

# Global convergence in the balance between leaf water supply and demand across vascular land plants

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**Abstract.** Coordination between the density of veins (water supply) and stomata (demand for water) has been found in the leaves of modern angiosperms and also in ferns. This suggests that this coordinated development is not a unique adaptation of derived angiosperms that enables their high productivity. To test this, we compiled leaf vein and stomatal density data from 520 land vascular plant species including derived angiosperms, basal angiosperms, gymnosperms and ferns. We found global coordination across vascular land plants, although the relationships were not significant in gymnosperms and vessel-less angiosperms. By comparing the evolution of xylem conduit elements with variation in the density of veins and stomata and theoretical stomatal conductance among plant lineages, we found that the physiological advantage of modern angiosperms is associated with the emergence of xylem with low intraconduit resistance and leaves with high vein and stomata densities. Thus our results indicate two major events associated with surges in xylem hydraulic capacity in angiosperms: (1) the origin of vessels and (2) the emergence of vessels with simple perforation plates, which diminished physical limitations on stomatal conductance. These evolutionary innovations may have enabled derived angiosperms to be more productive and adaptive to the changing climate.

**Additional keywords:** carbon uptake, hydraulic conductance, leaf photosynthetic rate, transpiration.

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## Introduction

Leaves form the end of the whole-plant hydraulic system and comprise a major part of the resistance to water flow through plants (Sack and Holbrook 2006; Sack and Scoffoni 2013). Thus leaf hydraulic conductance ( $K_{\text{leaf}}$ ) directly correlates with whole-plant hydraulic conductance and leaf photosynthetic rate (Scoffoni *et al.* 2016). Furthermore, plants with derived leaf hydraulic structure, which possess high  $K_{\text{leaf}}$  and thus low hydraulic resistance, typically have high capacity for photosynthesis and transpiration (Brodribb *et al.* 2007, 2010; Scoffoni *et al.* 2016).

Leaf venation forms the water supply network within leaves. Water flows through leaves via two pathways: the vein xylem pathway and the outside-xylem pathway (Sack and Holbrook 2006; Sack and Scoffoni 2013). The outside-xylem pathway is the distance from the end of the vein network

to sites of evaporation near the stomata, which is typically affected by vein positioning, mesophyll anatomy and biochemistry (e.g. aquaporin activity) in angiosperms (Brodribb *et al.* 2007; Scoffoni *et al.* 2016). Specifically, the depth of vein placement and the distance between veins in the leaf determine the distance from the vein to the epidermis and the maximum horizontal distance between the vein and stomata respectively, and directly affect the outside-xylem pathway (Zwieniecki and Boyce 2014). Because of the high resistance of the outside-xylem pathway, decreasing its length reduces the hydraulic resistance of the whole leaf and consequently increases whole-leaf  $K_{\text{leaf}}$  (Sack and Holbrook 2006). For angiosperms with reticular venation, extending vein length is usually associated with reduced outside-xylem pathway length. Thus an angiosperm plant with high vein density (VD;  $\text{mm mm}^{-2}$ ), which is the total vein length per

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leaf area, also tends to have high  $K_{\text{leaf}}$ , stomatal conductance ( $g_s$ ) and photosynthetic rate (Sack and Frole 2006; Brodribb *et al.* 2007, 2010; Boyce *et al.* 2009; McKown *et al.* 2010; Feild *et al.* 2011; Walls 2011).

Stomata are structures in the leaf surface through which water is exchanged for  $\text{CO}_2$ , controlling leaf–atmosphere gas exchange. Because of the strong correlation between  $g_s$  and stomatal density (SD;  $\text{mm}^{-2}$ ; total number of stomata per leaf area), SD is a key parameter for modelling maximum  $g_s$  ( $g_{s,\text{max}}$ ), which indicates leaf potential for C uptake and water loss (McElwain *et al.* 2016). It has been proposed that the physical size of the stomatal pore is one of the major limiting factors for  $g_s$  (de Boer *et al.* 2016).

Plants with high VD and SD, a condition associated with high rates of photosynthesis, have a competitive advantage over other plants in productive environments. However, only derived eudicots have leaves with high VD ( $>5 \text{ mm mm}^{-2}$ ). Leaf VD is lower in basal angiosperms ( $\sim 3.72 \text{ mm mm}^{-2}$ ) and even lower in ferns and conifers ( $\sim 1.59 \text{ mm mm}^{-2}$  and  $1.52 \text{ mm mm}^{-2}$  respectively) (Sack and Scoffoni 2013). The extraordinarily dense venation network of derived angiosperms contributes to their high productivity, which allowed them to outcompete their predecessors and dominate land vegetation (Feild *et al.* 2011; Feild and Brodribb 2013). In agreement with evolutionary patterns in VD, only derived angiosperms have small, densely packed stomata and, consequently, high  $g_s$ . Ferns and gymnosperms tend to have larger and fewer stomata, and lower  $g_s$  (de Boer *et al.* 2016). Recent studies suggest that evolutionary changes in leaf venation and stomata density were correlated with decreases in atmospheric  $\text{CO}_2$  concentrations in the Cretaceous (Franks and Beerling 2009; Brodribb *et al.* 2010; de Boer *et al.* 2012).

