Testing the Functional Implications of Photosynthetic Heterogeneity in Leaves of C₄ Plants: “Reductionism during Scale Expansion”

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With 8 Figures

Abstract

This chapter illustrates how experimental studies of the remarkable heterogeneity of photosynthetic properties in C₄ plants can determine if and when heterogeneity and diversity in complex systems matter. The studies reviewed here confirm that anatomical and biochemical heterogeneity among C₄ types seem to deliver an array of compensating responses that confer a similar and robust, leaf-level advantage with respect to water and nutrient economy of CO₂ assimilation at high light and temperature in a low CO₂, high O₂ atmosphere. The heterogeneity presumably reflects the independent origins of the C₄ pathway in different plant taxa, and other processes, unrelated to the pathway itself, may be responsible for distinctive patterns such as, for example, the differing distribution of NADP- and NAD-ME C₄ type grasses in relation to precipitation.

Zusammenfassung


1. Introduction

“Photosynthesis and other natural phenomena, especially evolved, biological phenomena, are very complex and difficult to comprehend. It is useful to formally organize our knowledge in hierarchical levels, knowing of the upscale and downscale effects that one hierarchical level has on the other” (Smith et al. 2004). These authors offered a comprehensive attempt

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to scale our understanding of photosynthetic processes from the molecule to the biosphere. An earlier focus on the still to be explained nexus between scale in size and relaxation times of key processes (Osmond et al. 1980) suggested that although the whole is clearly the sum of its parts (at any scale), for practical purposes in scaling up, the whole may become a good deal less than this. Reductionism thus takes on two meanings in relation to scaling: the commonly expressed (but semantically incorrect) fascination with ever increasing detail down scale, and the need to selectively discard much of this detail if one is to evaluate the “bigger picture” up scale.

There may be few general rules but with presently available technologies, the leaf is probably the most appropriate scale for experimental evaluation of the importance of photosynthetic heterogeneity. Speculations about the function of heterogeneity in cells of leaves, their specialization with respect to metabolism, their arrangement with respect to light absorption and to export of the products of CO₂ assimilation, date from the earliest microscopic studies. In this chapter we deal with one of the most striking and now possibly best understood cases of heterogeneity in the photosynthetic apparatus in leaves of plants that assimilate CO₂ by the C₄ pathway of photosynthesis. Most clearly anticipated by Haberlandt (1909) when he speculated “ob eine noch unbekannte Arbeitsteilung zwischen den Chloroplasten der Kranz- und jenen der Scheidenzellen dabei im Spiele ist”, we now know that this “division of labor” involves tight coordination of the partial reactions (both light and dark) to function as an internal CO₂-concentrating mechanism (Hatch and Osmond 1976, Edwards et al. 2001).

Until recently, it was thought that the CO₂-concentrating mechanisms of the C₄-photosynthetic pathway were distinctive in that they involve close collaboration of two adjacent highly differentiated photosynthetic cell types, the mesophyll (M) and bundle sheath (BS) cells (Edwards et al. 2001). However, now C₄ Chenopodiaceae have been discovered in central Asia in which all the components of the CO₂-concentrating mechanisms were found in spatially separated, clearly differentiated chloroplasts in the same cells. The biochemistry of the CO₂-concentrating mechanisms in these C₄ plants seems similar to those of the two-cell systems, but the symplastic connections sustaining the metabolite transfers are based on fascinating forms of intracellular compartmentation (Edwards et al. 2004).

Most common among plants well adapted to dry and hot environments, the biochemical heterogeneity of C₄ metabolism is thought to confer robust advantages of more efficient CO₂ assimilation, water use and nitrogen use, and productivity at high temperature, at the cost of less efficient light use in the prevailing low CO₂ and high O₂ atmosphere (Sage 2004). In focusing attention on this particular form of heterogeneity we will briefly review its role in molecular through physiological processes and describe experiments that explore whether it makes a difference to environmental responses at the leaf and larger scales.

