Physiological framework for adaptation of stomata to CO₂ from glacial to future concentrations

Peter J. Franks¹,²,* , Ilia J. Leitch³, Elizabeth M. Ruszala⁴, Alistair M. Hetherington⁴ and David J. Beerling²
¹Faculty of Agriculture, Food and Natural Resources, University of Sydney, Sydney New South Wales 2006, Australia
²Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK
³Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AD, UK
⁴School of Biological Sciences, University of Bristol, Bristol BS8 1UG, UK

In response to short-term fluctuations in atmospheric CO₂ concentration, cₐ plants adjust leaf diffusive conductance to CO₂, gₐ via feedback regulation of stomatal aperture as part of a mechanism for optimizing CO₂ uptake with respect to water loss. The operational range of this elaborate control mechanism is determined by the maximum diffusive conductance to CO₂, gₐ(max), which is set by the size (S) and density (number per unit area, D) of stomata on the leaf surface. Here, we show that, in response to long-term exposure to elevated or subambient cₐ plants alter gₐ(max) in the direction of the short-term feedback response of gₐ to cₐ via adjustment of S and D. This adaptive feedback response to cₐ, consistent with long-term optimization of leaf gas exchange, was observed in four species spanning a diverse taxonomic range (the lycophyte Selaginella uncinata, the fern Osmunda regalis and the angiosperms Commelina communis and Vicia faba). Furthermore, using direct observation as well as flow cytometry, we observed correlated increases in S, guard cell nucleus size and average apparent ¹C DNA amount in epidermal cell nuclei with increasing cₐ suggesting that stomatal and leaf adaptation to cₐ is linked to genome scaling.

Keywords: leaf gas exchange; photosynthesis; plant evolution; gas exchange capacity; transpiration; vein density

1. INTRODUCTION

As an important greenhouse gas and the substrate for photosynthesis, atmospheric CO₂ concentration, cₐ is one of several important components in the feedback interaction between vegetation and the atmosphere [1,2]. Throughout the history of plants (approx. 400 Myr) on land, cₐ is estimated to have fluctuated from as high as several thousand ppm in the Devonian [3], to as low as around 180 ppm in the Last Glacial Maximum (LGM) [4]. Currently, it stands at approximately 390 ppm (National Oceanic and Atmospheric Administration data) but could exceed 1000 ppm by the end of this century according to Intergovernmental Panel on Climate Change estimates. Prehistoric CO₂ fluctuations have been linked to alternating greenhouse/icehouse global climates [3,5] as well as major episodes of plant evolution and diversification [6,7]. Along with current concerns about the impact of future elevated atmospheric CO₂ concentrations on global climate, the direct effects of the ongoing rapid rise in cₐ on plant photosynthetic productivity and water use is likely to be significant [1,8,9].

Changes in cₐ have a direct and immediate effect on the rate of CO₂ assimilation [10–13]. To counteract or mitigate these effects in the short term (seconds to minutes), plants employ a physiological feedback mechanism involving interaction between the diffusive conductance of the leaf epidermis, governed by stomata, and the rate of photosynthesis occurring in the mesophyll (figure 1) [14–16]. This short-term feedback mechanism controls the operational stomatal conductance to CO₂, g(oper) at a value between zero and the maximum, g(oper) for ⁴P_g. However, it is the physical attributes of the stomata that determine g(oper) (figure 2). At any given average guard cell turgor pressure P_g, g(oper) will be a function of the average stomatal aperture, a, the depth of the stomatal pore, l, and the number of stomata per unit area (or density, D) [17,18]. Ultimately, as P_g approaches its maximum and the a versus P_g relationship saturates, average stomatal aperture will reach its maximum, a(max) (figure 2a), causing g(oper) to reach its maximum, g(oper)(max) (figure 2b). Because the overall size of stomata determine both a(max) and l, g(oper)(max) can be described conveniently as a function of stomatal size, S, and D [19,20]. When g(oper)(max) adapts to new
environmental conditions (figure 2c), it necessarily involves adaptation of $S$ and/or $D$ [19,20].

