2.2 C4 subgroups

2.2.1??Evolution of the C4 mode

Approximately 85% of all terrestrial plant species are C_3 plants, while about 10% are CAM plants and are usually found in highly xeric sites (deserts, epiphytic habitats). The remainder are C_4 plants, and these become more dominant with aridity. Naively, we could conclude that evolution of C_4 photosynthesis was a response to selection by high temperature and low water availability. More likely, a decline in atmospheric CO_2 concentration during past millennia provided an initial impetus.

One hundred million years ago (Mid-Cretaceous), atmospheric CO_2 was between 1500 and 3000 µL L^{-1}, or four to ten times postindustrial levels. Given such a large concentration of CO_2 at that time, photorespiration of C_3 plants was inhibited (Section 2.3) so that photosynthetic ef?ciency was higher than it is now. In addition, maximum photosynthetic rates were double twentieth century values, and the energy cost of photosynthesis would have been around three ATP and two NADPH per molecule of CO_2 ?xed. However, as atmospheric CO_2 concentrations declined to approximately 250–300 µL L^{-1}, photosynthetic rates were halved, photo-respiration increased substantially, photosynthetic ef?ciency declined and the energetic costs of photosynthesis increased to approximately ?ve ATP and 3.2 NADPH per CO_2 molecule ?xed. Such events would have generated a strong selection pressure for genetic variants with increased carboxylation ef?ciency and increased photosynthetic rates.

Angiosperms have a higher relative speci?city of Rubisco for CO_2 than ferns and mosses (see examples of other less evolutionarily advanced species in Figure 2.2). Such differences imply minor evolution in this highly conserved molecule of Rubisco and there is little variation between species of vascular plants. Consequently, alteration of Rubisco in response to a changing atmospheric CO_2 concentration has not been an option.

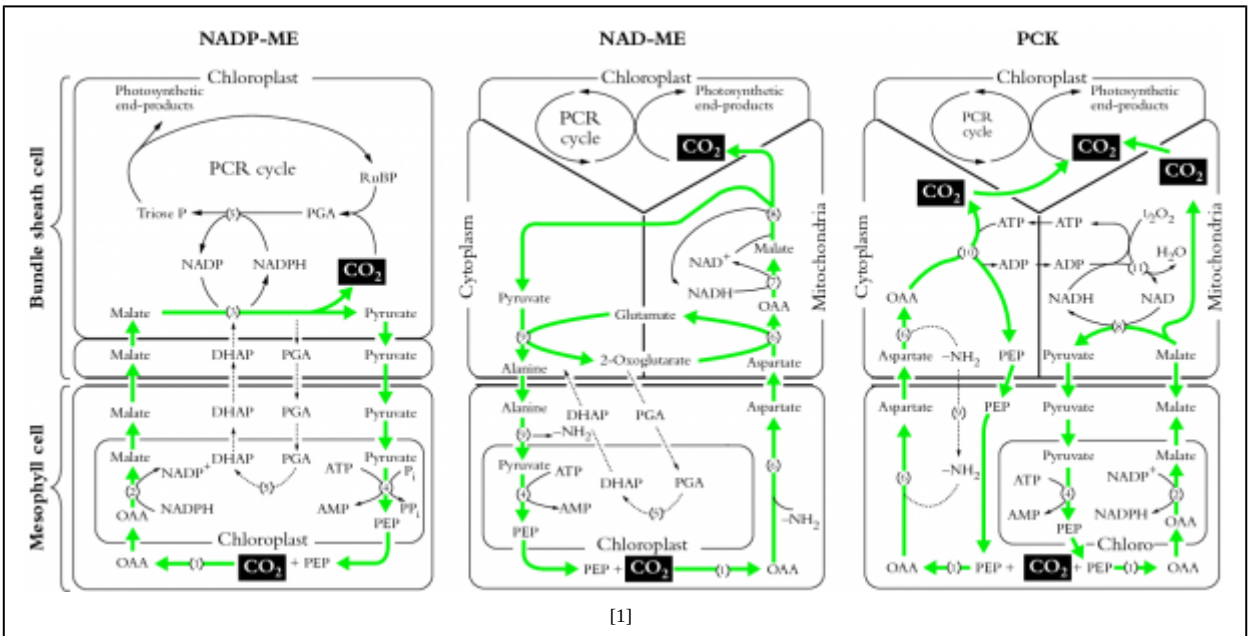
By contrast, evolution of a new photosynthetic pathway (C_4) has occurred independently and on several occasions in diverse taxa over 50 to 60 million years as CO_2 levels declined. The oldest identi?able fossils with pronounced bundle sheath layers are seven million years old, although necessary metabolic pathways could have evolved earlier, prior to this adaptation in anatomy. C_4 plants are known to differ from C_3 plants in their discrimination against atmospheric $^{13}CO_2$, and shifts in the stable carbon-isotope signature of soil carbonate layers that reflect emergence of C_4 plants have been dated at 7.5 million years bp. By inference, C_4 photosynthesis evolved in response to a signi?cant decline in atmospheric CO_2 concen-tration, from 1500–3000 µL L^{-1} to about 300 µL L^{-1}. By evolving a CO_2-concentrating mechanism, C_4 plants presented their Rubisco with an elevated partial pressure of CO_2 despite lower atmospheric CO_2. As a consequence, photorespiration was inhibited, maximum photosynthetic rates increased and energetic costs reduced.