The issues that bedevil efforts to deal with scaling and to bring accountability to plant sciences research were elegantly expounded by Passiouara (1979) in a brief but no longer easily accessible article. Passiouara’s “parable” of scaling, based on the dots in a screened image taken from Piaget (1971), is especially relevant to the Jülich symposium theme, and can be re-told using images of chlorophyll fluorescence from a shade leaf exposed to full sunlight through a photographic negative. The rather stable images stored in the leaf as differential chlorophyll fluorescence emission from active and inactive photosystem II centers in grana of chloroplast thylakoids are readily captured using a digital camera fitted with appropriate excitation and filters (Osmond et al. 1999). It is a simple matter to expand the image size to the extent that, although we can differentiate every pixel and its intensity on a scale of 1–256,
it remains a meaningless landscape of light and shade. One can only begin to make sense of
the image by reducing it so that mind’s eye no longer resolves the fine structure and instead
reveals the big picture as the ear of a distinguished plant biologist (Fig. 1).

Thus we can imagine our purpose to be the experimental evaluation of “operational struc-
turalism” in which Piaget ascribed primary importance to understanding the natural struc-
tures that comprise the whole, rather than the detail in the components that make up the
natural structures, or the emergent properties of the whole itself. We ask whether heteroge-
neous biochemical components that underlie the natural structures in C₄ plants (including
CO₂-concentrating mechanisms, water use and nutrient use efficiency, and thermal responses)
significantly influence the whole, as reflected in the ecological distribution of C₄ plants along
environmental gradients.

Fig. 1  A photoinactivation portrait of a well known plant biologist made on a shaded bramble leaf and imaged by
chlorophyll fluorescence (Osmond et al. 1999). The enlarged pixilated image on the left is precisely representative
of the heterogeneity of information in one of the images on the right. The left image is perhaps analogous to our
detailed molecular understanding of C₄ photosynthesis. At a larger scale it becomes recognizable as a “natural struc-
ture”, the left ear in the central image on the right. These are perhaps analogous to our broad understanding of C₄
plant ecophysiology; of the whole in different contexts. Photos prepared by Barry Osmond at the Botanical Institute,
Technical University Darmstadt, July 1999.
2. Dimensions of Heterogeneity in the Photosynthetic Apparatus of C₄ Plants

Rubisco, the primary CO₂-fixing enzyme of photosynthesis, is a poor catalyst (Andrews et al. 1971, Andrews and Lorimer 1981, Tcherkez et al. 2006). Many species, including unicellular algae (Badger and Price 1992) and crassulacean acid metabolism plants and C₄ plants (Leegood et al. 1997), have evolved CO₂-concentrating mechanisms that deliver high CO₂ at the site of carboxylation and enhance Rubisco catalysis. This reduces Rubisco oxygenation and photorespiration and allows Rubisco to operate close to its maximal activity.

Photosynthetic heterogeneity in the multicellular C₄ types is most commonly illustrated and analyzed in transverse leaf sections (Fig. 2A) showing the “Kranz” (wreath-like) radial arrangement (Haberlandt 1909, Hattersley and Watson 1975) to highlight the path-length for metabolite exchange (Hatch and Osmond 1976, von Caemmerer and Furbank 2003) between chloroplasts in mesophyll and bundle sheath cells. However, Figure 2B also shows a median paradermal section that emphasizes the large air spaces between loosely arranged mesophyll cells in which CO₂ is initially fixed into C₄ acids by phosphoenol pyruvate carboxylase (PEPC). These and other metabolites are then exchanged with bundle sheath cells tightly appressed to veins, and are decarboxylated there by three distinctly compartmented and different biochemical pathways that generate elevated [CO₂]. The structure of the bundle sheath wall (which has a low permeability to CO₂), the relative biochemical capacities of the C₃ cycle in the bundle sheath, and metabolite exchange across the mesophyll-bundle sheath interface, all contribute to achievement of elevated [CO₂] for Rubisco in the bundle sheath (von Caemmerer and Furbank 2003).

