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Is C_4 photosynthesis less phenotypically plastic than C_3 photosynthesis?*

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Abstract

C_4 photosynthesis is a complex specialization that enhances carbon gain in hot, often arid habitats where photorespiration rates can be high. Certain features unique to C_4 photosynthesis may reduce the potential for phenotypic plasticity and photosynthetic acclimation to environmental change relative to what is possible with C_3 photosynthesis. During acclimation, the structural and physiological integrity of the mesophyll–bundle sheath (M-BS) complex has to be maintained if C_4 photosynthesis is to function efficiently in the new environment. Disruption of the M-BS structure could interfere with metabolic co-ordination between the C_3 and C_4 cycles, decrease metabolite flow rate between the tissues, increase CO_2 leakage from the bundle sheath, and slow enzyme activity. C_4 plants have substantial acclimation potential, but in most cases lag behind the acclimation responses in C_3 plants. For example, some C_4 species are unable to maintain high quantum yields when grown in low-light conditions. Others fail to reduce carboxylase content in shade, leaving substantial over-capacity of Rubisco and PEP carboxylase in place. Shade-tolerant C_4 grasses lack the capacity for maintaining a high state of photosynthetic induction following sunflecks, and thus may be poorly suited to exploit subsequent sunflecks compared with C_3 species. In total, the evidence indicates that C_4 photosynthesis is less phenotypically plastic than C_3 photosynthesis, and this may contribute to the more restricted ecological and geographical distribution of C_4 plants across the Earth.

Key words: Acclimation, C_3, C_4 photosynthesis, phenotypic plasticity, sun–shade, temperature.

Introduction

Phenotypic plasticity refers to the ability of individual organisms to respond to environmental variation by altering their characteristics to compensate for, or acclimate to, variable environmental conditions (Pigliucci, 2001). Organisms considered to be generalists have highly plastic phenotypes and are suited for a wide variety of conditions. They are thus more likely to acclimate and survive in unpredictable environments than less phenotypically plastic species. Organisms with low phenotypic plasticity are less likely to acclimate fully to environmental change, and thus tend to occur in less variable conditions than highly plastic organisms. In part because of a more uniform selection pressure, these organisms are often highly specialized for a restricted set of conditions, and are more fit than generalists within that environment. Increased specialization, however, may result in a loss of plasticity or acclimation potential. As a result, the more evolutionarily derived and specialized species could be more restricted in ecological and geographical distribution.

Within angiosperms, the CO_2-concentrating mechanism of C_4 photosynthesis represents a specialized adaptation derived from C_3 ancestors. C_4 photosynthesis has independently arisen over 45 times in a wide range of advanced angiosperm taxa (Sage, 2004). In almost all known C_4 species, C_4 photosynthesis requires the development of Kranz anatomy (Figs 1, 2; Dengler and Nelson, 1999). Despite the polyphyletic nature of C_4 photosynthesis, certain anatomical features are common to most C_4 plants, including: (i) specialization of two distinct photosynthetic tissue types: bundle sheath (BS) and mesophyll (M) tissue; (ii) the arrangement of BS cells near vascular tissue, with M peripheral to BS cells and adjacent to intercellular spaces; (iii) high vein density and a low ratio of M to BS (often 1:1), resulting in short diffusion pathways for C_4

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* This paper is dedicated to Professor Nancy Dengler on the occasion of her retirement on 1 July, 2005.

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Abbreviations: BS, bundle sheath; IVD, interveinal distance; M, mesophyll.

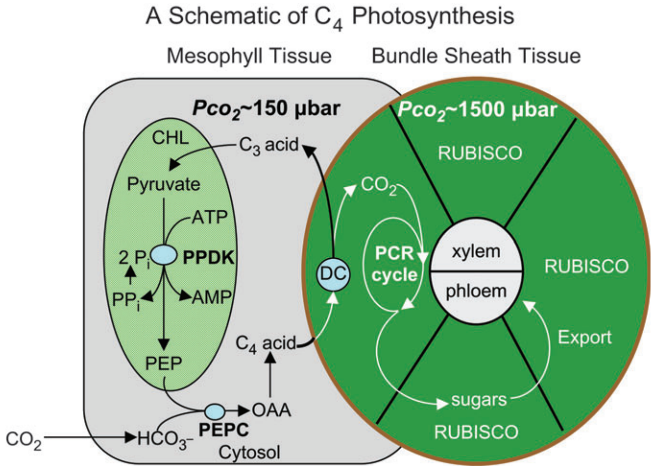

Fig. 1. A schematic diagram of the C_4 photosynthetic pathway showing the coupling of the C_4 cycle that originates in the mesophyll, and the C_3 cycle that originates with the fixation of CO_2 by Rubisco in the bundle-sheath compartment. Arrows indicate the path of molecule diffusion. Abbreviations: DC, decarboxylating enzyme; PEPC, PEP carboxylase; PCR, photosynthetic carbon reduction; PPDK, pyruvate, phosphate dikinase.

metabolites; and (iv) minimal CO_2 leakage from BS cells, reflecting extensive contact between M and BS cells. Within C_3 plants, the photosynthetic assimilation and reduction processes occur in both M and BS tissues, typically within a single photosynthetic cell; however, in C_4 plants, M tissue assimilates CO_2 to form organic acids that diffuse to the BS tissue (Fig. 1). In all cases, the evolution of the C_4 pathway involved the modification of pre-existing biochemistry in C_3 ancestors to enable the concentration of CO_2 into the BS tissue compartment where the CO_2-fixing enzyme Rubisco is localized (Hatch, 1987; Kanai and Edwards, 1999; Sage, 2004).

Together, the modifications of metabolism and anatomy that allow for CO_2 concentration represent a co-ordinated, specialized adaptation that enhances performance of C_4 plants during periods of low atmospheric CO_2 availability and in warm, often dry environments (Ehleringer *et al.*, 1991, 1997). As a derived specialization, however, the metabolic and structural requirements of the C_4 pathway may have decreased the ability of C_4 plants to modify the photosynthetic apparatus in order to acclimate and improve performance in altered environments. This paper explores the possibility that one cost of evolving C_4 photosynthesis is a reduced potential for phenotypic plasticity and acclimation.

Why should C_4 plants have less acclimation potential than C_3 plants?

The success of most plant species requires some ability to acclimate to environmental change, as environmental variation is inevitable (Schlichting, 1986). In addressing acclimation potential between generalists and specialists, or in this paper, C_3 versus C_4 plants, the issue is one of relative degrees of acclimation, and whether barriers exist that might prevent highly plastic acclimation responses in C_4 taxa. Specialist species may have inherent barriers that constrain or even prevent high phenotypic plasticity. The nature of these constraints is not well defined. Examination of C_3 and C_4 acclimation potentials may serve as a useful case study in how specialized adaptations affect phenotypic plasticity.

Conceptually, there are a number of reasons why C_4 plants might not have the same ability to acclimate to low light, temperature variation, or elevated CO_2 as C_3 species. The C_4 pathway requires close integration of distinct photosynthetic processes: PEP carboxylation and regeneration in M tissue with the Calvin cycle in BS tissue (Fig. 1). Failure to co-ordinate M and BS structure and function would reduce photosynthetic capacity and resource use efficiency (Leegood and Walker, 1999). At the structural level, disruption of M to BS tissue arrangements could increase diffusion distances, interfere with diffusion pathways, or enhance pathways for CO_2 leakage from the BS cells. At the metabolic level, ineffective acclimation could lead to a loss of co-ordination between the C_3 and C_4 biochemical cycles. For example, if the C_4 cycle reactions proceed faster than the C_3 cycle following acclimation, too much CO_2 would be pumped into the BS, building up the CO_2 concentration to a point where leakage of CO_2 out of the BS becomes substantial (von Caemmerer and Furbank, 1999). In effect, the C_4 pump would begin to resemble a futile cycle and lose photosynthetic efficiency. Alternatively, if C_4 cycle activity following acclimation is slow relative to the capacity of Rubisco and the C_3 cycle, then BS CO_2 levels would decline and photorespiration rates increase. To avoid these problems, acclimation of C_4 photosynthesis has to involve co-ordinated changes between the M and BS tissues in order to maintain functional stoichiometries. By contrast, in C_3 species, all photosynthetic cells are functionally equivalent, thereby allowing each cell to acclimate to a new environment in a more autonomous manner than should be possible in a C_4 leaf. The simplicity of the C_3 system relative to the C_4 system, therefore, allows photosynthetic plasticity to be concentrated at the cellular rather than tissue levels, potentially allowing for greater acclimation ability in C_3 leaves.

