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Allocation of the epidermis to stomata relates to stomatal physiological control: Stomatal factors involved in the evolutionary diversification of the angiosperms and development of amphistomaty

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ABSTRACT

The proportion of the leaf epidermis allocated to stomata ($EP_{\%}$) and stomatal function (the capacity to adjust stomatal pore area to regulate stomatal conductance: G_s) are key components in leaf gas exchange, and have likely played a major role in plant evolution. We examined the velocity of change in G_s ($G_{s50\%}$) during a transition from steady state conditions in the light to darkness and $EP_{\%}$ in 31 vascular plants with diverse evolutionary origins. Across all species, $EP_{\%}$ correlated to $G_{s50\%}$ and the magnitude of G_s reduction (G_{sLIGHT}-G_{sDARK}) after the cessation of illumination. Those species with higher absolute and relative $G_{s50\%}$ values tended to distribute stomata more evenly over the abaxial and adaxial leaf surfaces, whereas species with lower $G_{s50\%}$ utilised only one leaf surface for gas exchange. Groups that diverged at relatively early stages in plant phylogeny, including ferns, gymnosperms and basal angiosperms, exhibited lower $EP_{\%}$ and $G_{s50\%}$, and took longer to achieve the initial 50% reduction in G_s ($T_{50\%}$) than the more recently diverging angiosperms; in particular, the amphistomatous monocot grasses, which also showed higher absolute rates of photosynthesis and G_s. We propose that selective pressures induced by declining [CO_2] over the past 100 Myr have favoured greater allocation of the epidermis to stomata, increased amphistomaty (the presence of stomata on the abaxial and adaxial surfaces) and faster control of G_s in the more recently derived angiosperm groups. Modification of photosynthesis to enhance the carbon and water use efficiencies of C3 crops may therefore require concurrent increases in stomatal density and in the capacity of stomata to react quickly to environmental pressures.

1. Introduction

The origination of stomata 410 million years ago (Ma) enabled plants to colonise the land by facilitating the uptake of carbon dioxide (CO_2) for photosynthesis (A) while allowing the regulation of transpiration to minimise the risk of desiccation (Edwards et al., 1998; Duckett et al., 2009). Stomatal conductance (G_s) is controlled by physiological adjustment of the size of the stomatal pore and morphological alteration of the number and size of stomata on newly developing leaves. These morphological stomatal responses effectively set the limits for physiological control of G_s (Woodward, 1987; Fanourakis et al., 2015). The percentage of the leaf epidermis devoted to stomata ($EP_{\%}$), and therefore available for gas exchange, varies widely between species and has likely played a key role in plant evolution (Franks and Beerling, 2009; Assouline and Or, 2013; Boer et al., 2016). A diverse range of physiological stomatal behaviours are also observed, and whether these are associated with plant phylogeny remains a matter of debate (Brodribb et al., 2009; McAdam and Brodribb, 2012; Chater et al., 2013; Hasper et al., 2017). Physiological and morphological stomatal responses operate in tandem to control G_s following a change in the atmospheric concentration of carbon dioxide ([CO_2]) (Haworth et al., 2013; Haworth et al., 2015). However, despite the wide range of research undertaken into epidermal patterning and physiological stomatal behaviours, they are often considered in isolation, and it is not

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clear whether allocation of the epidermis to stomata is related to the physiological function of stomatal complexes. Any coordination between $EP_{\%}$ and stomatal function may have played a role in plant evolutionary history, and may also have implications for the modification of crops to optimise A and water use efficiency (WUE).

The allocation of the epidermis to gas exchange is determined by the stomatal pore area (SPA) and stomatal density (SD) on the abaxial and adaxial leaf surfaces (Cowan, 1977). Observations of living (Hetherington and Woodward, 2003) and fossil (Franks and Beerling, 2009) plants indicates that there is an inverse relationship between stomatal size and density. More recently diverged angiosperm groups tend to possess higher densities of smaller stomata than more basal groups with ancient evolutionary origins such as ferns and gymnosperms (Franks and Beerling, 2009). This trend towards higher densities of smaller stomata in groups that originated more recently may reflect the influence of declining [CO_2] over the past 100 million years (Myr) (Haworth et al., 2017). As the availability of CO_2 for A declined, stomatal morphologies that enabled a high rate of CO_2 diffusion, but also limited the structural and mechanical impact of large stomata being too closely spaced, might have been favoured (Assouline and Or, 2013; Dow et al., 2014; Boer et al., 2016). Large numbers of small stomata may also offer greater control of G_s, as small stomata are considered to be able to adjust SPA and regulate G_s more rapidly, thus optimising WUE over shorter time-scales (Giday et al., 2013; Raven, 2014).

The majority of plant species possess stomata only on the abaxial leaf surface (hypostomaty) (Peat and Fitter, 1994; Muir, 2015). Amphistomatous species have stomata on both the abaxial and adaxial surfaces, theoretically permitting greater $EP_{\%}$ by utilising the entire leaf epidermis. Hypostomaty is considered to represent the primitive form of stomatal arrangement (Mott et al., 1982). If amphistomaty represents the more derived status, the fact that it is not more widespread suggests that amphistomatic species do not experience a clear selective advantage in all environments, and that evolutionary costs (such as increased susceptibility to pathogens) may be incurred by possessing stomata on both leaf surfaces (Muir, 2015). Nevertheless, the increased occurrence of amphistomatic species with a herbaceous growth form in high-light, open habitats may indicate that the adaptive significance of amphistomaty relates to increased conductance to CO_2 (Parkhurst, 1978; Mott et al., 1982; Peat and Fitter, 1994; Muir, 2018). To the best of our knowledge, previous studies of stomatal distribution have not considered the role of physiological stomatal regulation (ie. the speed of stomatal aperture adjustment) in the occurrence of amphistomaty.

Physiological regulation of stomatal aperture ranges from active (where osmolytes are pumped across the cell membrane of the guard cells following a stimulus) to passive (where the turgor of the guard cells follows that of the leaf). Stomatal opening in response to sub-ambient [CO_2] has been observed in epidermal strips detached from the mesophyll layer, while closing to super-ambient [CO_2] involves a signal from the mesophyll layer (Fujita et al., 2013). An evolutionary transition from passive to active stomatal behaviour has been proposed to have contributed towards the expansion of the angiosperms (Brodribb et al., 2009; McAdam and Brodribb, 2012). However, genetic (Chater et al., 2011; Ruszala et al., 2011), guard cell membrane transporter protein (Chen et al., 2017) and gas exchange (Ruszala et al., 2011; Haworth et al., 2013; Franks and Britton-Harper, 2016; Hasper et al., 2017) analyses suggest that active physiological stomatal behaviours originated in early plant lineages. Nonetheless, the more recently derived Poaceae monocots (grasses) exhibit morphologically and mechanically divergent stomatal complexes (termed 'dumb-bell' stomata) in comparison to other angiosperms and gymnosperms (termed 'kidney-shaped' stomata). The stomata of grasses tend to be capable of more rapid adjustments of SPA (Franks and Farquhar, 2007). This enables grasses to adjust G_s more rapidly to changes in light and [CO_2] than other vascular plants which have kidney-shaped stomata (Haworth et al., 2013; Haworth et al., 2015).

To elucidate potential relationships between epidermal patterning and stomatal function, we assessed $EP_{\%}$, the time to achieve the initial 50% of the overall reduction in G_s ($T_{50\%}$) and the velocity in the change of G_s over time (hereafter termed $G_{s50\%}$) during a transition from light to darkness. The G_s response to darkness has been used to investigate evolutionary patterns in physiological stomatal behaviour (McAdam and Brodribb, 2012; Elliott-Kingston et al., 2016; Xiong et al., 2018) and stomatal function in plants grown under elevated [CO_2] (Haworth et al., 2016) and drought (Haworth et al., 2018). Stomatal closure during a transition from light to dark conditions has been shown to be more effective in differentiating plant groups (McAdam and Brodribb, 2012; Xiong et al., 2018) and characterising the impact of drought on physiological stomatal function (Haworth et al., 2018) than a transition from dark to light to stimulate stomatal opening. We hypothesise that those species that devote a larger proportion of their epidermis to gas exchange will exhibit more rapid control of G_s. This study specifically aimed to: i) examine $G_{s50\%}$, $T_{50\%}$ and $EP_{\%}$ in plants with diverse evolutionary origins; ii) investigate the influence of $EP_{\%}$, $T_{50\%}$ and $G_{s50\%}$ on A under steady-state conditions in the light, and; iii) explore possible evolutionary patterns in $EP_{\%}$ and stomatal function which may relate to the diversification of the angiosperms, and whether these attributes can be utilised to enhance the carbon and water use efficiencies of crop plants.