Fig. 2 Transverse (A) and (B) paradermal sections of the “Kranz” anatomy in Atriplex vesicaria leaves grown under warm conditions (28 °C day/20 °C night), showing marked differences in the size and vacuolation of mesophyll cells (M) and bundle sheath cells (BS). Light micrographs × 100 of thin sections prepared for transmission electron microscopy; for details see Caldwell et al. (1977).
The C₄ photosynthetic pathway has evolved a number of times in a large number of both dicot and monocot genera (SAGE 2004). Three major biochemical subgroups of C₄ plants have been characterized, that use different C₄-acid decarboxylases in different bundle sheath cell compartments to generate elevated [CO₂]; NADP-ME type in chloroplasts, NAD-ME type in mitochondria and PCK types primarily in the cytosol (DOWNTON 1971, HATCH et al. 1975). These biochemical variations are accompanied by a suite of anatomical features, such as the presence or absence of a suberized lamella in the cell wall between bundle sheath and mesophyll cells, and the centripetal and centrifugal orientation of chloroplasts in the bundle sheath. They are also accompanied by suites of complementary alterations in the light harvesting and photosynthetic electron transport capacities of chloroplasts in the two cell types (HATCH and OSMOND 1976, HATCH 1987).

Deficiency of PSII activity in the bundle sheath of sorghum and other NADP-ME types is one of the best documented examples of heterogeneity in thylakoid composition in chloroplasts of C₄ species ( Woo et al. 1970, MAYNE et al. 1974, EDWARDS et al. 1976, GHIRARDI and MELIS 1984). Agranal bundle sheath chloroplasts of sorghum have very limited PSII activity and CO₂ reduction is driven by complementary metabolite shuttles, not by PSII electron transport. The remarkably different PSII fluorescence emission of mesophyll and bundle-sheath cells of sorghum in vivo is clearly displayed by confocal microscopy (Fig. 3) which even resolves the strong point sources of PSII fluorescence in grana of mesophyll chloroplasts, and the weak diffuse fluorescence of agranal bundle-sheath chloroplasts (EDWARDS et al. 2001, GUNNING 2007). It is not yet entirely clear how the tight coupling between demands of CO₂ reduction and the bioenergetic capabilities of light reactions in chloroplasts in the adjacent cells is co-ordinated (MEIERHOFF and WESTHOFF 1993).

Here we confine ourselves to recent studies of the extent to which functional heterogeneity in the CO₂ assimilation machineries of bundle sheath and mesophyll cells, and the light reactions of their chloroplasts, is reflected in photosynthesis measured by leaf level gas exchange and chlorophyll fluorescence in different C₄ plants. We also examine whether heterogeneity in these attributes helps improve our understanding of the distribution of these plants in relation to gradients in temperature and water availability, nitrogen nutrition and light environments.

3. Heterogeneity of CO₂ Diffusion within Leaves and its Implications for Water Use Efficiency of C₄ Plants

Heterogeneity of photosynthesis in C₃ leaves arises from patchy stomatal closure that denies CO₂ supply to sectors in leaves isolated by vein extensions which prevent lateral diffusion of gases (heterobaric leaves; TERASHIMA et al. 1988), as well as from slow lateral diffusion of CO₂ between mesophyll cells in homobaric leaves (PIERUSCHKA et al. 2005, MORISON et al. 2005). Heterogeneity of photosynthesis is also evident at high internal CO₂ concentrations in CAM plants with very large, uniform mesophyll cells because these cells are tightly packed and have little intercellular air space for CO₂ diffusion which is much slower when confined to wet cell walls (RASCHER et al. 2001, NELSON et al. 2005, DUARTE et al. 2005).

The functional cooperation between mesophyll and bundle sheath cells puts quite different constraints on CO₂ diffusion in C₄ leaves. A prerequisite for high photosynthetic rates of C₄ photosynthesis is a high rate of CO₂ diffusion from intercellular airspace to the mesophyll cytosol. Since PEPC is located in the mesophyll cytosol, CO₂ has to diffuse through the cell
Fig. 3 Confocal microscope image of chlorophyll auto-fluorescence from mesophyll and bundle sheath cells of *Sorghum bicolor* in longitudinal view. Mesophyll cell chloroplasts (M, outer rows, left) have thylakoids with high activity of both photosystems show strong fluorescence from PSII in grana (resolved in lower chloroplasts, right). Adjacent bundle sheath cells contain larger chloroplasts (BS, inner rows, left) with PSII deficient thylakoids, lack grana and show diffuse fluorescence from PSI alone (upper chloroplasts, right). Photographs courtesy of B. E. S. Gunning; modified after Edwards et al. 2001.