Photosynthetic acclimation brings the costs of tissue construction and maintenance in line with the probable photosynthetic carbon gain that a new environment can support (Mooney and Gulmon, 1982; Bloom *et al.*, 1985; Sims *et al.*, 1998*a*; Poorter *et al.*, 2006). Because the photosynthetic unit in C_3 plants is localized within autonomous cells, individual cells can be enlarged or reduced in size and number (in newly developing leaves) without compromising metabolic integrity. In C_4 plants, the requirements to maintain functional relationships between the C_3 and C_4 cycles could constrain the extent to which tissue construction and maintenance costs are altered following environmental change. In addition, chloroplasts and other organelles in C_4 leaves are spatially localized to either the interior third, or the outer periphery, of BS cells depending on photosynthetic biochemical subtype (Fig. 2; Dengler and Nelson, 1999). Organelle localization in the BS is essential because the ability to trap and refix CO_2 before it can escape is enhanced by locating organelles near the vascular tissue (Kanai and Edwards, 1999). Localizing BS chloroplasts in such a manner restricts the total cell volume available to house Rubisco and the enzymes of the carbon reduction cycle. Changing the amount of these enzymes is an important part of the acclimation process in C_3 plants (Anderson *et al*., 1988; Evans and Seemann, 1989; Leegood and Edwards, 1996), but may be constrained in C_4 species by restrictions in organelle size and number. Modifications to organelle sizes and numbers in C_4 plants may be difficult as it could interfere with ultrastructural arrangements required for an effective C_4 pathway. In C_3 plants, by contrast, all chlorenchyma cells are functionally equivalent in that all contain Rubisco and carbon reduction cycle enzymes. The C_3 leaf is not restricted to packaging Rubisco into the relatively small space located at one end of the BS cells. If Rubisco activity becomes limiting in a C_3 leaf, for example, the plant can compensate by increasing Rubisco content per chloroplast, creating more chloroplasts, or producing more cells in new leaves (Oguchi *et al*., 2005).

Acclimation requires the ability to sense environmental change and transduce it into an effective response. Photosynthetic acclimation is controlled by three general mechanisms: (i) environmental perception by sensory proteins such as phytochrome that activate a signal-transduction pathway, (ii) chloroplast-specific control that is linked to redox state, and (iii) carbohydrate, nutrient, and phytohormone signals that co-ordinate leaf and whole plant responses (Stitt and Krapp, 1999; Malakhov and Bowler, 2001; Lin and Shalitin, 2003; Long *et al*., 2004; Walters, 2005). In C_3 plants, much of the control over the acclimation response is internal to the cell because redox state changes originate within chloroplasts and mitochondria (Anderson *et al*., 1995; Walters, 2005). Reliance on local command and control is problematic in C_4 plants because of the need to co-ordinate M and BS responses; hence, an additional layer of regulatory control is probably required for an effective acclimation response. Furthermore, different promoter systems are required for the development of C_4 tissue specialization (Dengler and Taylor, 2000; Matsuoka *et al*., 2001); therefore, acclimation responses may have to be transduced through additional promoter networks during development.

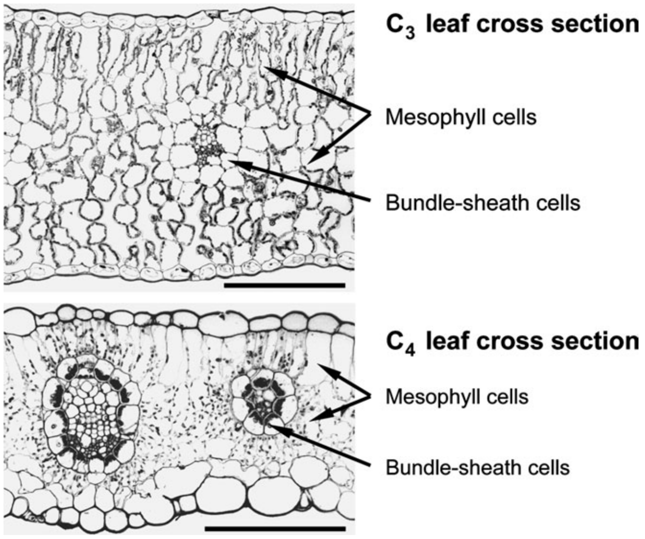

Fig. 2. Cross-sections of C_3 and C_4 leaves grown under high-light conditions. Mesophyll and bundle-sheath tissues are indicated by arrows. Note the centripetal arrangement of Rubisco-chloroplasts in the bundle-sheath cells of the C_4 species. Leaves were sampled from *Flaveria pringlei* (C_3) and *Flaveria trinervia* (C_4). Scale bars are 100 μm.

Alternatively, there may be no barriers associated with the photosynthetic pathway that inherently restrict phenotypic plasticity in C_4 plants relative to C_3 plants. Instead, low phenotypic plasticity may simply result from specialization for hot, high-light environments in the same manner that many C_3 species specialized for these environments have low phenotypic plasticity. Because the main advantage of the C_4 pathway occurs in conditions promoting photorespiration, it is probable that many C_4 species are specialized for hot, high-light conditions and thus they may not be appropriate for assessing hypotheses regarding varying potential for phenotypic plasticity. There are situations, however, where a high degree of phenotypic plasticity would be advantageous to C_4 plants. Numerous C_4 species develop dense canopies where self-shading of older leaves is extensive. Acclimation to low light within a canopy is thus required if interior leaves are to maintain high resource-use efficiency and significantly contribute to carbon gain. In addition, a number of C_4 species are successful in environments that are atypical for C_4 photosynthesis, namely low-light and cooler habitats (Long, 1999; Sage *et al*., 1999). Although some adaptive specializations may have occurred in C_4 species from cooler or low-light habitats, they may also show substantial phenotypic plasticity as most of these species occur in variable environments such as canopy gaps and high elevation (Brown, 1977; Smith and Martin, 1987*b*; Sage and Sage, 2002). A greater potential for phenotypic plasticity would probably be found in C_4 plants from these variable environments.

Acclimation of C_3 and C_4 photosynthesis to shade

Shade acclimation is the best-studied acclimation response of C_3 photosynthesis, such that it now serves as a model of phenotypic plasticity in classrooms and textbooks. Shade-acclimation demonstrates the range of acclimation responses present in leaves (Table 1; Lambers *et al*., 1998; Walters, 2005). At one level are the economic-type responses, where the activities of non-limiting processes are modulated to match activities of limiting processes (Bloom *et al*., 1985). Examples of economic-type responses are where the levels of Rubisco, carbon-reduction-cycle protein, enzymes for sucrose and starch synthesis, and electron-transport machinery are reduced following shading to match light-harvesting capacity (Table 1; Anderson *et al*., 1988; Evans, 1988; Bailey *et al*., 2004). At the leaf level, cell size, cell number, and leaf thickness are altered in newly formed leaves to bring construction and maintenance costs in line with the ability of the light environment to support the energetic costs (Björkman, 1981; Mooney and Gulmon, 1982; Evans and Seemann, 1989). Following shading, pigment levels shift as the levels of photoprotective carotenoids decline, while chlorophyll content increases (Bailey *et al*., 2004; Horton and Ruban, 2005). Acclimation also involves qualitative adjustments, such as the stacking of thylakoid membranes to enhance light capture and to create additional volume for proton storage (Sharkey *et al*., 1986). Some acclimation responses are rapid and reversible, such as chloroplast movements, while others are slow and largely irreversible, such as anatomical patterns established when leaves mature in a particular environment. With respect to C_3 and C_4 acclimation, the structural and economic-type responses are the most relevant for evaluating the potential for phenotypic plasticity. Acclimation responses involving changes in anatomy or enzyme complement are more likely to be linked to the photosynthetic pathway because C_3 and C_4 species inherently differ in these attributes.