2.2.2??Concentrating CO2 in bundle sheath cells

C_4 photosynthesis calls for metabolic compartmentation which is in turn linked to specialised anatomy (Figure 2.3). Three variants of C_4 photosynthesis have evolved which probably derive from subtle differences in the original physiology and leaf anatomy of their C_3 progenitors.

CO_2 assimilation by all three subgroups of C_4 plants (Figure 2.13) involves ?ve stages:

1. carboxylation of PEP in mesophyll cells, thereby generating four-carbon acids (malate and/or aspartate);
2. transport of four-carbon acids to bundle sheath cells;
3. decarboxylation of four-carbon acids to liberate CO_2;
4. re-?xation of this CO_2 via Rubisco within the bundle sheath, using the C_3 pathway;
5. transport of three-carbon acid products following decarboxylation back to mesophyll cells to enable synthesis of more PEP.



[1]

Figure 2.13 C_4 plants belong to one of three subgroups represented here (left to right) as NADP-ME, NAD-ME and PCK. Each subgroup has a distinctive complement and location of decarboxylating enzymes, and each differs with respect to metabolites transferred between mesophyll and bundle sheath. The path of

carbon assimilation and intracellular location of key reactions are shown for each of these biochemically distinct subgroups. Heavy arrows indicate the main path of carbon flow and associated transport of metabolites. Enzymes involved (numbers shown in parentheses) are as follows: (1) PEP carboxylase, (2) NADP-malate dehydrogenase, (3) NADP malic enzyme, (4) pyruvate P_i dikinase, (5) 3-PGA kinase and GAP dehydrogenase, (6) aspartate aminotransferase, (7) NAD-malate dehydrogenase, (8) NAD-malic enzyme, (9) alanine aminotransferase, (10) PEP carboxykinase, (11) mitochondrial NADH oxidation systems. In PCK-type C_4 plants, the PGA/DHAP shuttle would also operate between cells as indicated for NADP-ME and NAD-ME. Cycling of anaino groups between mesophyll and bundle sheath cells involves alanine and alanine aminotransferase.
(Original diagram courtesy M.D. Hatch)

However, recognising some systematic distinctions in whether malate or aspartate was transported to bundle sheath cells, C_4 plants were further subdivided into three subgroups according to their four-carbon acid decarboxylating systems and ultrastructural features (Hatch *et al*. 1975). Members of each group contain high levels of either NADP-malic enzyme (NADP-ME), phosphoenolpyruvate carboxykinase (PCK) or NAD-malic enzyme (NAD-ME) (so designated in Figure 2.13). High NADP-malic enzyme activity is always associated with higher NADP-malate dehydrogenase activity, while those species featuring high activities of either of the other two decarboxylases always contain high levels of aminotransferase and alanine aminotransferase activities. As a further distinction, each of the decarboxylating enzymes is located in bundle sheath cells; NAD-malic enzyme is located in mitochondria but phosphoenolpyruvate carboxykinase is not.

In all three subgroups, the primary carboxylation event occurs in mesophyll cytoplasm with PEP carboxylase acting on HCO_3^- to form oxaloacetate. However, the fate of this oxaloacetate varies according to subgroup (Table 2.2; Figure 2.13). In NADP-ME species, oxaloacetate is quickly reduced to malate in mesophyll chloroplasts using NADPH. By contrast, in NAD-ME and PCK species, oxaloacetate is transaminated in the cytoplasm, with glutamate donating the amino group, to generate aspartate. Thus, malate is transferred to bundle sheath cells in NADP-ME species and aspartate is transferred in NAD-ME and PCK species. The chemical identity of three-carbon acids returned to mesophyll cells varies accordingly.

