segregating glycine decarboxylase into the interior of a leaf. In so doing, high photorespiration is likely the stimulus that enabled C_4-evolutionary trends to commence in many taxa.

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