wall and the plasma membrane and cytosol, and the path length in the cytosol is difficult to estimate (Evans and von Caemmerer 1996). The mean value for mesophyll cell surface area to leaf area is 11.6 ± 1.1 for C₄ monocots and 15.6 ± 1.4 for C₄ dicots (von Caemmerer et al. 2007). These values are slightly lower than those reported for C₃ species but similar to values of chloroplast surface areas adjacent to intercellular airspaces in leaves of C₃ species (von Caemmerer et al. 2007). Given the need for rapid CO₂ diffusion across this interface one wonders whether the lower mesophyll cell surface areas in C₄ leaves are an anatomical constraint imposed by the need to stay connected with the bundle sheath cells. There are no studies we know of that have examined lateral photosynthetic heterogeneity in C₄ species which we suspect may be less pronounced than in C₃ leaves. It might be interesting to repeat the fluorescence imaging experiments of Morison et al. (2005) who occluded stomata with spots of grease to examine CO₂ diffusion within the mesophyll of C₃ species.

The requirement of high conductance for CO₂ diffusion, from intercellular airspace to the mesophyll, contrasts with the requirement of low CO₂ permeability across the mesophyll bundle sheath interface needed to achieve elevated [CO₂] for the CO₂-concentrating mechanism. In part this is achieved by low bundle sheath to leaf surface area ratios (1.8 ± 0.1) that vary little amongst species (von Caemmerer et al. 2007). One of the measures of the success of the cooperation between mesophyll and bundle sheath cells in C₄ photosynthetic CO₂ concentra-
tion mechanism is the CO₂ leakiness of the bundle sheath, defined as the rate of CO₂ leakage out of the bundle sheath relative to the rate of CO₂ supply of the C₄ cycle (Farquhar 1983). It has been suggested that NADP-ME grasses may be less leaky than NAD-ME grasses since they possess a suberized lamella in the bundle sheath cell wall which could increase the gas tightness of the bundle sheath. This hypothesis fits with the differences in the carbon isotope composition of leaf dry matter observed between NAD-ME and NADP-ME species (Hattersley 1982, Farquhar 1983, Ghannoum et al. 2002). However, short term measurements of photosynthetic carbon isotope discrimination made concurrently with gas exchange revealed no evidence for differences between the biochemical subtypes (Henderson et al. 1992, Cousins et al. 2007). Perhaps differences in dry matter carbon isotope discrimination are related to differences in respiratory metabolism, but this has yet to be explored.

The near constancy of the estimated leakiness in C₄ species from a range of evolutionary origins seems quite remarkable. It complements the convergence found in other gas exchange characteristics despite anatomical and biochemical heterogeneity observed in these species. For example, when grown under standard conditions there was a surprising similarity in photosynthetic rate per leaf area although NAD-ME species had slightly greater whole plant water used efficiency under drought (Ghannoum et al. 2002). It turned out that the key factor contributing to this difference was a higher catalytic turnover rate of Rubisco carboxylation in NADP-ME compared to NAD-ME species (Ghannoum et al. 2005). The geographic distribution of the different C₄ types with rainfall shows dramatically different correlations; species of the NADP-ME subtype are more abundant in high rainfall areas whereas the NAD-ME subtype is more abundant in drier habitats (Ellis et al. 1980, Hattersley 1983). Clearly, there is more to ecological success than water efficient CO₂ assimilation.