2. Materials and methods

The area of the epidermis allocated to stomata and physiological stomatal functionality was assessed in 31 species. These vascular plants represented species with diverse evolutionary lineages, and for the purposes of the present study were categorised as: ferns (*Osmunda regalis*, *Cyathea cooperi*, *Cyrtomium fortunei*, *Matteuccia orientalis* and *Dicksonia antarctica*), gymnosperms (*Lepidozamia peroffskyana*, *Cycas siamensis*, *Ginkgo biloba*, *Agathis australis*, *Nageia nagi* and *Podocarpus macrophyllus*), basal angiosperms (*Amborella trichopoda*, *Schisandra grandiflora*, *Magnolia stellata* and *Magnolia grandifolia*), eudicots (*Solanum lycopersicum*, *Moricandia moricandiodes*, *Coffea arabica*, *Helianthus annuus*, *Gossypium hirsutum*, *Chenopodium quinoa*, *Populus nigra*, *Capsicum frutescens* and *Salix alba*) and monocots (*Avena sativa*, *Triticum aestivum*, *Hordeum vulgare*, *Fargesia robusta*, *Phragmites australis*, *Typha latifolia* and *Arundo donax*). The term "basal angiosperm" refers in the present work to angiosperms whose lineages diverged, according to a consensus of recent molecular phylogenetic studies (Byng et al., 2016), prior to the most recent common ancestor of eudicots and monocots.

The magnitude of the overall G_s response (G_{sLIGHT}-G_{sDARK}), the time to achieve the first 50% of the total reduction in G_s ($T_{50\%}$) and the velocity of change in G_s ($G_{s50\%}$) as stomata close following a cessation of illumination were used to determine physiological stomatal functionality following Haworth et al. (2018). A PP-Systems Ciras-2 attached to a PLC6(U) leaf cuvette and LED light unit (PP-Systems, Amesbury, Massachusetts, USA) was used to measure G_s. The size of cuvette was chosen in relation to the shape/size of leaf. In all cases, the leaf filled the entire cuvette; the cuvette plate size for each species is provided in Supplementary data Table 1. Leaves were placed inside the cuvette under conditions of 400 ppm [CO_2], 25° C, 60–65% relative humidity and saturating photosynthetically active radiation (PAR) (values for each species are given in Supplementary data table S1) for 30 min until G_s was stable (G_{sLIGHT}). Stomatal conductance was then recorded every 10 s for a further 10 min. After ensuring stomatal stability, the lights within the cuvette were switched off and G_s was recorded every 10 s for a minimum of 60 min, or until the full extent of stomatal closure had been achieved and G_s had remained stable for 20 min (G_{sDARK}) (Fig. 1). The $G_{s50\%}$ was determined as the velocity by which 50% of the G_{sLIGHT}-G_{sDARK} reduction that occurred after the onset of darkness, this was expressed as absolute and relative values (assuming 100% G_s at the point where lights were switched off). This parameter, and the time needed to achieve the initial 50% reduction in

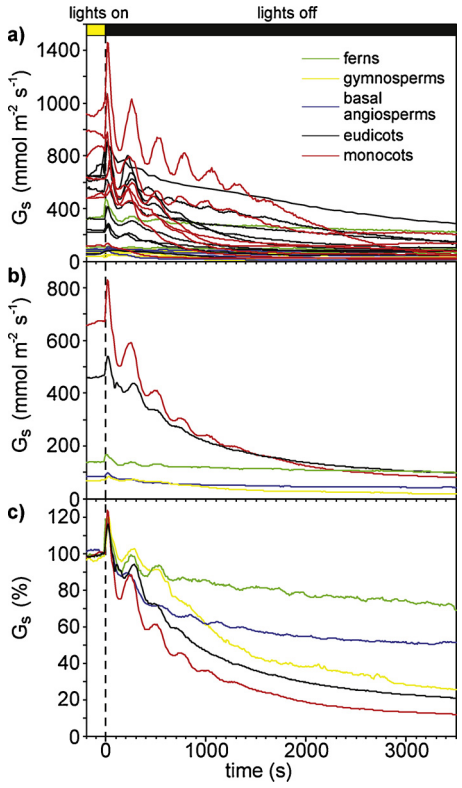

Fig. 1. The response of stomatal conductance (G_s) during a transition from steady state conditions in the light to darkness (indicated by a vertical dashed line and change from yellow to black in the upper horizontal bar) of the evolutionarily diverse species analysed in this study: a) each line represents the mean G_s response of each species (a minimum of four replicates for each species); b) the mean G_s response of each plant group (absolute values of G_s), and; c) the mean relative G_s response of each group assuming 100% G_s at the point when illumination ceased. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

G_s ($T_{50\%}$) were measured following the protocol of Haworth et al. (2018)(a schematic illustration is provided in Supplementary data Fig. S1). The G_s decrease is in effect a rate of change of velocity (ie. in this case a deceleration) and expressed as mmol $m^{-2} s^{-2}$. The $G_{s50\%}$ was measured on one leaf per plant from a minimum of four replicate plants per species.

Epidermal micro-morphology was examined on the same leaves used for gas exchange analysis. Dental impression gel was used to create negative impressions of the abaxial and adaxial leaf surfaces. Clear nail varnish was then applied to the dental impression gel (Weyers and Lawson, 1985). The nail varnish positives were then mounted on glass microscope slides and imaged using a Leica DM2500 microscope attached to a Leica DFX300FX camera (Leica Microsystems, Wetzlar, Germany). A 0.4 × 0.4 mm grid was digitally superimposed over each image to calculate SD. The mean of 10 images was used to determine SD for each leaf surface. Rarefaction analysis indicated that SD values stabilised after five to six images. The average SD for the abaxial and adaxial leaf surfaces was then determined. The SD for each replicate plant was then averaged to produce the mean value per species. Stomatal pore area during full stomatal opening was calculated assuming an ellipse shape where stomatal width is half stomatal pore length (Beerling and Chaloner, 1993). Stomatal pore length was measured from a minimum of 40 stomatal complexes per species (ie. 10 per replicate). The SD and SPA were then used to determine $EP_{\%}$ (see Haworth et al., 2015). The values of $EP_{\%}$ presented within the manuscript represent the maximum proportion of the epidermis devoted to gas exchange over both the abaxial and adaxial leaf surfaces.

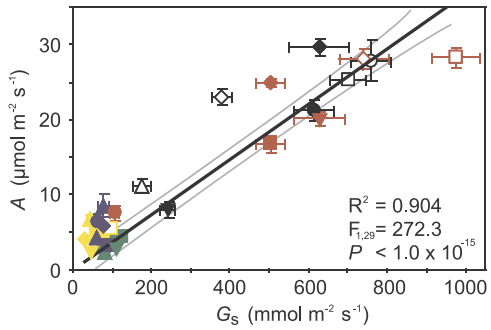

Fig. 2. The relationship between photosynthesis (A) and stomatal conductance (G_s) of vascular plants with diverse evolutionary origins. Ferns (green): *Osmunda regalis* (solid square), *Cyathea cooperi* (solid diamond), *Cyrtomium fortunei* (solid triangle), *Matteuccia orientalis* (solid circle) and *Dicksonia antarctica* (solid inverted triangle). Gymnosperms (yellow): *Lepidozamia peroffskyana* (solid square), *Cycas siamensis* (solid diamond), *Ginkgo biloba* (solid triangle), *Agathis australis* (solid circle), *Nageia nagi* (solid inverted triangle) and *Podocarpus macrophyllus* (square white-fill). Basal angiosperms (blue) *Amborella trichopoda* (solid square), *Schisandra grandiflora* (solid diamond), *Magnolia stellata* (solid triangle) and *Magnolia grandifolia* (solid circle). Eudicots (black): *Solanum lycopersicum* (solid square), *Moricandia moricandiodes* (solid diamond), *Coffea arabica* (solid triangle), *Helianthus annuus* (solid circle), *Gossypium hirsutum* (solid inverted triangle), *Chenopodium quinoa* (square white-fill), *Populus nigra* (diamond white-fill), *Capsicum frutescens* (triangle white-fill) and *Salix alba* (circle white-fill). Monocots (red): *Avena sativa* (solid square), *Triticum aestivum* (solid diamond), *Hordeum vulgare* (solid triangle), *Fargesia robusta* (solid circle), *Phragmites australis* (solid inverted triangle), *Typha latifolia* (square white-fill) and *Arundo donax* (diamond white-fill). The black line indicates the line of best fit and the two grey lines either side indicate the 95% confidence intervals of the mean. Linear regression was used to assess the significance of any relationship between G_s and A. Error bars indicate one standard error either side of the mean. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results