(a) Stomatal conductance and CO$_2$

Sustained exposure to elevated or subambient $c_a$ involves feedback regulation of the biochemical capacity for CO$_2$ assimilation [21–26]. Over a gradient of growth $c_a$ treatments from subambient to elevated CO$_2$ concentration, the maximum rate of carboxylation, $V_{c\text{-max}}$ typically declines [24,26–28], consistent with a mechanism of adaptive feedback regulation of photosynthesis via adjustment of the amount of key photosynthetic enzymes [21,23]. Regulation of CO$_2$ assimilation rate under changing $c_a$ therefore involves stomatal and non-stomatal feedbacks operating over several timescales. However, despite significant advances in understanding the non-stomatal feedbacks, much less is known about the long-term response of stomata to CO$_2$ (weeks to millennia).

Our hypothesis is that over the long term, the leaf epidermis adapts to keep $g_{c\text{(op)}}$ and $g_{c\text{(max)}}$ aligned optimally with the leaf’s biochemical capacity for carbon assimilation under the prevailing $c_a$. Taking the short-term feedback response to $c_a$ as an analogue for the long term, we predict that following sustained exposure to a significant change in $c_a$, new leaves or plants will exhibit altered $g_{c\text{(op)}}$ in the direction of the short-term $g_{c\text{(op)}}$ response, through adjustment of $S$ and/or $D$. This adjustment is a practical necessity, ensuring that normal $g_{c\text{(op)}}$ is within the high sensitivity region of the $g_{c\text{(op)}}$ versus $P_e$ curve (figure 2b). Furthermore, there is a strong body of evidence suggesting that both across and within species, $S$ versus $D$ forms a negative linear log–log relationship, such that higher $g_{c\text{(max)}}$ is achieved through smaller $S$ and higher $D$ [19,20,29,30]. We predict, therefore, that in plants grown under low $c_a$, $g_{c\text{(max)}}$ will be higher, and this will be attributed to smaller $S$ and higher $D$. If our hypothesis is correct, then over evolutionary timescales (millions of years), sustained shifts in $c_a$ could select for $g_{c\text{(max)}}$ on the basis of adaptation of $S$ and $D$. Reconstructions of $g_{c\text{(max)}}$ from $S$ and $D$ in fossil leaves show a significant negative correlation with reconstructed $c_a$ over geological time [20], suggesting that $g_{c\text{(max)}}$ and $c_a$ have covaried in the long term. However, the pattern of adaptation of $S$, $D$ and $g_{c\text{(max)}}$ has not been examined closely under independent control of $c_a$.

(b) Stomatal size and genome size

Although $S$ and $D$ can be modified in many different combinations to alter $g_{c\text{(max)}}$ there are constraints. For example, allocation of more epidermal space to stomata may be at the expense of other important epidermal structures [20]. The tradeoffs involved in altering $S$ and $D$ may therefore be complex. There is, however, a readily apparent constraint with regard to changing $S$. The size of the guard cell nucleus is proportional to the width of the guard cell (figure 3a) resulting in a correlation between the two across species [31]. Because guard cell nuclei are typically diploid [32], this scaling of the size of a guard cell and its nucleus leads to a correlation between stomatal size and plant genome size (or C-value, measured as haploid or 1C DNA amount) [31,33,34]. This suggests that the large changes in $S$ over geological time were accompanied by changes in plant genome size, and that both $S$ and plant genome size co-evolved with $c_a$. This of course would not mean that $c_a$ alone drove the evolution of $S$ and/or plant genome size. Other environmental factors, including drought and light intensity, are known to affect $S$ [19,35,36]. However, if $S$, $g_{c\text{(max)}}$, and 1C DNA respond to independent control of $c_a$, then a firmer physiological framework can be established for the adaptation, natural selection and evolution of plants in relation to past and future changes in global $c_a$. The objective of this study was to test for sensitivity, in the form predicted earlier, of $g_{c\text{(max)}}$ $S$ and 1C DNA amount to controlled, long-term changes in $c_a$, from a subambient concentration (180 ppm) approximating that in the LGM, to an elevated concentration (1000 ppm) predicted for the end of this century. The timescale of sensitivity in this case is that of plant growth and development, i.e. in the order of several weeks.