In most temperature transects examined, whether latitudinal or altitudinal, the frequency of C₄ plants in grass floras is positively correlated with growing season minimum temperature (Teeri and Stowe 1976, Vogel et al. 1978, Hattersley 1983, Taub, 2000). Since Ehleringer and Björkman (1977), this correlation has been attributed to the temperature insensitivity of quantum yield in C₄ plants, and the higher quantum yield of C₃ photosynthesis at about 10 °C. However, we need to remember that the quantum yield advantage is only likely to be selected in low light environments. The canopy of tall tropical grasslands may be light limited, but it is clear that the development of the photosynthetic apparatus in tropical C₄ grasses is impaired at low temperatures (Taylor and Craig 1971, Slack et al. 1974). Moreover, species with the same C₄ type (NADP-ME), but from different genetic lineages, show remarkably different responses to low temperature. Cold-tolerance in Miscanthus × giganteus is associated with maintenance of high levels of Rubisco and some C₄ enzymes, compared with cold-sensitive Zea mays (Naidu et al. 2003). Low temperature tolerance is widely known among NAD-ME type C₄ dicotyledons such as Atriplex spp. (Osmond et al. 1980), and the yet to be explained starch accumulation in both mesophyll and bundle sheath cells at low temperature (Caldwell et al. 1977) seems common across both pathway types and between species.

4. Heterogeneity, Nitrogen use Efficiency and Light Environment

The higher gross leaf nitrogen use efficiency of the C₄ pathway compared to the C₃ pathway is well known (Seemann et al. 1984, Hatch 1987, Long 1999). Recent detailed comparisons
of photosynthetic N allocation in C\textsubscript{3} and C\textsubscript{4} plants (Makino et al. 2003) have been refined to show that the higher efficiency in the latter arises from both reduced Rubisco content (less carboxylase is needed to sustain the same rate of CO\textsubscript{2} fixation at elevated [CO\textsubscript{2}]) and improved catalytic turnover rates of Rubisco in C\textsubscript{4} species (Ghannoum et al. 2005). The partitioning of leaf chlorophyll and nitrogen to the bundle sheath in four C\textsubscript{4} grasses examined by Ghannoum et al. (2005) is shown in Figure 4. The NAD-ME grasses had a lower photosynthetic nitrogen use efficiency than NADP-ME grasses because they contained more leaf N per area (Ghannoum et al. 2005). In the NAD-ME species 65\% of both leaf N and chlorophyll is found in the PSII-rich bundle sheath, whereas only 35\% of both chlorophyll and nitrogen is found the PSII-depleted bundle sheath of NADP-ME species. Despite containing 65\% of leaf chlorophyll less than 40\% of leaf functional PSII and PSI centers are located in the bundle sheath of the two NAD-ME species. Bundle sheath cells of the two NADP-ME species contain a similar amount of PSI activity but PSII activity is almost negligible.

There are few studies of the plasticity of chloroplast thylakoid organization in mesophyll and bundle sheath in C\textsubscript{4} species in relation to N nutrition and light environment. Henderson

![Figure 4](image-url)
et al. (1992) observed increased leakiness in NADP- and NAD-ME types at low light, and Wong and Osmond (1991) noted an increase in $\delta^{13}$C composition of *Echinochloa frumentacea* (NADP-ME type) and stimulation of growth by elevated [CO$_2$], indirectly indicating a partial failure of the CO$_2$-concentrating mechanism at low N. In the C$_4$ dicot *Amaranthus cruentus* (NAD-ME) low N and low light increased the investment of N in light harvesting systems and surprisingly, sustained a higher quantum yield of photosynthesis at low light, in spite of increased leakiness (Tazoe et al. 2006). In maize, Drozak and Romanowska (2006) reported that low light increased the ratio of PSII electron transport activity of bundle sheath chloroplasts relative to mesophyll from 0.15 to 0.20 and decreased the ratio of PSI electron transport from 1.94 to 1.70. Larger increases in the PSII-associated light harvesting components of mesophyll and bundle sheath cells were observed, with little change in PSI antennae. The range of photosystem plasticity seems limited, but seems to compensate the bioenergetics of CO$_2$ assimilation.

5. Distribution of Light Absorption in C$_4$ Leaves

Terashima and Saeki (1983) first showed that monochromatic light was attenuated in direct proportion to the cumulative chlorophyll content of a C$_3$ leaf. Careful paradermal sectioning of spinach leaves demonstrated that chloroplasts differed vertically through the leaf, both biochemically and ultrastructurally (Terashima and Inoue 1985a, b). Evans and Vogelmann (2003) measured profiles of light absorption more directly by quantifying chlorophyll fluorescence images of transversely cut faces obtained when blue or green or blue light was applied to the abaxial or adaxial surface of spinach leaves.