Photosynthesis was positively related to G_s under saturating PAR in the evolutionarily diverse vascular plants analysed in this study (Fig. 2). The ferns, gymnosperms and basal angiosperms exhibited similarly low levels of A and G_s, although the basal angiosperms showed a wider range of A values (Fig. 3a and b). Stomatal conductance under saturating PAR was on average 32.9% greater in the monocots than the eudicots, and G_s in the monocots were seven times greater than those observed in ferns, gymnosperms and basal angiosperms (Fig. 3b). The greater levels of G_s in the monocots were associated with higher SD (Fig. 3c), $EP_{\%}$ (Fig. 3d) and both relative (Fig. 3g) and absolute (Fig. 3h) $G_{s50\%}$ and lower $T_{50\%}$ (Fig. 2f) than the other plant groups. The absolute (Fig. 1b) and relative (Fig. 1c) reductions in G_s when averaged for each group were most rapid in the eudicots and monocots. The fast growing amphistomatous grasses *P. australis*, *T. latifolia* and *A. donax* exhibited the highest levels of $G_{s50\%}$, G_{sLIGHT}-G_{sDARK} and $EP_{\%}$, and the lowest levels of $T_{50\%}$. The eudicot angiosperms exhibited respective 60.8 and 54.7% lower average $G_{s50\%}$ and $EP_{\%}$ values, and 88.7% longer $T_{50\%}$ than the monocots. The ferns, gymnosperms and basal angiosperms exhibited the lowest rates of G_s response and maximum allocation of the epidermis to gas exchange (Fig. 3c and f). Data presented in Figs. 2 and 3 is given in Supplementary information Table 1.

The 31 species analysed in this study showed a negative relationship between SPA and SD (Fig. 4), similar to those reported for SD with guard cell length (Hetherington and Woodward, 2003) and the size of the stomatal complex (Franks and Beerling, 2009). The magnitude of the total G_s response (G_{sLIGHT}-G_{sDARK}) was not associated with SPA (Fig. 5a) or the SD:SPA ratio (Fig. 5c). However, G_{sLIGHT}-G_{sDARK} was positively related to SD (Fig. 5b) and $EP_{\%}$ (Fig. 5d). The time to achieve

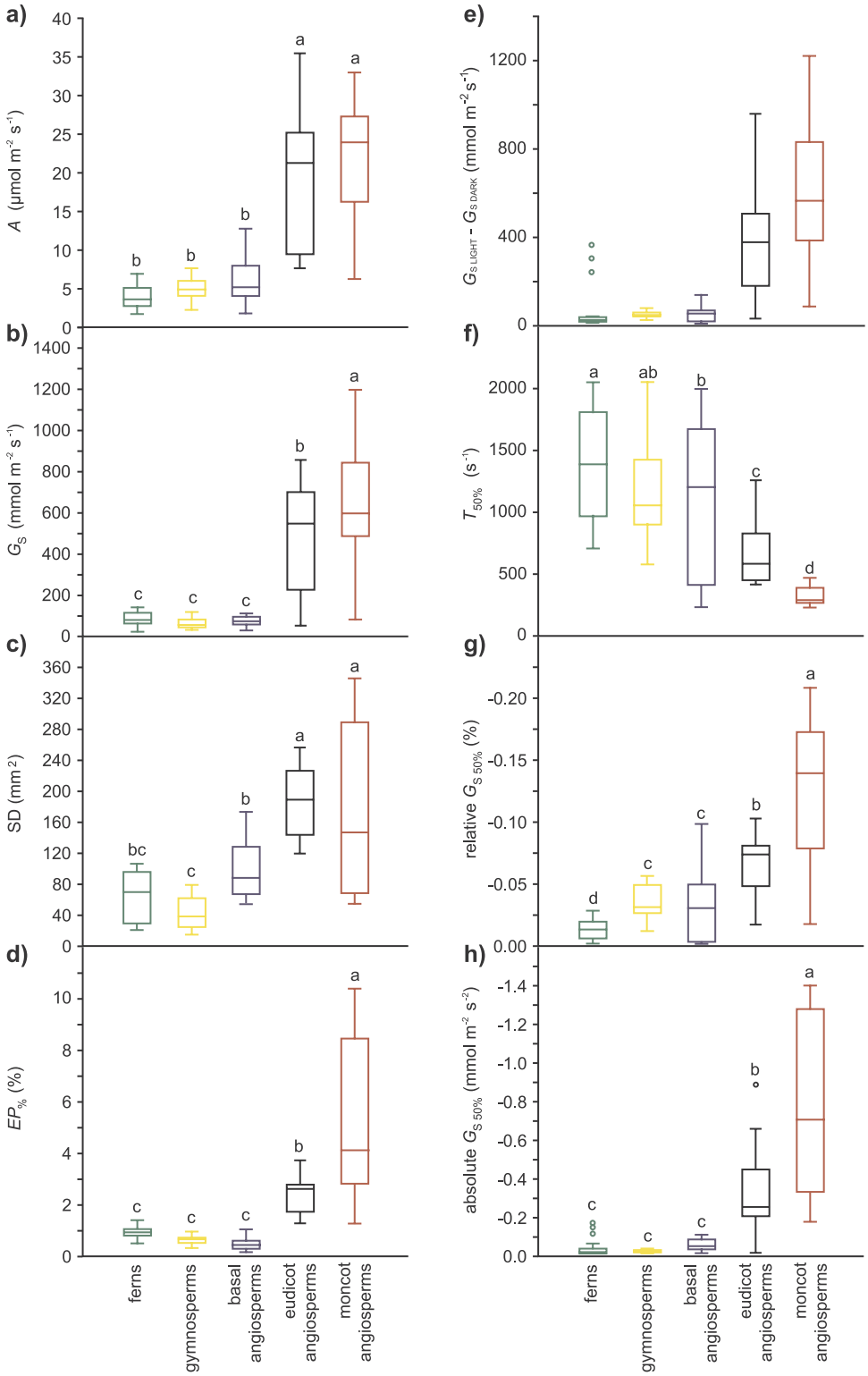

Fig. 3. Box plots representing the range of values of photosynthesis (a), stomatal conductance (b), stomatal density (c), the proportion of the epidermis allotted as stomata (d), the magnitude of the G_s response during the transition from light to dark conditions (e), the time to achieve the initial 50% of the overall reduction in stomatal conductance (f) and the relative (g) and absolute (h) velocity of stomatal conductance response during a transition from light to dark conditions observed in the fern (green), gymnosperm (yellow), basal angiosperm (blue), eudicot (black) and monocot (red) species analysed in this study. The box signifies the distribution of the 25–75% quartiles, the median is represented by a horizontal line within the box, horizontal bars either side of the box indicate minimum/maximum values. Circles indicate outlying data points. Letters above each box indicate significant difference at the 0.05 level using a one-way ANOVA with an LSD *post-hoc* test. A histogram showing the mean and standard error for each group is provided in Supplementary information Fig. S2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the initial 50% of the overall G_s response was negatively related to SPA (Fig. 5e), SD (Fig. 5f), the SD:SPA ratio (Fig. 5g) and $EP_{\%}$ (Fig. 5h). These relationships were statistically significant, although R^2 values ranged from 0.04 to 0.26 signifying comparatively low fit. Relative and absolute values of $G_{s50\%}$ were not related to SPA (Fig. 5i and m), but were positively correlated to SD (Fig. 5j and n). Relative (linear regression: $R^2 = 0.0005$; $F_{1,29} = 0.013$; $P = 0.910$) and absolute (linear regression: $R^2 = 0.032$; $F_{1,29} = 0.974$; $P = 0.332$) $G_{s50\%}$ was also not related to the length of the guard cell. The ratio of SD to SPA was weakly correlated to relative $G_{s50\%}$ ($R^2 = 0.174$; Fig. 5k), but not associated with absolute $G_{s50\%}$ in the species analysed (Fig. 5o), indicating that species with large numbers of small stomata did not alter G_s more rapidly than those with low densities of large stomata. Relative values of $G_{s50\%}$ were positively associated with $EP_{\%}$ (Fig. 5l); however, the hypostomatous monocot *F. robusta* diverged from this relationship, exhibiting low $EP_{\%}$ but comparatively a high relative $G_{s50\%}$. A strong positive correlation was observed between absolute values of $G_{s50\%}$ and $EP_{\%}$ across all species examined (Fig. 5p).