2. MATERIALS AND METHODS

(a) Plant material and treatments

Plants were grown in controlled environment chambers under standard conditions: 1000 µmol m$^{-2}$ s$^{-1}$ photosynthetically active radiation (500 µmol m$^{-2}$ s$^{-1}$ for
Stomatal adaptation to CO₂  P. J. Franks et al.  539

Selaginella uncinata); 10 h photoperiod; 25 °C; and well-watered, commercial compost soil. Growth chamber CO₂ concentration (cₐ) was controlled at 180 or 240 ppm for ‘subambient CO₂’, representing glacial atmospheric conditions, 450 ppm for ‘ambient CO₂’, representing current day conditions, and 1000 ppm for ‘elevated CO₂’, projected to occur this century. Commelina communis and Vicia faba were grown from seed sown directly into pots in their respective growth chambers; Osmunda regalis was grown from spores and S. uncinata cloned by layering and dividing parent plants. All measurements are reported as the mean ± s.e. Significance of difference between means, at the 0.05 level, was determined with one-way analysis of variance (ANOVA) and post hoc means comparison tests (Tukey test) using standard statistical software (OriginPro v. 8.0, OriginLab Corporation, Northampton).

(b) Stomatal size and density

Epidermal peels were dissected from leaves and mounted on glass slides for viewing with a light microscope. Digital images of stomata were recorded at 200 × and 630 × magnification. Guard cell length and width were measured using image analysis software (IMAGEJ), and stomatal size (S) was calculated as guard cell length multiplied by the width of the guard cell pair (not including aperture) [20]. Guard cell dimensions were measured on 20 stomata from three epidermal peels from three plants per CO₂ treatment (n = 60). Stomatal density (D) was calculated as the number of stomata per square millimetre of epidermis, measured in ten 0.09 mm² fields on epidermis from three plants (n = 30). The volume of a single guard cell was approximated as that of a cylinder the same width as the guard cell, with hemispherical ends. A simplified volumetric measure of guard cell size, guard cell width cubed, is also reported.

(c) Maximum stomatal conductance, gₛ(ₐₘₐₓ)

Maximum stomatal conductance to CO₂ (gₛ(ₐₘₐₓ)) mol m⁻² s⁻¹) was estimated using a modified version of the Brown and Escombe equation [17,18,20]:

\[ gₛ(ₐₘₐₓ) = \frac{dₖDₐₘₐₓ}{v(l + (\pi/2)\sqrt{aₘₐₓ/\pi})}, \]

where dₖ is the diffusivity of CO₂ in air (m² s⁻¹), D is the stomatal density (m⁻²), aₘₐₓ is the mean maximum stomatal pore area (m²), v is the molar volume of air (m³ mol⁻¹), l is the depth of the stomatal pore (m, approximated as the width of a single guard cell [37]) and π is the mathematical constant (typically rounded to 3.142).

Note that aₘₐₓ relates to fully inflated guard cells that, for many species, can form stomatal pores approximating circular dimensions (figure 3b). It is therefore convenient in some cases to estimate aₘₐₓ as the area of a circle, π(p/2)², where p is the stomatal pore length [20]. Alternatively, when images of stomata with fully inflated guard cells are available, either for the species of interest or for a species with similar guard cell geometry, aₘₐₓ can be estimated as a fraction α of S; i.e., aₘₐₓ = αS [19,20]. We adopted the latter approach here, estimating aₘₐₓ as 0.08S for

Figure 2. Guard cell pressure drives stomatal aperture and stomatal conductance. (a) Stomatal aperture, a and, consequently, (b) stomatal conductance to CO₂ diffusion, gₛ, increase with guard cell pressure Pₑ in a saturation fashion. Stomatal aperture control is achieved through osmotic regulation of Pₑ. The operational gₛ(ₒₜ₉) is typically regulated via Pₑ within the high sensitivity region of the curve (double arrow). Maximum conductance, gₛ(ₐₘₐₓ), is determined by the number and size of stomata (see equation (2.1)). Data are for Tradescantia virginiana, adapted from Franks & Farquhar [17]. (c) Illustrating how altering gₛ(ₐₘₐₓ) keeps gₛ(ₒₜ₉) in the high sensitivity region of the Pₑ versus gₛ curve. Starting at the initial operating point (point a), a sustained change in cₐ from ambient (amb.; solid line) to elevated CO₂ concentration results in adjustment of gₛ to a lower gₛ(ₒ₅₉) (point b). The plant then changes stomatal size and density in new leaves, altering the gₛ versus Pₑ curve (dashed line, subambient CO₂; sub.) to return gₛ(ₒ₅₉) to its optimal position (point c). Similarly, following a sustained shift from ambient to subambient cₐ the plant adjusts gₛ to a higher gₛ(ₒ₅₉) (point d). New leaves are produced with altered stomatal size and density, shifting to a new gₛ versus Pₑ curve (dotted line, subambient CO₂; sub.) and increasing gₛ(ₐₘₐₓ) to return gₛ(ₒ₅₉) to its optimal position (point c). Curves in (c) are representative, based on (b).