However, the anatomy of C$_4$ leaves means that bundle sheath chlorophyll is generally shielded by the surrounding mesophyll cells. Therefore, one would expect that relatively less light would be absorbed per chlorophyll in the bundle sheath compared with the mesophyll. To investigate this, Evans et al. (2007) used the above techniques to image chlorophyll fluorescence emitted from the cut transverse face of leaves *F. bidentis*. Three images are shown in Figure 5A. Under epi-illumination, light is directed onto the transverse face through the microscope lens, which also captures the fluorescence. The distribution of fluorescence represents that of chlorophyll. Bright fluorescence can be seen throughout the mesophyll. The fluorescence is less in the bundle sheath because of reduced PSII content (cf. Fig. 3). By applying light to the adaxial surface, the fluorescence image reveals the gradient in light absorption through the leaf. Blue light is strongly absorbed by chlorophyll and is rapidly scattered on entry into the leaf. This results in intense fluorescence near the adaxial surface, but little fluorescence from the lower half of the leaf. In contrast, green light penetrates further into the leaf and some fluorescence is still emitted from chloroplasts near the lower surface.

The profile of fluorescence across the leaf was quantified from *F. bidentis* images by sampling transects through mesophyll or vascular tissue (Fig. 5B). Depth was measured from the boundary between the epidermis and mesophyll. Under blue light, fluorescence declines rapidly, falling below 20% within 40 µm through the mesophyll. In contrast, it takes about 300 µm for blue light absorption to decline by a similar amount in leaves of *Spinacia oleracea* (Fig. 4B; Evans and Vogelmann 2003). Thus a C$_4$ leaf like *F. bidentis* absorbs light very efficiently over a short distance in comparison to the much thicker spinach leaves (Fig. 5).
Green light penetrated further and significant amounts of green light reached the bundle sheath chloroplasts compared with relatively little blue light. The differential penetration of blue and green light into *F. bidentis* leaves led us to investigate the consequent effect on photosynthesis. We compared steady-state rates of CO$_2$ assimilation and photochemical efficiency under white, green, or blue light with equivalent incident photon fluxes (Fig. 6). Despite giving the same incident photon irradiance for each colour, the rates of CO$_2$ assimilation were lower under blue light by about 25% for *Spinacia* (data not shown) and 50% for *F. bidentis* (EVANS et al. 2007). This was not reflected in the photochemical efficiency signal, which decreased by only 15% (Fig. 5). This illustrates the fact that gas exchange integrates the flux through the depth of the leaf over a given area, while fluorescence records from a layer of chloroplasts near the adaxial surface. The results presented in Figure 6 suggest that the poor penetration of blue light into the bundle sheath cells did not allow for sufficient ATP formation in the bundle sheath to match the rate of CO$_2$ pumping. One would predict that leakiness should be greater under blue light than under green light and this prediction remains to be investigated. Our results highlight that bundle sheath and mesophyll experience very different light environments.
6. Whole Leaf Chlorophyll Fluorescence, What Do We See?

A close relationship between chloroplast electron transport estimated from chlorophyll fluorescence and CO₂ assimilation rate was first demonstrated for Zea mays by Genty et al. 1992 and Edwards and Baker 1993. Surprisingly, the differences in chlorophyll and PSII distribution between mesophyll and bundle sheath cells amongst the different biochemical subtypes is not apparent when rates of chloroplast electron transport estimated from chlorophyll fluorescence are correlated with measurements of CO₂ assimilation rates (Krall and Edwards 1990). Likewise, the changes in PSII and PSI light harvesting and electron transport activity in Z. mays grown at different light intensities had little impact on light dependence of PSII efficiency estimated by fluorescence, although low light grown leaves had about 30% lower capacity for nonphotochemical quenching at high light (Drozak and Romanowska 2006).