Table 1. *General characteristics of sun and shade leaves in C_3 plants (after Björkman, 1981; Lambers* et al.*, 1998)*

	Sun grown	Shade grown
Structural characteristics		
Leaf thickness	Thick	Thin
Palisade parenchyma	Multilayered	1–2 cell layered
Chloroplasts per area	Many	Few
Thylakoids per granum	Few	Many
Interveinal distance	Low	High
Biochemical characteristics		
Chlorophyll per chloroplast	Low	High
Chlorophyll per area	Similar	Similar
Chlorophyll *a*/*b* ratio	Low	High
Light-harvesting complex per area	Low	High
Antennae size per photosystem	Low	High
Electron-transport protein	High	Low
Rubisco per area	High	Low
Nitrogen per area	High	Low
Xanthophylls per area	High	Low
Physiological characteristics		
Photosynthetic capacity per area	High	Low
Dark respiration rate	High	Low
Light-compensation point	High	Low

Using the well-described responses of C_3 plants to shade as a reference (Table 1), it can be evaluated whether C_4 plants have the same potential for shade acclimation as C_3 photosynthesis. Two parameters of particular value in evaluating structural and biochemical acclimation are leaf thickness and Rubisco activity, respectively. A common acclimation response to shading is the thinning of leaves; hence, the relative degree of leaf thinning can be compared to examine whether there are inherent differences between the photosynthetic pathways that might be associated with anatomical requirements to maintain the M-BS stoichiometry. Second, Rubisco contents decline markedly during shade acclimation in C_3 plants, on the basis of leaf area, chlorophyll, and leaf nitrogen content (Evans, 1988; Evans and Seemann, 1989). Because Rubisco activity is non-limiting in shaded C_4 leaves, significant reductions in Rubisco content should also occur during shade acclimation if resource use efficiency is to be maintained (von Caemmerer and Furbank, 1999).

No consistent differences are apparent in the ability of C_3 and C_4 species to reduce leaf thickness following shading. Comparisons of leaves produced at high light and low light generally show that leaf thickness declines by 30–50% in both C_3 and C_4 species (Louwerse and Zwerde, 1977; Ward and Woolhouse, 1986*a*). In a direct comparison of *Phaseolus vulgaris* (C_3) and *Zea mays* (C_4), the reduction in leaf thickness from high light to low light was 34% for the C_3 plants and 32% in maize (Louwerse and Vanderzweerde, 1977). Exceptions have been noted, for example, in *Amaranthus retroflexus*, a sun-adapted C_4 plant with extensive self-shading, there is little difference in the thickness of leaves from high- and low-light-grown plants (Tazoe *et al*., 2005).

Rubisco content in C_4 plants does not appear to be as responsive to changes in light availability as in C_3 plants, particularly in terms of the percentage of leaf nitrogen invested in Rubisco. In numerous C_3 species, Rubisco content or activity is reduced by over 55% in shaded compared to high-light grown leaves (Table 2). By contrast, the degree of reduction is generally less in C_4 species, being 10–54% when Rubisco content or activity is expressed on a chlorophyll basis (Table 2). In terms of the percentage of nitrogen allocated to Rubisco, there is modest (about 15%) reduction in Rubisco content per unit nitrogen in *Amaranthus retroflexus* between high- and low-light-grown plants (Tazoe *et al*., 2005). The fraction of nitrogen in Rubisco increases in maize and *Paspalum* leaves grown in low light (50 μmol photons m^{-2} s^{-1}) compared with high-light (1000 μmol photons m^{-2} s^{-1}; this observation is based on Rubisco: protein ratios derived from Ward and Woolhouse, 1986*b*). These results indicate that C_4 plants have a low ability to reduce their allocation of nitrogen to Rubisco following shading, in contrast to the typical C_3 response where the allocation of leaf nitrogen to Rubisco declines substantially (>50%) in low compared to high-light-grown leaves (Seemann *et al.*, 1987; Evans and Seemann, 1989).

An expensive component of a leaf is the vascular tissue, both from the greater investment costs (lignin is one of the most energetically-expensive molecules in plants), and because non-photosynthetic vascular tissue replaces photosynthetic cells, thereby reducing the light-absorbing capacity of the leaf. In low light, the rate of transpiration is reduced, and with it, the need for an extensive vascular network. Consequently, vein density can decline in shaded C_3 plants, allowing M cells to occupy a greater proportion of the leaf area (Wylie, 1939, 1951; Bjorkman *et al.*, 1972; Boardman, 1977; Jurick *et al.*, 1982). By contrast, C_4 plants must maintain a high vein density and tight vein spacing because of the requirement for close proximity of M and vein-associated BS cells. C_4 grasses have, on average, interveinal distance (IVD) values that are less than half that of C_3 grasses (Crookston and Moss, 1974; Morgan and Brown, 1979; Hattersley and Watson, 1975; Kawamitsu *et al.*, 1985; Dengler *et al.*, 1994). The average C_4 grass IVD is 120 μm, whereas the average IVD of C_3 grasses is 280 μm (values estimated from Dengler *et al.*, 1994; Ogle, 2003). Veins in C_4 grasses are spaced 50–200 μm apart, while in C_3 plants they are 200–400 μm apart (Ogle, 2003). The lower IVD values in C_4 grasses are correlated with decreases in M:BS tissue volume ratios compared with C_3 grasses (Hattersley, 1984; Dengler *et al.*, 1994). This has also been formalized for C_4 grasses where M cells are no more than 'one cell distant' from BS cells (Hattersley and Watson, 1975). Similar patterns in C_4 dicot species compared with C_3 dicot species have also been reported (Rao and Rajendrudu, 1989).

Table 2. *The ratio of Rubisco activity or content in plants grown in high versus low light conditions*

In all cases, Rubisco values used to generate the ratio were expressed on a chlorophyll basis. Ratios were derived from data presented in the given references, and reflect values for high nitrogen leaves.

Plant species	Rubisco in low versus high-light-grown leaves	Reference
C_3 species		
Atriplex patula	0.33	Evans, 1988
Alocasia macrorhiza	0.21	Seemann *et al.*, 1987
Oryza sativa	0.46	Evans, 1988
Phaseolus vulgaris	0.29	Seemann *et al.*, 1997
Solanum dulcamara	0.26	Osmond, 1983
Spinacea oleracea	0.43	Evans, 1988
Average for C_3 species	0.34	
C_4 species		
Amaranthus retroflexus	0.46	Tazoe *et al.*, 2005
Microstegium vimineum	0.81	Winter *et al.*, 1982
Paspalum conjugatum	0.66	Ward and Woolhouse, 1986*a*, *b*
Zea mays	0.90	Ward and Woolhouse, 1986*a*, *b*
Average for the C_4 species	0.71	

In low-light environments, there is an energetic cost associated with widely-spaced veins in C_4 plants, as indicated by surveys showing the quantum yield is lower in species with greater IVD (Fig. 3; Ogle, 2003). By contrast, quantum yield is independent of vein spacing in C_3 plants (Fig. 3). Carbon-isotope discrimination increases in shaded C_4 plants with wider vein spacing, indicating greater leakage of CO_2 out of the bundle sheath (Ogle, 2003). The increase in CO_2 leakage is probably responsible for the decline in the quantum yield of the C_4 species with greater vein spacing (Ogle, 2003). Reducing vein density as a means of shade-acclimation is not a restriction for C_3 leaves, but could be for C_4 leaves as it can compromise the efficiency of the C_4 apparatus.