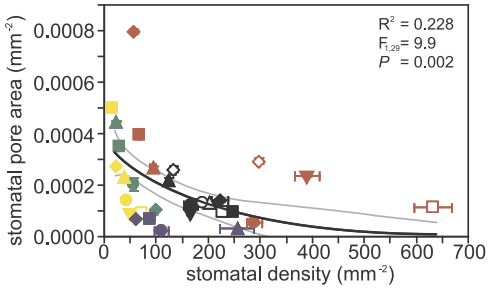

Fig. 4. Relationship between stomatal pore area and stomatal density of the species analysed in this study. The black line indicates a logarithmic best-fit line and the two grey lines either side indicate the 95% confidence intervals of the mean. Non-linear regression was used to assess the significance of any relationship between stomatal pore area and stomatal density. Symbols as in Fig. 2.

The majority of species analysed in this study were either perfectly hypostomatous (all stomata arranged on the abaxial surface) or amphistomatous (half of stomata on the adaxial surface). Three eudicots (*P. nigra*, *C. frutescens* and *H. annuus*) did not distribute stomata evenly over the leaf epidermis, with lower proportions of stomata on the adaxial surface. The relationship between the proportion of stomata on the adaxial surface and absolute $G_{s50\%}$ values (Fig. 6a) followed a rectangular hyperbola. The maximum $G_{s50\%}$ exhibited by a hyposotomatous species was 0.173 mmol m^{-2} s^{-2}, beyond this $G_{s50\%}$ threshold all species were amphistomatous. This pattern was retained but became less robust when the proportion of stomata on the adaxial surface was plotted against relative $G_{s50\%}$ values (Fig. 6b); possibly due to outliers such as the hypostomatous monocot *F. robusta*. A weaker negative relationship was observed between abaxial/abaxial distribution of stomata and $T_{50\%}$ (Fig. 6c). The allocation of the epidermis to stomata was positively related to the proportion of stomata distributed on the adaxial surface (Fig. 6d). Rates of G_s (Fig. 6e) and A (Fig. 6f) under saturating light were positively related to the proportion of stomata on the adaxial leaf surface.

Levels of A and G_s under saturating PAR exhibited positive relationships to G_{sLIGHT}-G_{sDARK} (Fig. 7a and 7f) and negative relationships with $T_{50\%}$ (Fig. 7b and g). Photosynthesis was positively related to both $G_{s50\%}$ (Fig. 7c and d) and $EP_{\%}$ (Fig. 7e); in all cases the response of A followed a rectangular hyperbola, reaching a plateau between 25 and 30 μmol m^{-2} s^{-1}, beyond which any further increase in $G_{s50\%}$ or $EP_{\%}$ was not associated with higher A. Stomatal conductance showed similar relationships, being linearly related to $G_{s50\%}$ (Fig. 7h and i) and $EP_{\%}$ (Fig. 7j) to a level of ~800 mmol m^{-2} s^{-1}. Further increases in $G_{s50\%}$ or $EP_{\%}$ were not associated with greater G_s in any of the species examined. A note of caution must be observed due to the occurrence of self-correlation in the relationships observed between steady-state G_s under saturating PAR and parameters such as G_{sLIGHT}-G_{sDARK} and $G_{s50\%}$ which utilise G_{sLIGHT} values in their calculation.

Principal component analysis of the physiological and morphological stomatal characteristics measured in this study indicated a high degree of overlap in the multi-variate space occupied by ferns, gymnosperms and basal angiosperms (one-way ANOVA with LSD post-hoc test of eigenvalues: component 1, $F_{2,52}$ = 1.197, P = 0.310; component

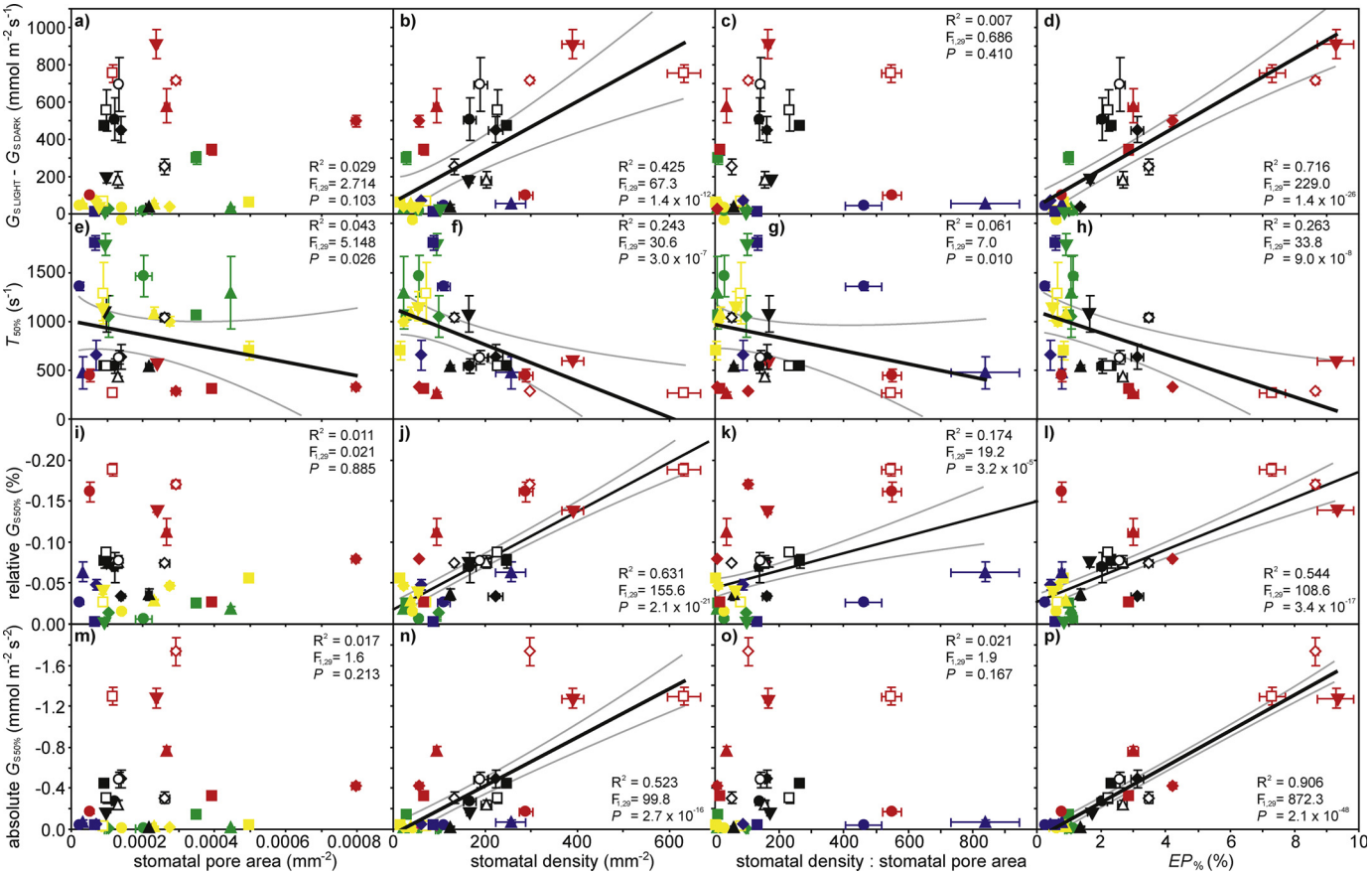

Fig. 5. Relationships between the magnitude of the G_s response (G_{sLIGHT}-G_{sDARK}) time to achieve the initial 50% of the overall G_s response ($T_{50\%}$) and the absolute and relative velocity of stomatal conductance response during a transition from light to dark ($G_{s50\%}$) and stomatal pore area (a, e, I, m), stomatal density (b, f, j, n), ratio of stomatal density to stomatal pore area (c, g, k, o) and the proportion of the epidermis devoted to stomata (d, h, l, p). The black line indicates the line of best fit and the two grey lines either side indicate the 95% confidence intervals of the mean. Linear regression was used to assess the significance of any relationships. Symbols as in Fig. 2.