Comparison of mass spectrometric measurements of gross O₂ evolution (originating form PSII) with measurements of chloroplast electron transport by chlorophyll fluorescence shows an almost one to one relationship (Fig. 7A; Siebke et al. 2003), whereas chloroplast electron transport measured with chlorophyll fluorescence overestimates gross CO₂ uptake slightly.
This can probably be explained by the significant amount of light dependent O₂ uptake via the Mehler reaction which was 18% of gross O₂ evolution (Fig. 8). Leaf discs of a number of C₄ grasses of either the NAD-ME or NADP-ME biochemical subtypes showed a linear dependence of ¹⁸O uptake on light intensity, and no differences between subtypes was observed (Siebke et al. 2003). These studies confirm earlier indications of uniformly lower quantum yield of O₂ evolution in different C₄ types compared with C₃ plants (Demmig and Björkman 1987).

7. Heterogeneity and the Ecology and Evolution of C₄ Plants

The above experiments suggest that we must search for other factors to explain clearly differentiated correlations of abundance in C₄ types with annual precipitation (Hattersley 1982, Henderson et al. 1992). For example, it now seems that the decline in relative abundance of C₄ grasses (relative to C₃) in response to decreasing rainfall in South Africa, even though C₄ plants have higher water use efficiency when water is available, may be attributed to the greater drought sensitivity of C₄ pathway metabolism (B. Ripley and C. Osborne, personal communication). Analogous constraints may explain the correlations between growing season minimum temperatures and C₄ grass distribution along longitudinal and altitudinal gradients (Teeri and Stowe 1976). It is already clear that these are not associated with different temperature-quantum yield relationships among C₄ types, but involve complex interactions with development of the whole photosynthetic apparatus. The NAD-ME C₄ dicots may be the most cool tolerant of all (Caldwell et al. 1977, Osmond et al. 1980, 1982). There have been few studies of nitrogen use efficiency and environment in C₄ plants, even in crop plants. Wong and Osmond (1991) explored some competitive interactions between wheat and pearl
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millet in relation to nitrogen use efficiency and elevated atmospheric [CO₂], with emphasis on carbon partitioning belowground in relation to nutrient uptake. Overall, enormous scope remains for exploration of these relationships in common garden and competition experiments.

We should not be dissuaded from further measurement and modeling of the functional significance of heterogeneity in these systems. Some unresolved molecular and cellular issues include accumulation of starch in mesophyll cells in response to low temperature and evaluation of lateral diffusion of CO₂ in C₄ monocots and dicots. Although heterogeneity in the C₄ pathway may not matter with respect leaf-level photosynthetic performance, it may well matter in relation to environmental tolerance limits during development and senescence.

Fig. 8 Light dependence of O₂ exchange in NAD-ME (open symbols) and NADP-ME (closed symbols) grasses. (A) Gross O₂ evolution at 1–2 % CO₂; (B) Gross O₂ uptake at 1–2 % CO₂ (circles) and O₂ uptake in the dark (triangles) measured on corresponding leaf discs at 30 °C and 21 % O₂. (NAD-ME species used were Eragrostis superba, Leptochloa dubia, Panicum coloratum; and NADP-ME species used were Dichanthium sericeum, Pennisetum alopecuroides, Panicum antidotale, Cenchrus ciliaris.) Symbols indicate average of 1–4 measurements per species and light intensity; error bars are standard errors calculated on the average of species. The Figure is adapted from Stueck et al. 2003.
as we scale up from the leaf to the landscape. At bottom, we need to remember that natural selection brings the whole environment to act upon the whole genome throughout the whole of development, survival and reproduction of the organism.

Crossing experiments with C3 and C4 Atriplex spp. at the Carnegie Department of Plant Biology in the 1970s yielded a huge array of anatomical and biochemical heterogeneity in some 300 F1-F3 hybrids. However, none showed the assembly of a functional “C4 syndrome”; all retained CO2 compensation points and δ13C values similar to the C3 parent (Osmond et al. 1980). Naturally occurring C3-C4 intermediates also show C3-like δ13C values, but a range of intermediate CO2 compensation points, suggesting a variety of partly functional C4-like CO2-concentrating mechanisms (Monson and Rawsthorne 2000). Recent studies of the genus Heliotropium have gone one step further, making detailed assessments of the relationships between intermediate CO2 compensation points, CO2 fixation rates under limiting and saturating CO2, water use efficiency and ecological niches (Vogan et al. 2007). These authors are thus closing on the mechanisms of evolution of C4 plants, and speculate that there may be “only a few viable levels of intermediacy” (i.e., only a few viable “natural structures”) and that natural selection from C3 to C4 proceeds in steps defined by a few functional assemblages of the elements of heterogeneity.