Direct tests of growth light intensity on vein spacing of closely-related C_3 and C_4 species are not apparent in the literature, so a study was established to examine shade responses of leaf anatomy and vein pattern in C_3 and C_4 species of the dicot genus *Flaveria. Flaveria* species are valuable for comparing the effect of C_4 evolution on various characteristics in plants, because the C_3 species is ancestral to the derived C_4 species (McKown *et al.*, 2005) and the weedy C_3 and C_4 species in this genus occur in roughly similar habitats (Powell, 1978). Leaf thickness, vein density, IVD, M tissue area, BS tissue area, and M:BS ratios were compared between *F. australasica* (C_4) and *F. robusta* (C_3) plants grown in a growth chamber at either 500 μmol photons m^{-2} s^{-1} or 100 μmol photons m^{-2} s^{-1}. Far red-to-red ratios were also lowered to mimic natural shading using plastic filters, thereby inducing the full range of shade acclimation responses seen in natural situations (Lee, 1985). Leaf thickness declined proportionally more in the C_3 than in the C_4 species (Figs 4, 5a). In C_3 *F. robusta*, vein density and IVD changed little while significant changes occurred in the C_4 *F. australasica* (Fig. 5b, c).

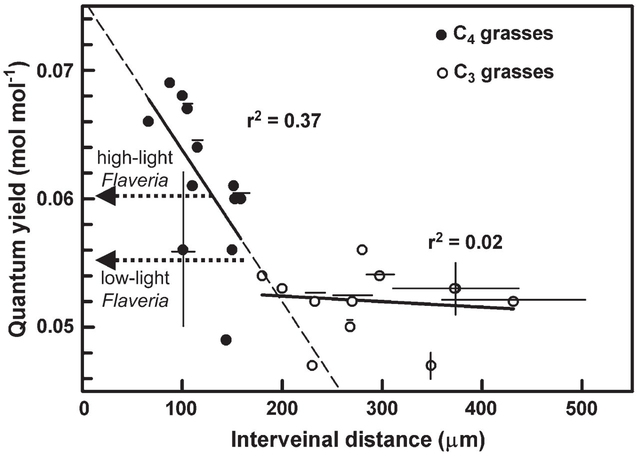

Fig. 3. The relationship between quantum yield and interveinal distance in a variety of C_4 grass species (from Ogle, 2003; with kind permission of Springer Science and Business Media). The horizontal arrows indicate the quantum yield that would correspond to the measured IVD values in Fig. 5C from *Flaveria australasica* sampled in high light or low light.

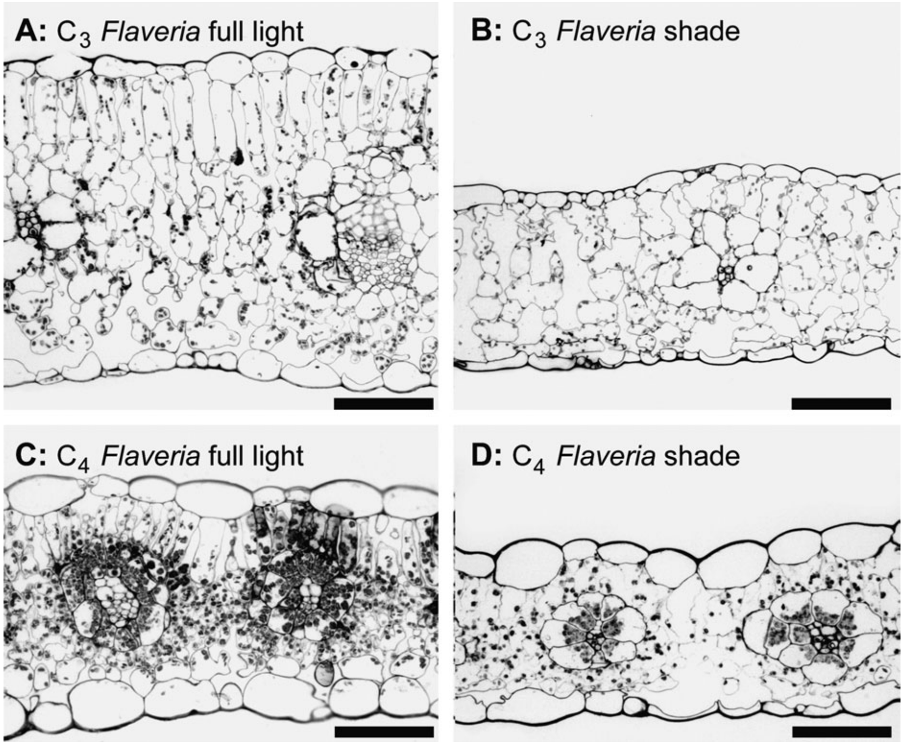

Fig. 4. Leaf cross-sections of *Flaveria robusta* (C_3) and *Flaveria australasica* (C_4) grown in illuminated (500 μmol m^{-2} s^{-1} at a red:far red ratio of 1.55) and shaded (100 μmol m^{-2} s^{-1} at a red:far red ratio of 0.5) treatments in a plant growth chamber. (A) C_3 *F. robusta* grown in full light conditions. (B) *F. robusta* grown under reduced light and red:far red conditions. (C) C_4 *F. australasica* in full-light conditions. (D) *F. australasica* grown under reduced light and red:far red conditions. Scale bars are 50 μm.

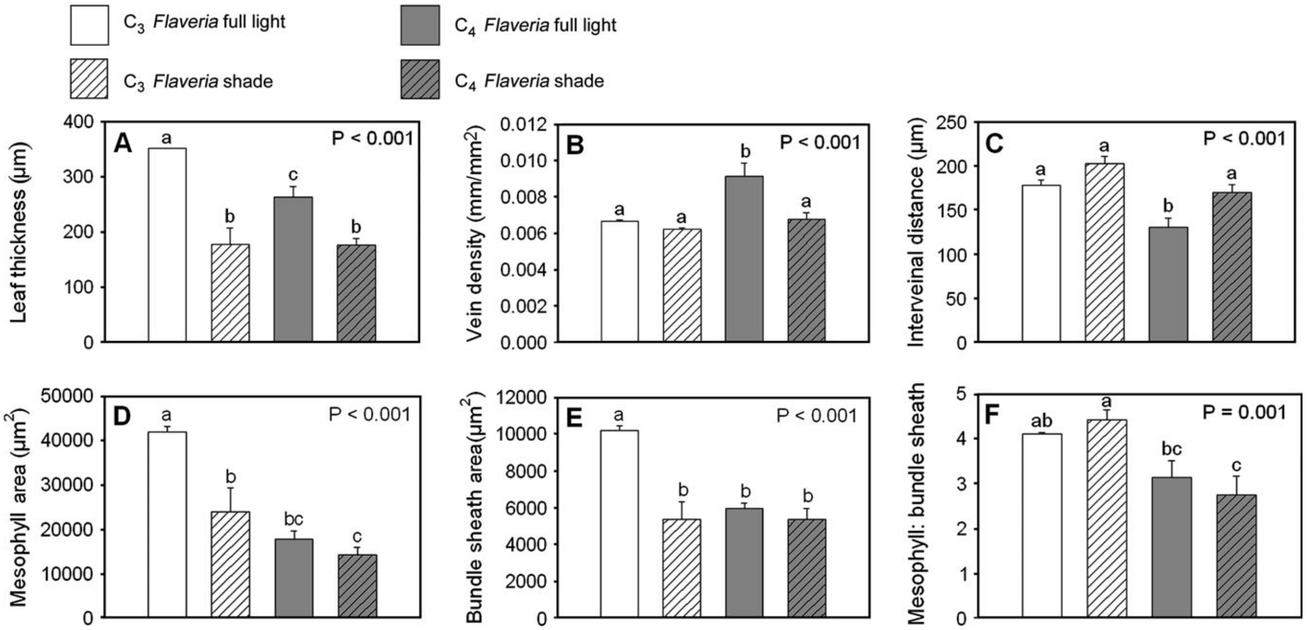

Fig. 5. Quantitative anatomical data from *Flaveria robusta* (C_3) and *Flaveria australasica* (C_4) grown under illuminated (500 μmol m^{-2} s^{-1}, red:far red ratio of 1.55) and shaded (100 μmol m^{-2} s^{-1}, red:far red ratio of 0.5) conditions in a plant growth chamber. All leaves sampled were at maturity, and had been initiated and fully developed under their respective light treatments. Measurements were conducted on cross-sectioned leaves and leaf clearings. Lower case letters indicate statistical groupings based on one-way ANOVA and Tukey's tests. Bars indicate standard errors. (A) Mean leaf thickness (μm); (B) vein density (mm mm^{-2}); (C) interveinal distance (μm); (D) mesophyll tissue area in cross-section, including intercellular space area ($μm^2$); (E) bundle sheath area in cross-section ($μm^2$); (F) mesophyll area to bundle sheath area ratio.