Phil. Trans. R. Soc. B (2012)
S. uncinata (based on the lycophyte Huperzia prolifera [37]), 0.1S for O. regalis (based on the fern Nephrolepis exaltata [37]) and 0.33S for both C. communis and V. faba (based on the herbaceous angiosperm Tradescantia virginiana [37]). Importantly, $a_{\text{max}}$ and hence $g_{\text{c(\text{max})}}$ occur only under the somewhat artificial conditions that promote fully inflated guard cells, such as the combination of low atmospheric CO$_2$ concentration (below that at which plants grew), high (photosynthesis-saturating) light intensity and 100 per cent humidity, or for certain in vitro preparations using epidermal peels [37]. Under more typical conditions, the highest operating stomatal conductance, $g_{\text{c(op)}}$, is likely to be around half of $g_{\text{c(\text{max})}}$ [19]. Owing to the infinite combination of natural conditions under which $g_{\text{c}}$ could be operating, $g_{\text{c(\text{max})}}$ is a more informative standard measure of the capacity of the leaf epidermis to support the diffusion of CO$_2$ from the atmosphere to the leaf interior, as it is determined solely on the basis of static anatomical dimensions.

### 3. RESULTS AND DISCUSSION

#### (a) Stomatal size, density and $g_{\text{c(\text{max})}}$ response to CO$_2$

The results show a consistent pattern of correlated adaptation of $S$ and $D$ to $c_a$ that may serve as a physiological framework for adaptation, selection and evolution of $g_{\text{c(\text{max})}}$ under changing global $c_a$ regimes. In response to growth across a gradient of increasing $c_a$, all four species developed leaves with increasingly large guard cells and lower stomatal density (figure 4). This translated into lower $g_{\text{c(max)}}$ with increasing $c_a$, as calculated from $S$ and $D$ measurements using equation (2.1) (figure 5). In all cases, $g_{\text{c(max)}}$ and $D$ were significantly lower, and $S$ significantly larger, at elevated relative to subambient $c_a$. The consistent response across these four species, representing a broad taxonomic range (lycophyte, fern and angiosperm), suggests that this mode of adaptation to $c_a$ is widespread in vascular plants and its genetic basis may therefore be highly conserved through plant lineages.

The CO$_2$-induced shift in $g_{\text{c(max)}}$ is in the direction of the short-term feedback adjustment of operational stomatal conductance resulting from stomatal aperture changes in response to CO$_2$ [14]. This correction of $g_{\text{c(max)}}$ via anatomical adjustment of $S$ and $D$ indicates a realignment of the $g_{\text{c}}$ versus $P_g$ relationship (figure 2b) that keeps the new operational $g_{\text{c}}$ in the same region of the curve as before the CO$_2$ change. This adaptive feedback response is consistent with a general mechanism of feedback adaptation of operational stomatal conductance and $g_{\text{c(max)}}$ to environmental changes. For example, in response to growth under treatment with the drought hormone abscisic acid, Tradescantia virginiana leaves altered $S$ and $D$, which lowered $g_{\text{c(max)}}$ and increased water-use efficiency, while maintaining operational $g_{\text{c}}$ in the same region of the $g_{\text{c}}$ versus $P_g$ curve [17].