Table 2.2 C_4 plants belong to one of three subgroups which differ in identity and location of enzymes responsible for decarboxylation

	Type of C_4 plant		
	NADP-ME	**NAD-ME**	**PCK**
Decarboxylating enzyme	NADP-dependent malic enzyme	NAD-dependent malic enzyme	Phosphenol-pyruvate carboxykinase
Location of decarboxylating enzyme	Chloroplast	Mitochondria	Cytoplasm
Main four-carbon acid moved to bundle sheath	Malate	Aspartate	Aspartate
Main three-carbon acid returned to mesophyll	Pyruvate	Alanine	Alanine/pyruvate

(Based on Hatch 1992)

[2]

Note also (Figure 2.13) that in NADP-ME species, only chloroplasts are involved in decarboxylation

and subsequent carboxylation via the PCR cycle. By contrast, in NAD-ME and PCK species, chloroplasts, cytoplasm and mitochondria are all involved in moving carbon to the PCR cycle of bundle sheath chloroplasts. In NAD-ME and PCK species, aspartate arriving in bundle sheath cells is reconverted to oxaloacetate in either mitochondria (NAD-ME) or cytoplasm (PCK) (Table 2.2). Reduction and decarboxylation of oxaloacetate occurs in mitochondria of NAD-ME species and CO_2 is thereby released for ?xation by chloroplasts of bundle sheath cells. In PCK species, oxaloacetate in the cytoplasm is decarboxylated by PCK, thereby releasing CO_2 for ?xation in bundle sheath chloroplasts (Figure 2.13).

Transport of metabolites to bundle sheath cells

Table 2.3 A high density of plasmodesmata significantly increases the permeability of bundle sheath cells to small metabolites such as aspartate and malate. Permeability to CO_2 is much reduced by a suberin layer in the walls of bundle sheath–mesophyll cell junctions. In NAD-ME types of C_4 plants, centripetal distribution of chloroplasts within bundle sheath cells helps restrict outward diffusion of CO_2

Plant type	Permeability coefficient (μmol min^{-1} (mg Chl)$^{-1}$ mM^{-1}) for small metabolites	Permeability coefficient for CO_2
C_3	<0.3	>2000
C_4	2–5	≈ 15

(Based on Hatch 1992)

[3]

A rapid transfer of malate and aspartate to bundle sheath cells from mesophyll cells is required if the CO_2 concentration in bundle sheath cells is to stay high. A very high density of plasmodesmata linking bundle sheath cells to mesophyll cells facilitates this traf?c. Consequently the permeability coef?cient of C_4 bundle sheath cells to small metabolites such as four-carbon acids is about 10 times larger than that of C_3 mesophyll cells (Table 2.3). However, coupled with this need for a high permeability to metabolites moving into bundle sheath cells is a low permeability to CO_2 molecules so that CO_2 released through decarboxylation in the bundle sheath does not diffuse rapidly into mesophyll airspaces. For some species, a layer of suberin in the cell wall of bundle sheath–mesophyll junctions (suberin lamella) signi?cantly reduces CO_2 efflux (Table 2.3).

Centrifugal versus centripetal chloroplasts

Not all species contain a suberin layer, but all C_4 plants have a need to prevent CO_2 from diffusing quickly out of bundle sheath cells, so that the location of chloroplasts of bundle sheath cells becomes critical in those species lacking a suberin layer (Figure 2.14). Where species have a suberin layer, chloroplasts are located in a centrifugal position, that is, on the wall furtherest away from the centre of the vascular bundle lying in the middle of the bundle sheath (Figure 2.14E, F). In contrast, in those C_4 species lacking a suberin layer, chloro-plasts are located centripetally, that is, on the wall closest to the centre of the vascular bundle lying within the bundle sheath (Figure 2.14A, B). Such a location would help restrict CO_2 diffusion from bundle sheath to mesophyll cells.