In the C_4 species, the reduction in vein density was approximately 20%. Mesophyll and BS tissue areas in leaf cross-sections decreased in both species, but the change in cross-sectional area (which indicates changes in tissue volume) was greater in the C_3 *F. robusta* (Fig. 5d, e). Intercellular spaces (ICS) increased and BS exposure to ICS doubled from 4.6% to 10.5% ($P < 0.0001$) in the shaded C_4 *F. australasica* plants (data not shown), indicating there is a greater potential for CO_2 leakage out of the BS directly into the intercellular air spaces. This hypothesis is supported by carbon isotope measurements. *Flaveria australasica* plants grown in the shade had a lower carbon isotope ratio (–16.0 per mil) than plants grown in bright light (–15.2 per mil; $P < 0.05$: the plant were grown as described in the legend of Fig. 5). The more negative isotopic values for the shade-grown C_4 leaves indicate a greater degree of CO_2 leakage from the bundle sheath (von Cammerer *et al.*, 1997). Similarly, *Amaranthus retroflexus* grown in shade and at high nitrogen also shows a reduction in vein density that is correlated with a decrease in the carbon-isotope ratio (Tazoe *et al.*, 2005). When the mean IVD values from the C_4 *Flaveria* species are plotted on the quantum yield versus IVD relationship developed for grasses (Fig. 3), then the predicted reduction in quantum yield is modest (about 9%); however, the importance of this decline is substantial, since the quantum yield advantages of C_4 over C_3 dicot species at 30 °C (0.061 mol CO_2 mol^{-1} photons for C_4 versus 0.052 mol mol^{-1} for C_3 dicots; Ehleringer and Pearcy, 1983) would be halved. Quantum yield differences between C_3 and C_4 species are important for understanding the relative performance of C_3 versus C_4 photosynthesis in low-light and variable thermal environments (Ehleringer and Pearcy, 1983; Ehleringer *et al.*, 1997).

Flaveria, *Zea mays*, and *Amaranthus* are sun-adapted plants; they may acclimate to low light (as during self-shading), but would not be able to complete their life cycle in the shade of a forest canopy (Björkman, 1981). Sun-adapted plants are generally considered specialists for open environments and, as such, comparisons involving only sun-plants may not show the full range of acclimation that could be seen in a true generalist species. Hence, generalist species should also be considered if the potential of C_4 plants to acclimate to low light is to be fully evaluated. Many C_4 species persist in heavily shaded environments (Brown, 1977; Pearcy and Calkin, 1983; Long, 1999) indicating they either possess a substantial capacity for shade acclimation, or they are truly shade-adapted (Horton and Neufeld, 1998; Sage and Pearcy, 2000). Based on floristic descriptions, it does not appear that the majority of these species are shade specialists, as they also occur along canopy openings such as paths or tree-fall gaps, and most can also occur in partially open habitats. For example, in the *Flora of the Guianas* (Judziewicz, 1990; the Guianas occur in the wet-tropics of northern South America), no C_4 grass species is described as being localized in forest interiors, while 34% of the C_3 grass flora is described as such (Sage, 2000). One per cent of the C_4 grass flora of the Guianas is described as occurring along forest margins. These particular species represent the sun–shade generalists, and would be the best group to examine shade acclimation potentials in the C_4 flora.

In contrast to sun-adapted C_4 species (such as *Flaveria*), shade-tolerant C_4 grasses are able to maintain close vein spacing under shaded conditions, for example, in *Microstegium vimineum* (Winter *et al.*, 1982), *Muhlenbergia frondosa*, *M. sobolifera*, *M. schreberi* (Smith and Martin, 1987*a*), *Paspalum conjugatum* (Ward and Woolhouse, 1986*a*, *b*), and *Rottboellia exaltata* (Paul and Patterson, 1980). Instead of increasing, IVD decreases in *Muhlenbergia frondosa* and *Rottboellia exaltata* as M and BS cells do not expand to normal size. Reductions in the size of BS and M cells have also been observed in *Paspalum conjugatum* relative to *Zea mays* grown in shade (Ward and Woolhouse, 1986*a*, *b*). Ogle (2003) suggested that surviving shade conditions with sufficient quantum yield involves maintaining a threshold IVD lower than that observed in most C_4 species. The shade-adapted species of *Microstegium*, *Muhlenbergia*, and *Paspalum* have much lower IVD values than the average reported for C_4 grasses, respectively, 72 μm (Winter *et al.*, 1982), 73 μm (Smith and Martin, 1987*a*), and 78 μm (Kawamitsu *et al.*, 1985).

A particularly interesting case of maintaining low IVD is observed in the shade-tolerant dicot, *Chamaesyce herbstii* (formerly *Euphorbia forbesii*) from the Hawaiian Islands (Herbst, 1972; Pearcy, 1983). *Chamaesyce herbstii* is a small-to-medium stature tree that is scattered in the understory of mesic Hawaiian forests (Koutnik and Huft, 1990). During normal leaf development in shaded *C. herbstii*, a number of 'disjunct' veins arise, consisting of isolated xylem tracheids (Herbst, 1972). There is no physical connection between the vein 'islands' and the rest of the leaf venation, yet normal BS develops around these disjunct veins. C_4 dicots are generally not shade-tolerant, so this unique solution to the problem of maintaining close vein spacing and M:BS ratios exemplifies the challenge posed by the C_4 pathway during low-light acclimation. In a direct comparison of physiological acclimation to shade in *C. herbstii* with the co-occurring understory C_3 tree, *Claoxylon sandwicense*, grown under identical high- and low-light conditions, Pearcy and Franceschi (1986) observed that the shade-grown C_3 species reduced the dark respiration and electron-transport rates to a greater relative degree than shade-grown *Chamaesyce herbstii* (Table 3). Leaf chlorophyll content declined little in *Chamaesyce herbstii*, while it rose in *Claoxylon sandwicense*. Increased chlorophyll content is indicative of a greater ability to harvest photons in low light (Evans, 1988; Evans and Seemann, 1989). The result of these changes is that the C_3 species in low-light environments has a lower light-compensation point than the C_4 species, indicating a greater tolerance for shaded conditions (Table 3).

The shade-tolerant C_4 grass *Microstegium vimineum* is a summer-active species that occurs in gaps and understoreys in deciduous forests (Horton and Neufeld, 1998). In the eastern US, *M. vimineum* is a serious invasive species that can displace native C_3 herbs in shaded habitats within the forest. Shade-acclimation of *M. vimineum* has not been directly compared with that of C_3 species, but a number of patterns stand out that indicate it has less potential to acclimate to low-light environments than similar C_3 species. Winter *et al*. (1982) observed typical patterns of shade acclimation in terms of leaf thickness and leaf area responses, but not carboxylating enzymes (Table 4). Rubisco activity rose slightly from high to low light, while PEPCase activity was unchanged. The failure of carboxylating enzymes to adjust to low-light conditions is a sign of limited acclimation potential in this shade-tolerant grass.