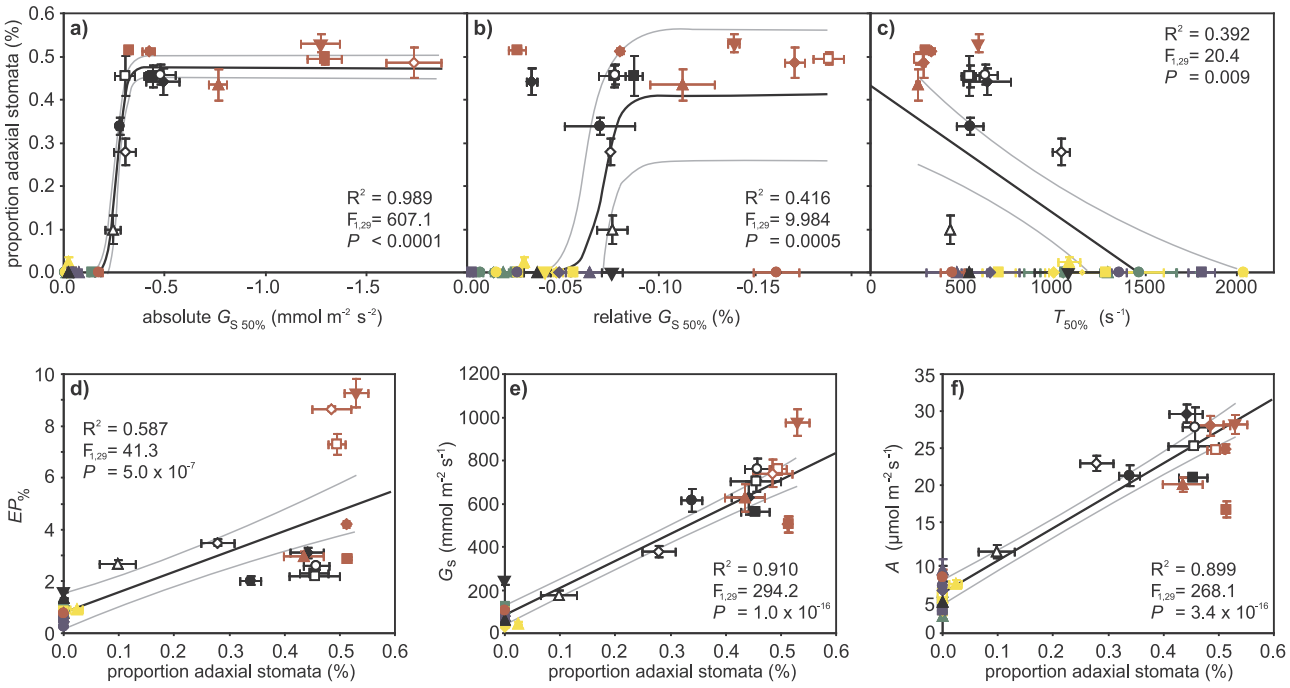

Fig. 6. The relationships between the proportion of stomata distributed on the adaxial surface to absolute $G_{s50\%}$ (a), relative $G_{s50\%}$ (b), $T_{50\%}$ (c), $EP_{\%}$ (d) G_s (e) and A (f). The black line indicates the line of best fit and the two grey lines either side indicate the 95% confidence intervals of the mean. Linear (c, d and e) and non-linear (a and b) regression was used to assess the significance of any relationships. Symbols as in Fig. 2.

2, $F_{2,52} = 1.6$, $P = 0.207$)(Fig. 8). The more recently evolved eudicots and monocots had statistically identical eigenvalues for the first component but not for the second (one-way ANOVA: component 1, $F_{1,66} = 0.172$, $P = 0.682$; component 2, $F_{1,66} = 34.4$, $P = 2.043 \times 10^{-7}$). Moreover, despite partly over-lapping with the space occupied by ferns, gymnosperms and basal angiosperms, the eudicots and monocots occupied significantly distinct multi-variate space (one-way ANOVA: component 1, $F_{1,133} = 133.1$, $P = 6.227 \times 10^{-21}$; component 2, $F_{1,133} = 14.7$, $P = 0.0002$).

4. Discussion

The proportion of the epidermis allotted to stomata is a major factor controlling leaf gas exchange and photosynthetic capacity (Kaiser, 2009). Photosynthesis was positively related to G_s (Fig. 2) in the 31 species studied, with the highest rates of A observed in eudicots and monocots, rather than the basal angiosperm, gymnosperms and ferns (Haworth et al., 2011; McAdam and Brodribb, 2012). Modelling of theoretical maximum G_s on the basis of epidermal micro-morphology often correlates to observed G_s using gas-exchange (Ohsumi et al., 2007; Kaiser, 2009). Scaling relationships based on physical characteristics (eg. Brown and Escombe, 1900; Cowan, 1977) will largely account for the strong correlation between G_s and $EP_{\%}$ found within this study (Fig. 5p). These relationships between leaf micro-morphology (Fig. 5l and p) and gas exchange characteristics (Fig. 7) were also closely related to the physiological ability of stomata to regulate G_s via adjustment of SPA.

The highest $EP_{\%}$ values were found in the amphistomatous eudicots and monocots (Fig. 6d). The capacity to utilise both the abaxial and adaxial leaf surfaces for gas exchange was associated with greater conductance to CO_2 and A (Fig. 3 and 6) (Parkhurst, 1978; Mott et al., 1982). Interference between adjacent stomatal complexes (Parlange and Waggoner, 1970) and structural constraints (Franks and Farquhar, 2007) may prevent hypostomatous species (including hyperstomatous species possessing only adaxial stomata) from achieving equivalent levels of $EP_{\%}$ to those observed in amphistomatous species. Amphistomaty generally occurs in fast growing species in open, high-light habitats where uptake of CO_2 may limit A (Mott et al., 1982; Peat and Fitter, 1994). The results of the present study are consistent with this interpretation (Fig. 6e), but also indicate that a high level of physiological stomatal regulation of G_s is a necessary component of amphistomaty (Fig. 6a). The selective pressures that have led to amphistomaty are complex and multifactorial (Parkhurst, 1978; Muir, 2015, 2018). It is noteworthy that there were comparatively few 'intermediate' species over a narrow range of $G_{s50\%}$ and $EP_{\%}$ values (Fig. 6a), consistent with selective pressures to optimise $G_{s50\%}$ and the proportion of stomata on the adaxial surface resulting in a restricted range of 'optimal' outcomes (Muir, 2015). This would suggest that selective pressures favouring increased $EP_{\%}$ through amphistomaty would also induce increased physiological regulation of SPA. Differential abaxial and adaxial stomatal responses to the same environmental signals in amphistomatous plants (Pospisilova and Solarova, 1980) also likely play a major role in the adaptation of amphistomatous plants to growth in high light environments. The hypostomatous monocot, *F. robusta*, was the exception to this pattern, displaying relative levels of $G_{s50\%}$ consistent with the amphistomatous monocots and eudicots, but lower $EP_{\%}$ (Fig. 6b). The leaves of closely related members of the genus *Fargesia* are amphistomatous (eg. Wang, 2017), raising the possibility that the costs of amphistomaty, such as pathogens and occlusion with water in a humid habitat (see Muir, 2015), have resulted in hypostomaty in *F. robusta*.

A number of studies have suggested that evolutionary patterns in leaf epidermal micro-morphology (Hetherington and Woodward, 2003; Franks and Beerling, 2009; Boer et al., 2016) and stomatal function (Brodribb et al., 2009; Haworth et al., 2011; Elliott-Kingston et al., 2016) have been driven by declining [CO_2] from the Cretaceous to the present. The results of this study (Fig. 3) show that the phylogenetically basal ferns, gymnosperms and basal angiosperms exhibited lower levels of $G_{s50\%}$ and $EP_{\%}$ than the more recently derived eudicots and monocots that originated during periods characterised by lower atmospheric [CO_2] (Haworth et al., 2011; Elliott-Kingston et al., 2016). Nonetheless, there is overlap in the values of A, G_s, $EP_{\%}$, $T_{50\%}$ and $G_{s50\%}$ between the more recently derived eudicot and monocot groups and the more basal lineages (ie. greater $EP_{\%}$ necessitates more rapid adjustment of G_s), suggesting a degree of scaling in these relationships that may preclude