Although the response to $c_a$ was significant, the change in $g_{\text{c(max)}}$ resulting from adaptation of $S$ and $D$ was less than 10 per cent in three of the four species (S. uncinata, V. faba and C. communis) over the subambient to elevated CO$_2$ gradient. In O. regalis, it was closer to 40 per cent. The sensitivity of $g_{\text{c(max)}}$ to...
growth at different $c_a$ is therefore variable and may, in some cases, be small or negligible. This is reflected in a survey of stomatal density response [26], where the change from ambient (mean 363 ppm) to elevated (mean 571 ppm) $c_a$ was a mean reduction of 5 per cent, with the most frequent response being a reduction of less than 10 per cent. The same survey reported a reduction in operating stomatal conductance of 22 per cent, suggesting partial stomatal closure in addition to a reduction in $D$. However, the magnitude of the $c_a$ treatment or change should be considered when assessing the sensitivity of $S$, $D$ and $g_{c(max)}$ to $c_a$. The survey by Ainsworth & Rogers [26] spanned a 1.5-fold range of $c_a$, averaging 2.4 per cent change in $D$ per 100 ppm; in this study the $c_a$ treatment spanned a fivefold range, averaging 3.2 per cent change in $D$ per 100 ppm; and for the survey of fossils in Franks & Beerling [20], where $c_a$ spanned a greater than eightfold range over 400 Myr, there was an average $-4.2$ per cent change in $D$ per 100 ppm increase in $c_a$ (noting that the response over large ranges of $c_a$ appears to be nonlinear [20]). The sensitivity of $D$ to $c_a$ is therefore typically in the order of minus a few per cent per 100 ppm increase in $c_a$ but this may be difficult to detect over smaller ranges of $c_a$, both in studies of the geological record and in experimental treatments. Furthermore, the response appears to be nonlinear over a large range in $c_a$ [20,39], with greater sensitivity at low $c_a$. The study of herbarium samples by Woodward [40], spanning the approximately 100 ppm rise in $c_a$ over the last 200 years (i.e. subambient conditions), reports a $-41$ per cent change in $D$ and a $-61$ per cent change for growth chamber experiments simulating similar conditions. This is supported by a recent study of leaf material preserved in peat deposits, together with herbarium samples, for the same period [30], which shows an average $-28$ per cent change in $D$.

A negative relationship between $S$ and $D$ appears to characterize the general adaptation, plasticity or variability in $g_{c(max)}$ in response to various environmental variables. It is expressed within the canopy of individual species as part of the natural variability in $g_{c(max)}$ [19] and holds for the pooled data of hundreds of fossil species across the geological record and the present [20]. Adaptation or selection of $g_{c(max)}$ by large

---

**Figure 4.** Stomatal size and density covary with CO$_2$. (a–d) Negative relationship between stomatal size ($S$) and density ($D$) in (a) *S. uncinata* (lycophyte), (b) *O. regalis* (Fern), (c) *V. faba* (angiosperm) and (d) *C. communis* (angiosperm), respectively, when grown at three different atmospheric CO$_2$ concentrations. Treating $c_a$ as a continuous variable, each datum point (mean ± s.e.m.) is connected by lines in the order of highest to lowest $c_a$ treatment. (a–d) Stomata were significantly smaller and more numerous at subambient compared with elevated atmospheric CO$_2$ concentration. Means with different adjacent letters, along respective axes, are significantly different. Filled circles, subambient CO$_2$; unfilled squares, ambient CO$_2$; filled triangles, elevated CO$_2$.
global changes in $c_a$ appears to operate within a general framework of down- or upregulation along a negative $S$ versus $D$ relationship in order to optimize plant carbon/water balance.

(b) Stomatal size and nucleus response to CO$_2$

Direct observations of guard cell nucleus size, made following fluorescent DNA staining, indicate significant increases in nucleus volume at elevated relative to subambient CO$_2$ concentration in all three species (figure 6a–c). Accompanying these changes were correlative increases in the volume of the guard cells (figure 6d), with guard cell volume and nucleus volume both significantly larger at elevated compared with subambient $c_a$. This pattern is reflected as an increase in the apparent 1C DNA amount in epidermal tissue with increasing growth $c_a$ (figure 7a–c). In all three species, 1C DNA amount in epidermal tissue was significantly greater at elevated relative to subambient CO$_2$ concentration. This suggests that the physiological adaptation of stomatal size to $c_a$ involves a coordinated change in guard cell nucleus size and genome size.

In contrast to the clear physiological consequences of adaptation of $S$, $D$ and $g_{c(max)}$ to $c_a$, the physiological benefit of an accompanying change in nucleus size or genome size is unclear. The minimum size of guard cells is of course constrained physically by the size of the guard cell nucleus (small guard cells have small nuclei), but the close coupling between guard cell size, nucleus size and apparent genome size in figures 6 and 7 appears to relate to subcellular scaling processes rather than the diffusion physics of leaf gas exchange. It has long been observed that there is a fundamental positive correlation between cell size (specifically the cytoplasm volume) and the size of the cell nucleus, corresponding broadly to a correlation between cell size and genome size [41–43]. The underlying mechanism for this has been the subject of considerable debate [43,44], but one model with an advanced biophysical basis is the ‘skeletal DNA theory’ [43]. The principle of the skeletal DNA theory is that the correlation of cell cytoplasm and nucleus volume maintains a balance between the rate of protein synthesis (cytoplasm-based) and the rate of RNA synthesis and processing (nucleus-based). It is possible that this energetic balancing of cell size and genome size applies to small, plastic changes in cell size within species, as observed in this study with guard cells. However, considerably more work is required to prove this. Until recently, even the possibility of variation in genome size within species (variation between populations and within individuals, as observed in this study) was hotly debated, but there is now overwhelming evidence to show that...
variability and plasticity in genome size is common within species [45]. Assuming that within-species differences in genome size are linked to the fitness of the phenotype, as shown here in relation to the feedback adjustment of \( g_{c_{\text{max}}} \) involving guard cell size, then it is possible for natural selection to act on these individuals, i.e. for \( c_a \) to select for genome size [31].