Horton and Neufeld (1998) characterized the ability of *Microstegium vimineum* to utilize sunflecks (short episodes of high light that shine through small canopy gaps). Most sunflecks last between a few seconds to a few minutes, and often represent the major source of photons in the understorey. The ability to capture and store light energy in a sunfleck is an important component of shade-tolerance (Sharkey *et al*., 1986; Pearcy *et al*., 1996, 1997). Utilization of sunflecks is related to the ability of a leaf to keep its photosynthetic apparatus in an active, induced state, ready to use the photonic energy in a sunfleck. Normally, photosynthetic enzymes deactivate following shading and, once deactivated, several minutes are required for reactivation (Sharkey *et al*., 1986; Sage *et al*., 1993; Pearcy *et al*., 1996). In the deactivated state, the photosynthetic apparatus cannot use most of the photons in a light fleck. If induction requires more than a minute or so, most sunflecks would not be exploited. Shade-adapted C_3 plants are able to maintain the leaf in a partially induced state for the better part of an hour after the last sunfleck has passed. In *Alocasia macrorrhiza*, an understorey C_3 species from northern Australia, the half-time for relaxation of the induction state of photosynthesis is 30 min (Chazdon and Pearcy, 1986). By contrast, it is just 2.4 min in the C_4 *Microstegium vimineum* (Horton and Neufeld, 1998). This result indicates that shade-tolerant C_4 plants do not maintain the ability to exploit sunflecks for more than a few minutes after the last sunfleck has passed, while in understorey C_3 species, the photosynthetic apparatus remains primed and ready for action for a considerably longer period. Failure of C_4 plants to maintain leaves in an induced state as well as shade-adapted C_3 plants could be associated with the additional requirement to maintain high activation of the C_4 cycle in addition to the C_3 cycle. High activation of the C_4 cycle would entail maintaining high gradients between the metabolite pools of the M and BS compartments, as well as maintaining activated forms of PEP carboxylase, pyruvate-phosphate dikinase and other C_4 cycle enzymes.

The sun-plant *Zea mays* also uses sunflecks less efficiently than C_3 plants such as soybean and *Alocasia*, particularly short duration sunflecks (<10 s; Krall and Pearcy, 1993). In C_3 plants, the photosynthesis rate increases as lightfleck duration falls below 10 s, while in maize it decreases. Much of the stored energy in short-duration lightflecks is apparently not used in C_4 plants due to a breakdown in the co-ordinated metabolism of the C_3 and C_4 cycles. Krall and Pearcy (1993) propose that the decline in maize photosynthesis during short duration sunflecks results from a burst of CO_2 leaving the BS cells. This is caused by the C_4-cycle reactions moving CO_2 into the BS faster than the deactivated C_3-cycle reactions can utilize it. The inability to maintain a high activation state of the C_3 cycle in maize appears to create conditions favouring the futile cycling of CO_2 during short-duration sunflecks (Krall and Pearcy, 1993).

Table 3. *Selected photosynthetic properties in C_4* Chamaesyce herbstii *and C_3* Claoxylon sandwicense *grown at high light (HL, 1200 μmol photon m^{-2} s^{-1}) or low light (LL, 55 μmol photons m^{-2} s^{-1}) (Pearcy and Franceschi, 1986)*

Parameter	*C. herbstii* (C_4)		*C. sandwicense* (C_3)	
	HL	LL	HL	LL
Dark respiration rate (μmol m^{-2} s^{-1})	1.5	0.9	1.1	0.5
Light-compensation point (μmol m^{-2} s^{-1})	41	18	31	15
Leaf chlorophyll content (mg m^{-2})	630	510	350	550
Electron transport rate (μmol O_2 mg^{-1} chl hr^{-1})	185	147	337	144

Table 4. *Selected parameters from the shade-tolerant C_4 grass* Microstegium vimineum *grown at high- or low-light conditions (Winter* et al.*, 1982)*

	Growth conditions	
	Full sun	5% of full sun
Area per leaf (cm^2)	3.9	8.2
Leaf thickness (μm)	122	66
Rubisco activity (μmol m^{-2} s^{-1})	6.4	7.6
PEPCase activity (μmol m^{-2} s^{-1})	37.1	37.8

Temperature acclimation

Research on temperature acclimation has emphasized responses to thermal extremes. Responses to thermal extremes do not obviously vary between photosynthetic pathways, so there is little reason to expect acclimation to extreme temperatures to be inherently different between ecologically similar C_3 and C_4 plants. There have been hypotheses that C_4 species are more prone to chilling injury because C_4-cycle enzymes can be cold-labile (Long, 1983; Potvin *et al.*, 1986). Recent studies show that the C_4-cycle enzymes from cold-tolerant C_4 plants are stable in chilling conditions (Simon and Hatch, 1994; Matsuba *et al.*, 1997; Long, 1999; Pittermann and Sage, 2000), demonstrating that chilling sensitivity is not an inherent feature of the C_4 pathway. In contrast to responses to thermal extremes, there may be inherent differences in the acclimation response of C_3 and C_4 photosynthesis to non-stressful temperature variation. C_4 plants exhibit a different pattern of biochemical limitation across a range of temperatures than C_3 species (von Caemmerer, 2000; Sage, 2002; Kubien *et al.*, 2003) which should alter the nature of the acclimation response between the two pathways.

Thermal acclimation to low temperature in C_3 plants often involves an enhancement of the photosynthetic rate below the thermal optimum (Slatyer, 1977; Berry and Raison, 1981; Mawson and Cummings, 1989; Savitch *et al.*, 1997; Strand *et al.*, 1999; Yamasaki *et al.*, 2002; Yamori *et al.*, 2005). In C_4 plants, early acclimation studies observed an enhancement in photosynthesis at cooler temperatures in desert species grown in moderate conditions (Pearcy, 1977; Berry and Raison, 1981). However, these studies often compared plants grown near 20 °C with species grown under hot (>40 °C) conditions, so that the thermal acclimation observed may have been more a case of heat acclimation than low-temperature acclimation. Recent studies of C_4 performance below 20 °C indicate little change in the photosynthetic rate of cold-tolerant C_4 plants upon growth in cool conditions (Matsuba *et al.*, 1997; Pittermann and Sage, 2001; Cavaco *et al.*, 2003; Naidu *et al.*, 2003; Naidu and Long, 2004; Kubien and Sage, 2004*a*).

C_4 photosynthesis is well recognized to be inhibited by low temperatures to a greater degree than C_3 photosynthesis (Berry and Raison, 1981). Three leading hypotheses have been proposed to explain poor photosynthetic performance at low temperature in C_4 leaves. First, the activity of the C_4-cycle enzymes PEPCase and PPDK decline due to a cold-induced lability of these enzymes (Long, 1983, 1999). This hypothesis may explain poor photosynthetic performance in species from warm regions, but C_4 species that are naturally cold-tolerant do not show declines in PPDK or PEPCase activity with prolonged cold exposure (Simon and Hatch, 1994; Usami *et al.*, 1995; Matsuba *et al.*, 1997; Pitterman and Sage, 2000). Hence, this limitation is not the obvious problem that necessarily prevents the C_4 pathway from performing in cool climates. Second, the maximum quantum yield of C_4 photosynthesis is less than that of C_3 species in cooler environments, due to the additional energy cost of running the C_4 pump (Ehleringer and Pearcy, 1983). This is proposed to be an inherent limitation on C_4 plants in the cold (Ehleringer *et al.*, 1997), but this constraint would mainly be an issue in low-light environments. At high light, where most C_4 species are found (including most of the cold-tolerant C_4 species), the quantum yield differences are not directly relevant, because there is an excess of photons, and much of the absorbed light energy is given off as heat (Kubien and Sage, 2004*b*). Therefore, quantum yield differences can contribute, but are not the main cause of poor C_4 photosynthetic performance in low temperature conditions (Sage and Kubien, 2003).