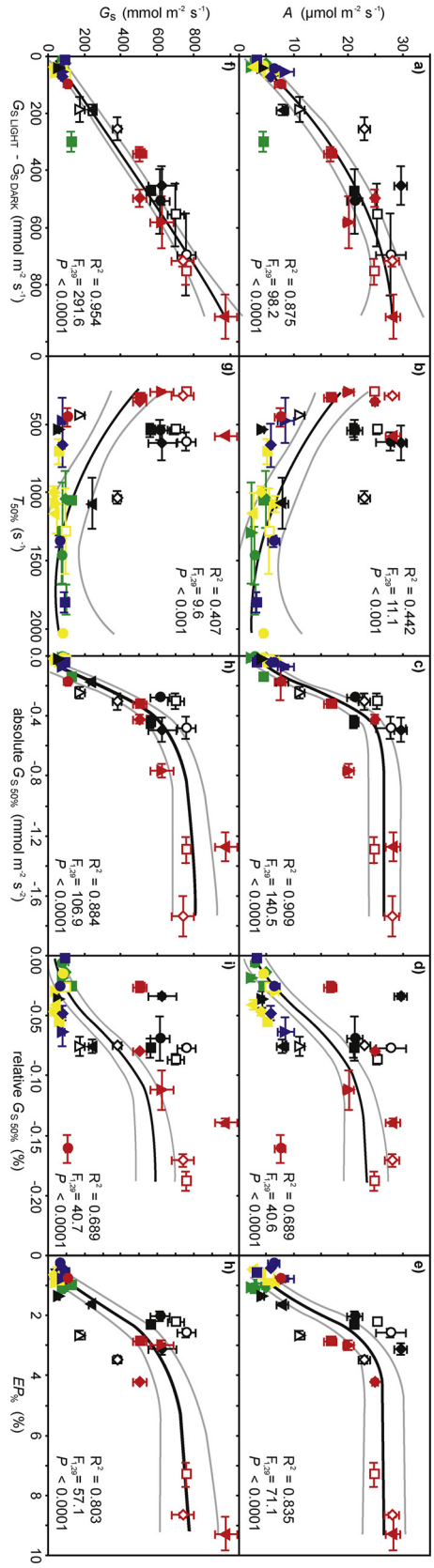

Fig. 7. The relationships between photosynthesis and stomatal conductance with G_{sLIGHT}-G_{sDARK} (a, f), $T_{50\%}$ (b, g), absolute $G_{s50\%}$ (c, h), relative $G_{s50\%}$ (d, i) and $EP_{\%}$ (e, j). The black line indicates a logarithmic line of best fit and the two grey lines either side indicate the 95% confidence intervals of the mean. Non-linear regression was used to assess the significance of any relationship between stomatal pore area and stomatal density. Symbols as in Fig. 2.

phylogenetic separation on the basis of these characteristics (Fig. 3 and 8).

Higher densities of small stomata may facilitate diffusion of CO_2 into the leaf (Boer et al., 2016) and allow for more rapid adjustment of G_s (Giday et al., 2013; Raven, 2014). The SD:SPA ratio and SPA were negatively related to $T_{50\%}$ (Fig. 5e and g), suggesting that smaller stomata can regulate G_s more rapidly. However, these relationships should be treated with caution as they were comparatively weak and were not reflected in the magnitude (G_{sLIGHT}-G_{sDARK}; Fig. 5a and c) or velocity ($G_{s50\%}$; Fig. 5i, m, k and o) of G_s adjustment. Positive relationships have been observed between $G_{s50\%}$ and stomatal size during stomatal opening in five closely related *Banksia* species (Drake et al., 2013) and in the dehydration response of *Rosa hydrida* (Giday et al., 2013), but not during a transition from light to dark conditions in a more diverse range of species (Fig. 5i and m) (Haworth et al., 2015; Haworth et al., 2016). This disparity may reflect the differential mechanical (Franks and Farquhar, 2007) and signalling (Haworth et al., 2016) mechanisms that operate in the stomata of the species represented in the present study. As observed in the genus *Banksia* (Drake et al., 2013), a positive correlation was also found between SD and $G_{s50\%}$ in this study (Fig. 5j and n), suggesting that the number of stomata, and not stomatal size, is the dominant factor in the relationship between the velocity of G_s response and $EP_{\%}$ (Fig. 5p).

The higher $G_{s50\%}$ found in many of the more recently derived eudicot and monocot groups, in comparison to ferns and gymnosperms, may be associated with differential mechanisms of stomatal movement (Franks and Farquhar, 2007). It is noteworthy, that the lowest relative $G_{s50\%}$ values occurred in the ferns, consistent with observations of comparatively slower G_s responses to light and [CO_2] (McAdam and Brodribb, 2012; Haworth et al., 2015; Franks and Britton-Harper, 2016). This may be indicative of a difference in the physiological function between the stomata of fern and seed plants (eg. McAdam and Brodribb, 2012); however, this would not be consistent with genetic, gas exchange and biochemical analyses (Ruszala et al., 2011; Chater et al., 2013). A comprehensive review of the hypotheses regarding the evolution of physiological stomatal function can be found in Franks et al. (2017). Increased $G_{s50\%}$ and lower $T_{50\%}$ (Fig. 3), alongside greater complexity in leaf vein architecture (Roth-Nebelsick et al., 2001), higher rates of water transport in xylem vessels (as oppose to xylem tracheids) (Sperry et al., 2006; Meinzer et al., 2009) and higher responsiveness of leaf hydraulic conductance to light transitions (Xiong et al., 2018) may have enabled greater allocation of the epidermis towards gas exchange in the more recently derived angiosperm groups (Fig. 5p). As [CO_2] declined over the past 100 Myr, increased $EP_{\%}$ would have facilitated diffusion of CO_2 into the leaf (Fig. 7j). Previous gas exchange analyses (Ruszala et al., 2011; Haworth et al., 2013; Haworth et al., 2015; Franks and Britton-Harper, 2016; Hasper et al., 2017) are not consistent with an evolutionary transition from generally passive physiological stomatal behaviours in more ancient plant groups to stomatal behaviours considered to be active in angiosperms (Brodribb et al., 2009; Brodribb and McAdam, 2011; McAdam and Brodribb, 2012). However, the results of this study would suggest that an increase in stomatal functionality, allowing more rapid G_s adjustment and greater allocation of the cuticle to gas exchange may have conferred a selective advantage to more recently derived eudicot and monocot angiosperm groups in terms of greater photosynthetic capacity (Figs. 5p and 7c). This difference in stomatal functionality and epidermal allocation to gas exchange is evident from the principal component analysis presented here (Fig. 8), in which differential groupings of the more recently derived eudicots and monocots, with respect to the more phylogenetically basal groups, were observed. The increased capacity for gas exchange in the more recently derived angiosperm groups may have contributed to their expansion since the Late Cretaceous (Haworth et al., 2011; de Boer et al., 2012). Despite the absence of a clear phylogenetic delineation in active and passive physiological stomatal behaviours between the more recently evolved angiosperm

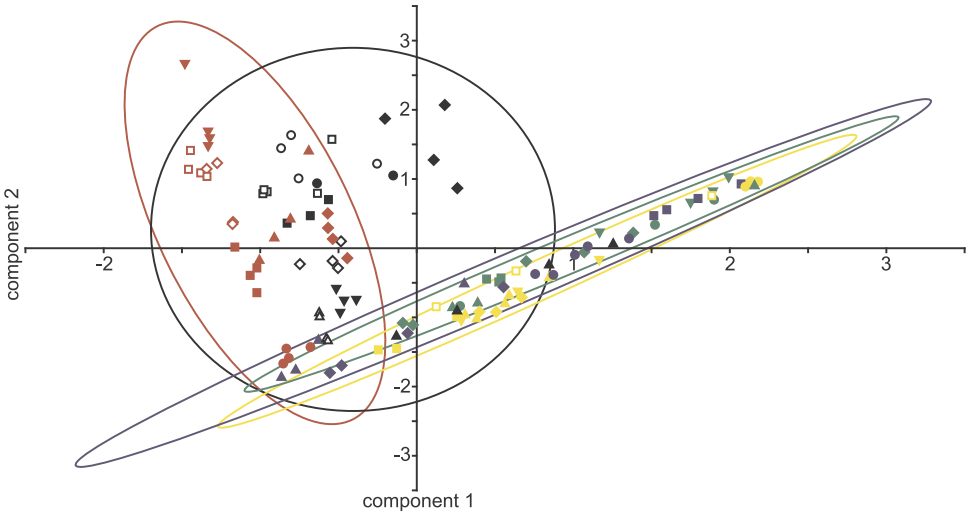

Fig. 8. Principal component analysis of the physiological and morphological stomatal parameters of the species analysed in this study − symbols denoting species are identical to Fig. 2. Ellipses represent 95% confidence intervals for fern (green), gymnosperms (yellow), basal angiosperms (blue), eudicot (black) and monocot (red) species. Statistical analysis indicates that the ferns, gymnosperms and early diverging angiosperms occupy the same position in multi-variate space, while the eudicots and monocots occupy statistically distinct multi-variate space. Component 1 accounts for 82.0% of the variance within the dataset (weighting extractions: $G_s = 0.918$; $EP_{\%} = -0.371$), and component 2 accounts for 15.5% of variance (weighting extraction: $EP_{\%} = 0.896$; SD = 0.203). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