8. Conclusions

Spatial and functional heterogeneity is a feature of the photosynthetic apparatus at all scales, from the molecular ecology of antennae and reaction centers responsible for solar energy transduction in thylakoid membranes of chloroplasts, to the structure of leaf tissues, to the display of leaves and to canopy architecture (Osmond et al. 1999). There is a remarkable range of heterogeneity in the leaves of C4 plants considered here, but is it reflected in functional diversity at larger scales; in selective advantage in the way different types of C4 plants respond to gradients of water, nutrient and light availability in the environment?

– In spite of substantial differences in the anatomical and biochemical heterogeneity in different C4 types their CO2-concentrating mechanisms confer similar improvements in water use efficiency under comparable conditions (Ghannoum et al. 2002).
– Higher nitrogen use efficiency is also a feature of all C4 types, and is reflected in different patterns of nitrogen allocation between thylakoid and soluble proteins in mesophyll and bundle-sheath chloroplasts in leaves of different types.
– The heterogeneity of chlorophyll and photosystem distribution in different C4 types is integrated by chlorophyll fluorescence measurements from the thin leaves. However, the relationship between photosynthetic electron transport and CO2 and O2 exchange rates that emerges from these measurements is similar, in spite of the heterogeneity among components of the light and dark reactions of photosynthesis in NADP- and NAD-ME C4 types (Siebke et al. 2003). Substantial light dependent electron flow to O2 (Mehler reaction) is a feature of both NADP- and NAD-ME C4 types.

The studies reviewed here confirm that the anatomical and biochemical heterogeneity among C4 types reflects natural selection of diverse pathways that confer a similar and robust leaf-level advantage with respect to water and nutrient economy of CO2 assimilation and light use in a low CO2, high O2 atmosphere. In terms of Piaget (1971) these leaf-level “natural
structures” represent the essence of “operational structuralism” within C₄ plants otherwise known as the “C₄ syndrome”. In spite of having arisen multiple times in unrelated taxa, there has been remarkable functional convergence such that we cannot associate specific selective advantages with heterogeneity among C₄ types in relation to leaf-environment interactions. In scaling up to the leaf level and beyond, for most practical purposes, the C₄ pathway becomes somewhat less than the sum of its parts.

Passioura (1979) argued for better communication between molecular, organismal and ecological levels of enquiry in plant sciences to achieve more accountable outcomes. Shortly afterwards, the first steps were taken to apply integrative leaf-level methods, such as stable isotopes to scale up CO₂ and H₂O exchange (the dark reactions of photosynthesis), and chlorophyll fluorescence (the light reactions) to evaluate the significance of heterogeneity in photosynthetic systems. Today, the physiological bases of C₃ and C₄ photosynthesis (Farquhar and Richards 1984) have assisted selection of more water use efficient varieties of wheat now released to Australian farmers, and now inform “big leaf” models of regional and global carbon and water vapor fluxes using δ¹³C and ¹⁸O signatures in atmospheric CO₂ (Lloyd and Farquhar 1993, Lin et al. 1998). Although the CO₂-concentrating mechanisms of the C₄ pathway clearly mitigates O₂ uptake by Rubisco, substantial O₂ uptake involving photosynthetic electron transport in the Mehler reaction persists in these plants (Siebke et al. 2003). The δ¹⁸O signature of photosynthetic electron flow to O₂ might help refine global insights into the steady state difference between the isotopic composition of atmospheric oxygen and its ultimate source in water (the “Dole effect”, Guy et al. 1993, Bender et al. 1994), particularly in the face of accelerating elevated atmospheric [CO₂]. Moreover, the leaf-level advantages conferred by the heterogeneity of the C₄ pathway also need careful further evaluation in relation to rising [CO₂] (Wong and Osmond 1991, Henderson et al. 1992, Ghannoum et al. 2001). Clearly, the functional importance of photosynthetic heterogeneity in leaves may yet have much to offer in scaling from the molecule to the biosphere.

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References


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