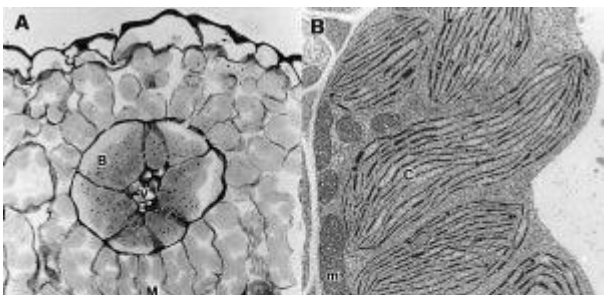


Figure 2.14 C_4 plants belong to one of three subgroups shown here in cross-section as light micrographs (left side) and electron micrographs (right side).Top to bottom, these subgroups are designated NAD-ME (A, B) PCK (C, D) and NADP-ME (E, F). Features common to all subgroups include a vascular bundle (V), bundle sheath (B), mesophyll tissue (M) and chloroplasts (C). See text and Hatch *et. al.* (1975) for detailed explanation of subgroups.

Subgroup NAD-ME (A, B) is represented by *Amaranthus edulis* and shows bundle sheath cells with centripetally located chloroplasts containing small starch grains and surrounded by mesophyll cells. The accompanying electron micrograph of a cytoplasmic region of a bundle sheath cell shows chloroplasts and numerous large mitochondria. Scale bar in A = 50 µm; in B = 2 µm. Subgroup PCK (C, D) is represented by *Chloris gayana* with chloroplasts arranged around the periphery of bundle sheath cells and adopting a centrifugal position. Mitochondria show well-developed internal membrane structures. Scale bar in C = 25 µm; in D = 3 µm.

Subgroup NADP-ME is represented by *Zea mays* where the bundle sheath contains centrifugally located chloroplasts with numerous starch grains, but lacking grana. Chloroplasts in adjacent mesophyll cells are strongly granal. Bundle sheath cells contain few mitochondria and these show only moderate development of internal membrane structures. Scale bar in E = 25 µm; in F scale bar = 2 µm
(Micrographs courtesy Stuart Craig and Celia Miller)

2.2.3??Regulation of C4 photosynthesis

Fixation of CO_2 by C_4 plants involves the coordinated activity of two cycles in separate anatomical compartments (Figure 2.13). The ?rst cycle is C_4 (carboxylation by PEP carboxylase), the second is C_3 (carboxylation by Rubisco). Given this biochemical and anatomical complexity, close regulation of enzyme activities is a prerequisite for ef?cient coordination.

PEP carboxylase, NADP-malate dehydrogenase and pyruvate orthophosphate dikinase are all light regulated and their activities vary according to irradiance. NADP-malate dehydrogenase is regulated indirectly by light via the thioredoxin system.

PEP carboxylase in C_4 plants exists in the same homo-tetramer in light- and dark-acclimated leaves. This is in marked contrast to CAM species where different forms exist in light- and dark-acclimated leaves. In C_4 plants, PEP carboxylase has extremely low activity at night, thus preventing uncontrolled consumption of PEP. Such complete loss of activity in darkness is mediated via divalent metal ions, pH plus allosteric activators and inhibitors. As a consequence, and over a period of days, C_4 plants can increase or decrease PEP carboxylase in response to light regime.

2.2.4??Environmental physiology

C_4 species frequently occur in regions of strong irradiance. For example, the C_4 grasses of savannas of northern Australia are relatively unshaded because of the low tree density and sparse canopy. Light is abundant and since the CO_2 con-centration inside C_4 leaves is high, a potentially high rate of light-saturated assimilation can be exploited. Most C_3 species reach light saturation in the range of one-eight to one-half full sunlight (Figure 2.6). In C_4 species, canopy assimilation might not become light saturated even in full sunlight. C_4 plants thus maintain a competitive advantage over C_3 plants in tropical locations, where average daily light receipt is much larger than in temperate zones, and associated with warmer conditions that also favour C_4 photosynthesis (Figure 2.6).

Given strong sunlight, warmth and seasonally abundant water, biomass production by C_4 plants is

commonly double the rate for C_3 plants. Typically, C_3 plants produce 15–25 t ha^{-1} but C_4 plants easily produce 35–45 t ha^{-1}. Added to this superior light-saturated capacity, C_4 plants achieve higher nitrogen and water use ef?ciencies due to their CO_2-concentrating mechanism and absence of photorespiration. Accordingly, C_4 plants are advantaged relative to C_3 plants in hot and nitrogen-poor environments with short growing seasons, hence their great abundance in wet/dry tropics such as Northern Territory savannas.

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