groups and plants with more ancient evolutionary histories, those gymnosperms used in this study with active physiological stomatal behaviours (the cycads and the conifer *N. nagi*: Haworth et al., 2013) altered G_s over a narrower range (Fig. 3e) and more slowly (Fig. 3h) than the more recently evolved eudicots and monocots. The selective advantage possessed by more recently derived angiosperm groups in a 'low [CO_2]' world (ie. < 600 ppm [CO_2] in the context of [CO_2] over the preceding 400 Myr: Berner, 2006) may be the capacity for more rapid stomatal movements, allowing greater $EP_{\%}$ through amphistomaty to sustain A (Fig. 7e), optimise WUE and protect against excessive water-loss when water availability is low (eg. Robinson, 1994) or evapotranspirative demand is high (eg. Schulze et al., 1974). The greater $EP_{\%}$ and $G_{s50\%}$ over a wider range of G_s values in the monocots may also act as a selective advantage in allowing more rapid alteration of G_s to exploit fluctuations in growth conditions over brief time-scales and optimise carbon gain in the short-term. It is noteworthy that leaf-level A does not increase any further beyond an $EP_{\%}$ of 4% (Fig. 7e). This suggests that high rates of carbon gain are restricted on a leaf area basis by diffusion resistance to CO_2-uptake in the mesophyll (eg. Veromann-Jürgenson et al., 2017) or photosynthetic biochemical and/or photochemical capacity (eg. Gu et al., 2014). The fast growing monocots (*P. australis*, *T. latifolia* and *A. donax*) may possess levels of $EP_{\%}$ and $G_{s50\%}$ sufficient to accompany greater mesophyll conductance and improved biochemical efficiency of CO_2 assimilation, whereas the eudicot angiosperms with lower rates of G_s and A may not exhibit sufficient levels of $EP_{\%}$ to cope with enhanced A (eg. Flexas, 2016). A common relationship is observed between the flux of gases and the total surface area of chloroplasts in plants (Evans and Loreto, 2000), suggesting that an increase in light harvesting (Sakowska et al., 2018) may allow for greater gas exchange in the monocots.

Declining [CO_2] over much of the Cretaceous and Tertiary may have favoured species with rapid stomatal movements and higher $EP_{\%}$ (Fig. 5p). However, rising [CO_2] over the past 200 years (Keeling et al., 2005; Monastersky, 2013), and the predicted increases over the coming century (Prentice et al., 2001; Meinshausen et al., 2009), may reduce the influence of selective pressures favouring high $EP_{\%}$ and fine control of G_s. Nevertheless, high G_s is associated with increased yield in C3 crop species (Fischer et al., 1998; Roche, 2015). Attempts to increase food security via modification of A have been restricted by the interconnectivity of photosynthetic biochemistry and other physiological and morphological traits (Flexas, 2016). The results of this study suggest that modification of epidermal patterning to increase $EP_{\%}$ and improved physiological stomatal functionality would be required alongside any modification in the photosynthetic physiology of C3 species. Improved $G_{s50\%}$ may be particularly relevant in the utilisation of drought prone dry-lands in the production of food and biomass crops (Turner, 2004). The fast growing monocots (*P. australis*, *T. latifolia* and *A. donax*) and eudicots (*Chenopodium quinoa*, *Populus nigra* and *Salix alba*) may serve as useful case studies in the analysis of species with G_s responses and epidermal patterning conducive to increased yield.

Author contributions

MH conceived the study. MH, GM, TMGG, CD, MC, JF and CPS conducted the experiment. All authors contributed to the writing of the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.envexpbot.2018.04.010.

References

Assouline, S., Or, D., 2013. Plant water use efficiency over geological time −evolution of leaf stomata configurations affecting plant gas exchange. PLoS One 8, e67757.
Beerling, D.J., Chaloner, W.G., 1993. Evolutionary responses of stomatal density to global CO_2 change. Biol. J. Linn. Soc. 48, 343–353.
Berner, R.A., 2006. GEOCARBSULF: a combined model for phanerozoic atmospheric O_2 and CO_2. Geochim. Cosmochim. Acta 70, 5653–5664.
Boer, H.J., Price, C.A., Wagner-Cremer, F., Dekker, S.C., Franks, P.J., Veneklaas, E.J., 2016. Optimal allocation of leaf epidermal area for gas exchange. New Phytol. 210 (4), 1219–1228.
Brodribb, T.J., McAdam, S.A.M., 2011. Passive origins of stomatal control in vascular plants. Science 331, 582–585.
Brodribb, T.J., McAdam, S.A.M., Jordan, G.J., Feild, T.S., 2009. Evolution of stomatal responsiveness to CO_2 and optimization of water-use efficiency among land plants. New Phytol. 183, 839–847.
Brown, H.T., Escombe, F., 1900. Static diffusion of gases and liquids in relation to the assimilation of carbon and translocation in plants. Philos.Trans. R. Soc. B − Biol. Sci. 193, 233–291.
Byng, J., Chase, M., Christenhusz, M., Fay, M., Judd, W., Mabberley, D., Sennikov, A., Soltis, D., Soltis, P., Stevens, P., 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Bot. J. Linn. Soc. 181, 1–20.
Chater, C., Kamisugi, Y., Movahedi, M., Fleming, A., Cuming, A.C., Gray, J.E., Beerling, D.J., 2011. Regulatory mechanism controlling stomatal behaviour conserved across 400 million years of land plant evolution. Curr. Biol. 21, 1025–1029.
Chater, C., Gray, J.E., Beerling, D.J., 2013. Early evolutionary acquisition of stomatal control and development gene signalling networks. Curr. Opin. Plant Biol. 16, 638–646.
Chen, Z.-H., Chen, G., Dai, F., Wang, Y., Hills, A., Ruan, Y.-L., Zhang, G., Franks, P.J.,