The size of the nucleus comprises the total amount of DNA in the nucleus (i.e. genome size, or ‘C-value’) and the degree of chromatin packing, and our results suggest that \( c_a \) may play a role in influencing either one or both of these. Over the short time frames of the CO\(_2\) experiments (up to three months), the observed increase in guard cell nuclear volume at elevated \( c_a \) (figure 6a–c) is likely mediated through changes in the states of euchromatin and/or heterochromatin, which are known to be dynamic and responsive to developmental and environmental cues [46,47]. The results may indicate an epigenetic response [48], whereby adaptation of guard cell size to \( c_a \) to facilitate the optimization of leaf gas exchange invokes chromatin repackaging possibly mediated via RNA interference [49]. The qualitatively consistent results across species suggest at least that \( c_a \) is an external cue to which the plant nucleus responds, either directly or as a correlated character with guard cell size. An alternative possibility is that the differences in the amount of DNA detected result from altered binding of the fluorochrome to the DNA owing to changes in the condensation state of chromatin [50], rather than absolute changes in genome size. However, it is noteworthy that the same result, as predicted from physiological and biophysical theory, was observed across three species.

### 4. CONCLUSIONS

The results provide a physiological framework for analysing and predicting the adaptation of stomata and maximum leaf diffusive conductance to atmospheric CO\(_2\) concentrations ranging from glacial to the elevated levels forecast for this century. The results suggest that for many species \( g_{c_{\text{max}}} \) exhibits developmental plasticity through a negative relationship between \( S \) and \( D \) that shifts the operational range of leaf diffusive conductance in the direction of the short-term feedback response of stomatal aperture to \( c_a \). Stomata therefore appear to exhibit both short-term dynamic and long-term adaptive feedback responses to \( c_a \). The plasticity in \( S \) appears also to be linked to correlated plasticity in the size of the guard cell nucleus as well as the apparent

---

Figure 6. Atmospheric CO\(_2\) influences the size of guard cell nuclei. Guard cell nuclei are significantly larger in plants grown under elevated compared with subambient atmospheric CO\(_2\) concentration for (a) \( O.\) regalis, (b) \( C.\) communis and (c) \( V.\) faba. (d) Guard cell nucleus volume increasing with guard cell volume for all three species grown under the different atmospheric CO\(_2\) concentrations. Symbols are as given for figure 4; in (d) the solid line is \( O.\) regalis, dashed line is \( C.\) communis and dotted line is \( V.\) faba; mean ± s.e.m.
of years, in which natural selection and evolution can operate \([44, 51–53]\), our results suggest that large changes in \(c_a\) may, in selecting for the phenotypic benefits of optimal \(\delta_c(\text{max})\) select also for correlated changes in \(S, D\) and genome size.

We thank T. Cavalier-Smith and M. W. Chase for helpful comments and discussion on plant genome size, and S. Pearce, G. Nicholson and B. Palmer for technical assistance with the plant growth chambers. The work was supported by funding from the Australian Research Council and the University of Sheffield (P.J.F. and D.J.B.). D.J.B. gratefully acknowledges a Royal Society–Wolfson Research Merit Award. E.M.R. is grateful to the Gatsby Charitable Foundation for the Award of a Sainsbury post-graduate studentship.

**REFERENCES**


9 Betts, R. A. et al. 2007 Projected increase in continental runoff due to plant responses to increasing carbon dioxide. *Nature* 448, 1037–1041. (doi:10.1038/nature06045)


14 Farquhar, G. D., Dubbe, D. R. & Raschke, K. 1978 Gain of feedback loop involving carbon-dioxide and stomata:
Stomatal adaptation to CO$_2$  P. J. Franks et al. 545