In cold-tolerant C_4 species, Rubisco capacity becomes limiting at low temperature and imposes a ceiling on photosynthetic rate below 20 °C (Pearcy, 1977; Pittermann and Sage, 2000; Sage, 2002; Kubien *et al.*, 2003). Rubisco capacity *in vitro* and gross photosynthesis become the same in a variety of C_4 species below 20 °C which should be the case if Rubisco controls the rate of CO_2 assimilation in C_4 plants (Fig. 6). Fluorescence and gas exchange measurements show that the ratio of Φ_{PSII}/Φ_{CO2} increases at low temperature where Rubisco capacity and the gross photosynthesis rate are equivalent (Fig. 7; Kubien *et al.*, 2003, Kubien and Sage, 2004*a*). Φ_{PSII}/Φ_{CO2} should rise with increasing leakiness of CO_2, because leakage of CO_2 does not affect the photochemical yield of PSII, but does reduce

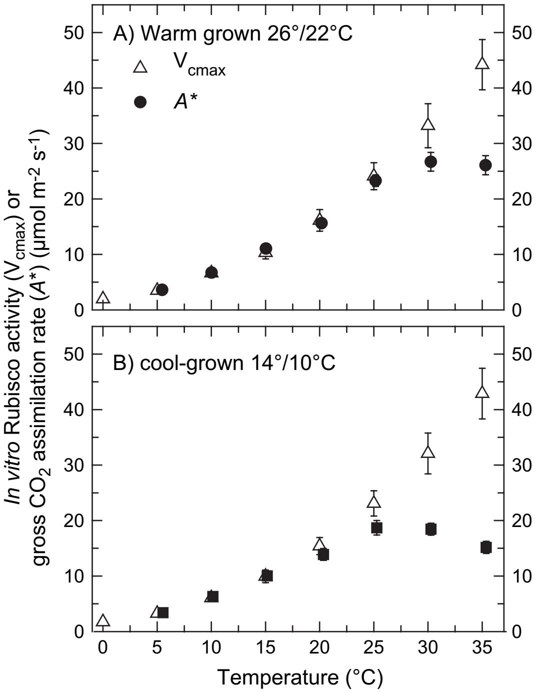

Fig. 6. The response of the maximum Rubisco activity (V_{cmax}) determined *in vitro* as a function of assay temperature, and the gross photosynthesis rate (**A*) measured with gas exchange in the C_4 grass *Muhlenbergia glomerata*. (A) *M. glomerata* grown at 26 °C during the day and 22 °C at night in a plant growth chamber. (B) *M. glomerata* grown at 14/10 °C day/night temperature. Plants were grown at 800 µmol photons m^{-2} s^{-1}. Note: photosynthesis and Rubisco activities are the same below 20 °C, but not at the thermal optimum. Rubisco contents for each treatment are not statistically different. From Kubien and Sage (2004*a*); with kind permission of Blackwell Publishing.

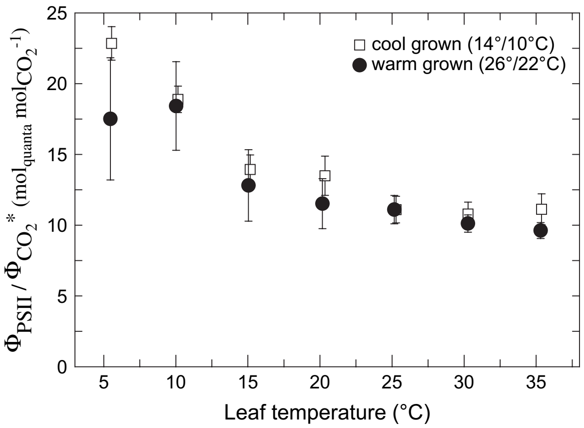

Fig. 7. The ratio of the quantum yield of photosytem II over the quantum yield of CO_2 fixation (Φ_{PSII}/ Φ_{CO2}) as a function of temperature in the C_4 grass *Muhlenbergia glomerata* grown at 26/22 °C or 14/10 °C day/night temperature as described for Fig. 6 (Kubien and Sage, 2004*a*); reproduced with kind permission of Blackwell Publishing.

the quantum yield of CO_2 fixation (von Caemmerer *et al*., 1997). Increased CO_2 leakage from the C_4 BS cells is consistent with Rubisco dominating the control of photosynthesis at low temperature. When Rubisco capacity is limiting, the C_4 cycle pumps CO_2 into the BS faster than it can be fixed by Rubisco, causing the BS CO_2 concentration and the leak rate to increase (Kubien *et al*., 2003).

If Rubisco is the leading limitation on C_4 photosynthesis at low temperature, then the main way to improve photosynthesis would be to increase Rubisco content by packing more Rubisco into the fraction of the BS where chloroplasts are localized. There is little evidence that this occurs. In most C_4 species examined, cold-acclimation is not accompanied by increased or varied Rubisco capacity. In the montane grass *Muhlenbergia montanum*, which grows above 3000 m in the Rocky Mountains of the USA, and the boreal-zone C_4 grass *Muhlenbergia glomerata*, Rubisco content is unchanged in warm- and cold-grown plants (Fig. 6; Pittermann and Sage, 2001; Kubien and Sage, 2004*a*). Shortly after transferring *M. montanum* to low temperature, there is a marked inhibition of photosynthesis, but only at warm measurement temperatures where Rubisco is not limiting (Pittermann and Sage, 2001). Within a few days of the transfer, the assimilation rate at elevated measurement temperatures has fully recovered to similar rates as before the transfer, indicating acclimation repairs inhibitions in PEP regeneration, PEP carboxylation, or electron transport that arise shortly after transfer to cool conditions (Pittermann and Sage, 2001). In *Miscanthus giganteus*, a montane C_4 grass from eastern Asia that exhibits high productivity in cooler environments, Rubisco capacity changes little in plants grown at cool relative to warm temperature (Naidu and Long, 2004). Similar patterns have been observed in the cold-tolerant C_4 grasses *Spartina anglica* and *Paspalum dilatatum* (Matsuba *et al*., 1997; Cavaco *et al*., 2003). By contrast, in the chilling-sensitive species *Zea mays* and *Zoysia japonica*, Rubisco contents decline substantially with prolonged exposure to chilling conditions, along with the photochemical capacity and the activity of C_4-cycle enzymes (Matsuba *et al*., 1997; Naidu *et al*., 2003; Naidu and Long, 2004).

In summary, there is little evidence that C_4 species compensate for low-temperature exposure by building up Rubisco content to overcome a strong limitation caused by low Rubisco activity. Instead, cold-tolerant C_4 plants are able to maintain Rubisco content and photosynthetic capacity, in contrast to cold-sensitive C_4 species where numerous components of the photosynthetic apparatus degrade with prolonged exposure to cool conditions. Cold-tolerant C_4 grasses have a pronounced ability to acclimate to chilling conditions qualitatively, as indicated by carotenoid changes that show they have well-developed mechanisms that protect against photoinhibition at low temperature (Kubien and Sage, 2004*a*).

In contrast to C_4 species, C_3 species show substantial acclimation to low temperature that involves increases in enzyme content. In C_3 plants, the ability to regenerate P_i for photophosphorylation becomes a major limitation at low temperature (Sharkey, 1985; Sage and Sharkey, 1987; Falk *et al*., 1996; Strand *et al*., 1999). Acclimation to low temperature involves a partial, if not complete, removal of the P_i-regeneration limitation. This is brought about by increasing enzyme capacity for starch and sucrose synthesis relative to Rubisco capacity and the capacity for RuBP regeneration, or a change in the internal P_i status in leaves which improves P_i regeneration in low-temperature conditions (Leegood and Edwards, 1996; Stitt and Hurry, 2002; Hendrickson *et al*., 2004). Improving P_i-regeneration capacity often increases photosynthetic capacity at low temperature (Savitch *et al*., 1997; Strand *et al*., 1999). The limitation that dominates the rate of photosynthesis after acclimation increases the P_i-regeneration capacity is unclear. At lower CO_2 levels than at present, Rubisco capacity can become limiting at cooler temperatures, and hence acclimation may involve an increase in Rubisco content (Sage, 2002). Consistently, Rubisco levels often increase at low temperature, and this is associated with increased rates of CO_2 assimilation in cold-acclimated leaves (Strand *et al*., 1999; Yamori *et al*., 2005). Electron-transport capacity also increases at low temperature, such that limitations caused by a deficient electron transport capacity are alleviated (Mawson and Cummings, 1989; Savitch *et al*., 1997). This limitation could be particularly important in CO_2-enriched atmospheres when the capacity for RuBP regeneration is the primary limitation.