Nevo, E., Blatt, M.R., 2017. Molecular evolution of grass stomata. Trends Plant Sci. 22, 124–139.
Cowan, I.R., 1977. Stomatal behaviour and environment. Adv. Bot. Res. 4, 117–128.
de Boer, H.J., Eppinga, M.B., Wassen, M.J., Dekker, S.C., 2012. A critical transition in leaf evolution facilitated the Cretaceous angiosperm revolution. Nat. Commun. 3, 1221–1232.
Dow, G.J., Berry, J.A., Bergmann, D.C., 2014. The physiological importance of developmental mechanisms that enforce proper stomatal spacing in *Arabidopsis thaliana*. New Phytol. 201, 1205–1217.
Drake, P.L., Froend, R.H., Franks, P.J., 2013. Smaller, faster stomata: scaling of stomatal size, rate of response, and stomatal conductance. J. Exp. Bot. 64, 495–505.
Duckett, J.G., Pressel, S., P'ng, K.M., Renzaglia, K.S., 2009. Exploding a myth: the capsule dehiscence mechanism and the function of pseudostomata in Sphagnum. New Phytol. 183.
Edwards, D., Kerp, H., Hass, H., 1998. Stomata in early land plants: an anatomical and ecophysiological approach. J. Exp. Bot. 49, 255–278.
Elliott-Kingston, C., Haworth, M., Yearsley, J.M., Batke, S.P., Lawson, T., McElwain, J.C., 2016. Does size matter?: Atmospheric CO_2 may be a stronger driver of stomatal closing rate than stomatal size in taxa that diversified under low CO_2. Front. Plant Sci. 7, 1253.
Evans, J.R., Loreto, F., 2000. Acquisition and Diffusion of CO_2 in Higher Plant Leaves, Photosynthesis. Springer, pp. pp p321–351.
Fanourakis, D., Giday, H., Milla, R., Pieruschka, R., Kjaer, K.H., Bolger, M., Vasilevski, A., Nunes-Nesi, A., Fiorani, F., Ottosen, C.O., 2015. Pore size regulates operating stomatal conductance, while stomatal densities drive the partitioning of conductance between leaf sides. Ann. Bot. 115, 555–565.
Fischer, R., Rees, D., Sayre, K., Lu, Z.-M., Condon, A., Saavedra, A.L., 1998. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate and cooler canopies. Crop Sci. 38, 1467–1475.
Flexas, J., 2016. Genetic improvement of leaf photosynthesis and intrinsic water use efficiency in C3 plants: why so much little success? Plant Sci. 251, 155–161.
Franks, P.J., Beerling, D.J., 2009. Maximum leaf conductance driven by CO_2 effects on stomatal size and density over geologic time. Proc. Nat. Acad. Sci. U.S.A. 106, 10343–10347.
Franks, P.J., Britton-Harper, Z.J., 2016. No evidence of general CO_2 insensitivity in ferns: one stomatal control mechanism for all land plants? New Phytol. 211, 819–827.
Franks, P.J., Farquhar, G.D., 2007. The mechanical diversity of stomata and its significance in gas-exchange control. Plant Physiol. 143, 78–87.
Franks, P.J., Berry, J.A., Lombardozzi, D.L., Bonan, G.B., 2017. Stomatal function across temporal and spatial scales: deep-time trends: land-atmosphere coupling and global models. Plant Physiol. 174, 583–602.
Fujita, T., Noguchi, K., Terashima, I., 2013. Apoplastic mesophyll signals induce rapid stomatal responses to CO_2 in *Commelina communis*. New Phytol. 199, 395–406.
Giday, H., Kjaer, K.H., Fanourakis, D., Ottosen, C.-O., 2013. Smaller stomata require less severe leaf drying to close: a case study in *Rosa hydrida*. J. Plant Physiol. 170, 1309–1316.
Gu, J., Yin, X., Stomph, T.J., Struik, P.C., 2014. Can exploiting natural genetic variation in leaf photosynthesis contribute to increasing rice productivity? A simulation analysis. Plant Cell Environ. 37, 22–34.
Hasper, T.B., Dusenge, M.E., Breuer, F., Uwizeye, F.K., Wallin, G., Uddling, J., 2017. Stomatal CO_2 responsiveness and photosynthetic capacity of tropical woody species in relation to taxonomy and functional traits. Oecologia 184, 43–57.
Haworth, M., Elliott-Kingston, C., McElwain, J.C., 2011. Stomatal control as a driver of plant evolution. J. Exp. Bot. 62, 2419–2423.
Haworth, M., Elliott-Kingston, C., McElwain, J., 2013. Co-ordination of physiological and morphological responses of stomata to elevated [CO_2] in vascular plants. Oecologia 171, 71–82.
Haworth, M., Killi, D., Materassi, A., Raschi, A., 2015. Co-ordination of stomatal physiological behavior and morphology with carbon dioxide determines stomatal control. Am. J. Bot. 102, 677–688.
Haworth, M., Killi, D., Materassi, A., Raschi, A., Centritto, M., 2016. Impaired stomatal control is associated with reduced photosynthetic physiology in crop species grown at elevated [CO_2]. Front. Plant Sci. 7, 1568.
Haworth, M., Marino, G., Centritto, M., 2017. The impact of atmospheric composition on the evolutionary development of stomatal control and biochemistry of photosynthesis over the past 450 ma. In: Nuno de la Rosa, L., Müller, G. (Eds.), Evolutionary Developmental Biology: A Reference Guide. Springer, Amsterdam, pp. 1–12.
Haworth, M., Cosentino, S.L., Marino, G., Brunetti, C., Riggi, E., Avola, G., Loreto, F., Centritto, M., 2018. Increased free abscisic acid during drought enhances stomatal sensitivity and modifies stomatal behaviour in fast growing giant reed (*Arundo donax* L.). Environ. Exp. Bot. 147, 116–124.
Hetherington, A.M., Woodward, F.I., 2003. The role of stomata in sensing and driving environmental change. Nature 424, 901–908.
Kaiser, H., 2009. The relation between stomatal aperture and gas exchange under consideration of pore geometry and diffusional resistance in the mesophyll. Plant Cell Environ. 32, 1091–1098.
Keeling, D., Piper, S.C., Bacastow, R.B., Wahlen, M., Whorf, T.P., Heimann, M., Meijer, H.A., 2005. Atmospheric CO_2 and $^{13}CO_2$ exchange with the terrestrial biosphere and oceans from 1978 to 2000: observations and carbon cycle implications – http://www.esrl.noaa.gov/gmd/ccgg/trends/. In: Ehleringer, J.R., Cerling, T.E., Dearing, M.D. (Eds.), A History of Atmospheric CO_2 and Its Effects on Plants, Animals, and Ecosystems. Springer Verlag, New York, pp. 83–113.
McAdam, S.A.M., Brodribb, T.J., 2012. Stomatal innovation and the rise of seed plants. Ecol. Lett. 15, 1–8.
Meinshausen, M., Meinshausen, N., Hare, W., Raper, S.C.B., Frieler, K., Knutti, R., Frame, D.J., Allen, M.R., 2009. Greenhouse-gas emission targets for limiting global warming to 2 °C. Nature 458, 1158–1162.
Meinzer, F.C., Johnson, D.M., Lachenbruch, B., McCulloh, K.A., Woodruff, D.R., 2009. Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. Funct. Ecol. 23, 922–930.
Monastersky, R., 2013. Global carbon dioxide levels near worrisome milestone. Nature 497, 13–14.
Mott, K.A., Gibson, A.C., O'Leary, J.W., 1982. The adaptive significance of amphistomatic leaves. Plant Cell Environ. 5, 455–460.
Muir, C.D., 2015. Making pore choices: repeated regime shifts in stomatal ratio. Proc. R. Soc. B 282 (1813) p. 20151498.
Muir, C.D., 2018. Light and growth form interact to shape stomatal ratio among British angiosperms. New Phytol. 218 (1), 242–252.
Ohsumi, A., Kanemura, T., Homma, K., Horie, T., Shiraiwa, T., 2007. Genotypic variation of stomatal conductance in relation to stomatal density and length in rice (*Oryza sativa* L.). Plant Prod. Sci. 10, 322–328.
Parkhurst, D.F., 1978. The adaptive significance of stomatal occurrence on one or both surfaces of leaves. J. Ecol. 66, 367–383.
Parlange, J.-Y., Waggoner, P.E., 1970. Stomatal dimensions and resistance to diffusion. Plant Physiol. 46, 337–342.
Peat, H.J., Fitter, A.H., 1994. A comparative study of the distribution and density of stomata in the British flora. Biol. J. Linn. Soc. 52, 377–393.
Pospisilova, J., Solarova, J., 1980. Environmental and biological control of diffusive conductances of adaxial and abaxial leaf epidermes. Photosynthetica 14, 90–127.
Prentice, I.C., Farquhar, G.D., Fasham, Goulden, M.L., Heimann, M., Jaramillo, V.J., Kheshgi, H.S., Le Quéré, C., Scholes, R.J., Wallace, D.W.R., 2001. The carbon cycle and atmospheric carbon dioxide. In: Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K., Johnson, C.A. (Eds.), Climate Change 2001: The Scientific Basis. Cambridge University Press, Cambridge, pp. 881.
Raven, J.A., 2014. Speedy small stomata? J. Exp. Bot. 65, 1415–1424.
Robinson, J.M., 1994. Speculations on carbon dioxide starvation, late Tertiary evolution of stomatal regulation and floristic modernization. Plant Cell Environ. 17, 345–354.
Roche, D., 2015. Stomatal conductance is essential for higher yield potential of C3 crops. Crit. Rev. Plant Sci. 34, 429–453.
Roth-Nebelsick, A., Uhl, D., Mosbrugger, V., Kerp, H., 2001. Evolution and function of leaf venation architecture: a review. Ann. Bot. 87, 553–566.
Ruszala, E.M., Beerling, D.J., Franks, P.J., Chater, C., Casson, S.A., Gray, J.E., Hetherington, A.M., 2011. Land plants acquired active stomatal control early in their evolutionary history. Curr. Biol. 21, 1030–1035.
Sakowska, K., Alberti, G., Genesio, L., Peressotti, A., Vedove, G.D., Gianelle, D., Colombo, R., Rodeghiero, M., Panigada, C., Juszczak, R., Celesti, M., Rossini, M., Haworth, M., Campbell, B.W., Mevy, J.-P., Vescovo, L., Cendrero-Mateo, M.P., Rascher, U., Miglietta, F., 2018. Leaf and canopy photosynthesis of a chlorophyll deficient soybean mutant. Plant Cell Environ (in press).
Schulze, E.-D., Lange, O., Evenari, M., Kappen, L., Buschbom, U., 1974. The role of air humidity and leaf temperature in controlling stomatal resistance of *Prunus armeniaca* L. under desert conditions: i a simulation of the daily course of stomatal resistance. Oecologia 17, 159–170.
Sperry, J.S., Hacke, U.G., Pittermann, J., 2006. Size and function in conifer tracheids and angiosperm vessels. Am. J. Bot. 93, 1490–1500.
Turner, N.C., 2004. Agronomic options for improving rainfall-use efficiency of crops in dryland farming systems. J. Exp. Bot. 55, 2413–2425.
Veromann-Jürgenson, L.-L., Tosens, T., Laanisto, L., Niinemets, Ü., 2017. Extremely thick cell walls and low mesophyll conductance: welcome to the world of ancient living!. J. Exp. Bot. 68, 1639–1653.
Wang, S., 2017. Bamboo sheath—A modified branch based on the anatomical observations. Sci. Rep. 7, 16132.
Weyers, J.D.B., Lawson, L.G., 1985. Accurate estimation of stomatal aperture from silicone rubber impressions. New Phytol. 101, 109–115.
Woodward, F.I., 1987. Stomatal numbers are sensitive to increases in CO_2 from pre-industrial levels. Nature 327, 617–618.
Xiong, D., Douthe, C., Flexas, J., 2018. Differential coordination of stomatal conductance, mesophyll conductance, and leaf hydraulic conductance in response to changing light across species. Plant Cell Environ. 41, 436–450.