Limitations controlling photosynthesis at elevated temperature remain unclear. Rubisco activase is reported to dissociate above the thermal optimum in both C_3 and C_4 species, creating a limitation on photosynthesis from a low activation state of Rubisco (Crafts-Brandner and Salvucci, 2002; Salvucci and Crafts-Brandner, 2004). Acclimation to elevated temperature in C_3 plants involves stabilization of Rubisco activase, in part by the increased presence of a longer, more heat-stable isoform of activase (Law *et al.*, 2001; Portis, 2003). Similar mechanisms appear to be present in maize, as acclimation to elevated temperature is associated with expression of a larger isoform of Rubisco and partial recovery of the Rubisco activation state (Crafts-Brandner and Salvucci, 2002). Electron-transport capacity can also become limiting for photosynthesis at elevated temperature in numerous C_3 species adapted to warm climates (Bukhov *et al.*, 1999; Schrader *et al.*, 2004; Wise *et al.*, 2004; Sharkey, 2005; Cen and Sage, 2005). The relative importance of limitations in electron transport capacity versus activation state remain uncertain. In C_4 species, the uncertain nature of the limiting processes at elevated temperature is a large part of the overall problem in understanding acclimation of C_4 plants to heat. In addition to the Rubisco activase and activation state limitations, photosynthesis may be limited by electron transport, PEP carboxylation, and PEP regeneration at elevated temperature (Sage, 2002; Kubien *et al.*, 2003). Without a clear picture of the limitations on C_4 photosynthesis at elevated temperature, it is difficult to assess how C_4 leaves acclimate to heat in terms of the biochemical reactions that determine photosynthetic capacity.

In summary, the limited amount of work on low-temperature acclimation in C_4 photosynthesis shows there is little enhancement of Rubisco capacity, as should be the case if a widespread limitation in Rubisco capacity is to be overcome. C_3 species do show substantial acclimation, and this is often explained by increases in P_i regeneration capacity and Rubisco content. The difference in thermal acclimation between C_3 and C_4 species is consistent with the hypothesis that the relatively low volume of leaves devoted to Rubisco-containing chloroplasts restricts the ability of C_4 species to compensate for low temperature by increasing Rubisco content. By contrast, C_3 species lack this restriction, and appear to have a greater ability to pack in extra enzyme as needed. Thus, there is evidence indicating that C_4 species may be constrained by their unique structural requirements to have a lower potential to acclimate to cooler temperatures than C_3 leaves. This could have consequences for the overall performance of C_4 species in environments where cool temperatures are common throughout the growing season.

Acclimation to elevated atmospheric CO_2 partial pressure

Acclimation of photosynthesis to atmospheric CO_2 variation deserves brief mention, largely because C_3 and C_4 plants respond differently to increases in atmospheric CO_2 content, although neither C_3 nor C_4 species show acclimation responses that are directly linked to CO_2 level. Instead, the CO_2 effect on the photosynthetic biochemistry is largely mediated by carbohydrate accumulation in leaves under conditions where carbon sinks in the plant are also experiencing high carbon supply (Sims *et al.*, 1998*b*; Long *et al.*, 2004). C_3 species show a greater degree of acclimation to elevated CO_2 partial pressure than C_4 species, largely because C_3 photosynthesis is stimulated more by rising CO_2, and hence the degree to which carbohydrate supply becomes excessive is potentially greater in the C_3 species (Sage, 1994). In C_3 species, there is a general decline in photosynthetic enzyme content with prolonged exposure to high CO_2; Rubisco is preferentially reduced during early phases of acclimation, but most photosynthetic genes are switched off after long-term exposure, particularly when sink limitations are substantial (Sage and Coleman, 2001; Long *et al.*, 2004). In C_4 plants, acclimation is often negligible due to the lack of a strong response to increased CO_2 partial pressure that is common in C_4 plants (Sage and Kubien, 2003). C_4 photosynthesis is CO_2-saturated, or almost CO_2-saturated at current atmospheric CO_2 levels, so a strong response is not expected. This feature of the C_4 photosynthetic pathway largely explains the relative lack of acclimation to rising CO_2 level in C_4 plants. However, certain C_4 species show a slight to modest short-term stimulation of photosynthesis by increased CO_2 availability, particularly under certain environmental conditions such as higher temperature and reduced mineral nutrition that increase the CO_2 saturation point of photosynthesis (Ziska *et al.*, 1999; Ghannoum *et al.*, 2000; Sage and Kubien, 2003). Where CO_2 stimulates photosynthesis, CO_2 acclimation can be observed, typically as a slight reduction in photosynthesis at both high and low levels of CO_2 (Tissue *et al.*, 1995; LeCain and Morgan, 1998; Watling *et al.*, 2000; Sage and Kubien, 2003). Acclimation may preferentially reduce the C_4 cycle, as indicated by a reduction of PEPCase but not Rubisco content in *Sorghum bicolor*, *Zea mays*, and *Flaveria* species grown at elevated CO_2 (Watling *et al.*, 2000; Gascoigne-Owens *et al.*, 2002; Snowdon *et al.*, 2002).

The consequence of reduced phenotypic plasticity in C_4 plants

Compared with C_3 species, C_4 plants have a restricted ecological and biogeographical distribution (Sage *et al.*, 1999). C_4 species are absent from polar biomes, rare if not absent in cool temperate to boreal biomes, rare in alpine and montane elevations at all latitudes, and uncommon in forest understoreys. C_4 photosynthesis is absent from certain plant life-forms, notably, canopy-forming forest trees. It is uncommon in short-stature trees, and in most shrub species, such that the woodland vegetation of the planet is almost exclusively C_3 plants. The only regions where C_4 photosynthesis is common in woody vegetation are in desert shrubs of saline soils and sand dunes. The reasons for the infrequency of C_4 photosynthesis in these regions and life-forms has never been adequately explained, although it is recognized that there are a number of contributing factors (Sage and Pearcy, 2000). For example, in low-light environments, a higher energy requirement for C_4 photosynthesis is an important consideration. This does not fully explain why there are so few shade-tolerant C_4 species, because the quantum yield of C_4 species is greater in warm environments (Ehleringer and Pearcy, 1983; Pearcy and Ehleringer, 1984). To the list of factors contributing to a lack of C_4 plants in cool or shaded environments, it is proposed that a reduced potential for phenotypic plasticity should be added. In many ways, C_4 plants show potential for acclimation, indicating that they are not too specialized for hot, high-light conditions to adjust to other environments. When the full suite of acclimation characteristics to low light or elevated temperature are considered, however, C_4 plants appear to be deficient in one or more traits when compared with C_3 species. For example, C_4 plants do not seem to have the same capacity to modulate Rubisco content, nor do they appear to be able to keep the leaf biochemistry as induced following sunflecks as well as C_3 plants. At the structural level, a requirement for high vein density may restrict acclimation potential to low light intensity. C_4 species can modulate leaf thickness as well as C_3 species, but appear to have extra costs when vein spacing is modified. Furthermore, a limited bundle-sheath volume may impose a space constraint on enzyme content, thus restricting the ability to increase Rubisco and other enzymes in situations where they may become limiting, such as low temperature. In summary, there is substantial evidence that C_4 plants have inherent constraints that prevent them from acclimating to environmental change as well as C_3 species, and this may have consequences for the range of life forms and landscapes where C_4 photosynthesis can occur.

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