Proportional relationships between VD and SD have been observed in tropical lianas, ferns, the *Proteaceae*, the genus *Paphiopedilum*, and trees of tropical and subtropical mountain forests, as well as within fern and angiosperm species grown under different environmental conditions (Brodribb and Jordan 2011; Carins Murphy *et al.* 2012; Zhang *et al.* 2012; Brodribb *et al.* 2013; Ding *et al.* 2014; Zhang *et al.* 2014a, 2014b, 2015a, 2018; Zhao *et al.* 2016, 2017). The correlations between liquid- and vapour-phase conductance predicted by these relationships have been confirmed in woody angiosperms, suggesting that correlation between leaf VD and SD results in balance between leaf demand for water and water supply in plants with similar leaf morphology and anatomy (Brodribb and Jordan 2011; Carins Murphy *et al.* 2016, 2017). This relationship may be a result of geometric relationships with cell size (i.e. the correlation between epidermal cell size and both VD and SD) (Carins Murphy *et al.* 2016; Brodribb *et al.* 2017). However, it is unclear whether this VD–SD correlation exists in all vascular land plant groups. Leaf shape and anatomy (e.g. the structure of the venation network, the type of vein conduit elements and the presence of transfusion tissue) vary among different plant groups. These differences affect the leaf hydraulic pathway and consequently influence the nature of the SD–VD relationship. It is uncertain whether coordination between SD and VD is common to all vascular land plants despite

enormous variation in leaf morphology and anatomy, or whether it is a unique feature of angiosperms to maximise photosynthetic uptake for minimal investment in vein and stomatal infrastructure.

In gymnosperm needle leaves, for instance, VD may be uncorrelated or only weakly correlated with  $K_{\text{leaf}}$  because of unique anatomical features (e.g. the accessory transfusion tissue in gymnosperm leaves may function like an extension of the vein network (Sack *et al.* 2015). This would result in a weaker relationship between VD and SD in gymnosperms. It is also unknown how massive transitions in the physiological and structural properties of basal angiosperm leaves impacted the relationship between VD and SD. Conduits in this group transitioned from tracheids to vessels with scalariform perforation plates and then to vessels with simple perforation plates, suggesting that the latter significantly improved xylem hydraulic efficiency (Feild *et al.* 2009). This evolutionary process is complex, with the gain and loss of vessels occurring independently at least twice in a relatively short time period (Feild and Brodribb 2013). These complex evolutionary patterns may result in weak coordination between SD and VD within basal angiosperms. In fact, in some basal angiosperms, SD is higher than expected with a proportional relationship with VD (Zhang *et al.* 2014b; Zhao *et al.* 2016). This suggests that there may have been asynchronous evolution of VD and SD resulting in a mismatch between water supply and demand for water, or that unique anatomical features in these species contribute more to  $K_{\text{leaf}}$  than VD. Despite this evidence that VD and SD coordination is weak in some basal angiosperms, the relationships between VD and SD in ferns are very similar to those observed among more derived angiosperms (Carins Murphy *et al.* 2017). This implies that VD and SD coordination is not an adaptation unique to derived angiosperms that supports their high productivity.

Instead, it has been proposed that a reduction in within-vein xylem conduit resistance in derived angiosperms enhanced stem hydraulic conductance and enabled increased leaf VD, increasing water supply to leaves and thus photosynthesis (Feild and Wilson 2012; Jansen and Nardini 2014). The strong coordination between VD and SD in derived angiosperms would suggest that increases in VD associated with the appearance of xylem conduits with low individual resistance were accompanied by proportional increases in SD and miniaturisation of the stomata, and thus increases in  $g_{s,\text{max}}$ . However, exactly how the evolution of xylem conduit elements affected SD, guard cell length (GCL) and  $g_{s,\text{max}}$  is unknown. Furthermore, there is also uncertainty about how the evolution of xylem conduit elements affected operational  $g_s$  ( $g_{s,\text{op}}$ ), which represents the  $g_s$  under natural conditions limited by leaf hydraulic supply, usually at some point between approximately zero (closed stomata) and  $g_{s,\text{max}}$  (Franks *et al.* 2014; Simonin and Roddy 2018). By modelling  $g_{s,\text{max}}$  and  $g_{s,\text{op}}$  from VD, SD and GCL for a range of vascular land plants with differing xylem conduit anatomies, we can provide evidence as to whether the evolution of xylem conduit elements in angiosperms is associated with the evolution of small, densely packed stomata (as is the case for venation networks) and,

consequently, elevated  $g_s$ . We hypothesise that the evolution of xylem conduit elements from tracheids to vessels with simple perforation plates in angiosperms is linked with increases in both  $g_{s,max}$  and  $g_{s,op}$  because of increases in VD and SD and decreases in GCL. Thus, VD–SD coordination may be a universal feature of vascular land plants (i.e. it is not linked to the high productivity of derived angiosperms) but the evolution of more efficient xylem conduits is likely to have enabled the high gas exchange capacity of derived angiosperms.

To test these hypotheses, we compiled the VD and SD data of land vascular plants from the literature and our unpublished data. We examined the relationship between SD and VD in vascular land plants to test the hypothesis that VD–SD coordination is unique to derived angiosperms. We then tested the effect of evolutionary changes to xylem conduit elements on VD and SD in land vascular plants, specifically in angiosperms, based on genus-level xylem conduit element types from the InsideWood database. In addition, by modelling both  $g_{s,max}$  and  $g_{s,op}$ , we tested the hypothesis that the evolution of xylem conduit elements was associated with increases in gas exchange capacity. Thus it could be tested whether the evolution of xylem conduit elements with low individual resistance rather than VD–SD coordination *per se* was a unique feature of derived angiosperms that enabled a higher gas exchange capacity.

## Materials and methods

### Data collection

We compiled the data on SD, VD and stomata GCL for vascular land plants from published and unpublished data (Table S1, available as Supplementary Material to this paper). So we could consider the intraspecific physiological plasticity, only data with both VD and SD from a single study were compiled. An average value was used when the data were derived from single studies involving different genotypes or growth conditions for one species.

Stem xylem conduit element data of angiosperms were collected from the Inside Wood database (<http://insidewood.lib.ncsu.edu/>, accessed 22 June 2020) and the book *Atlas of Stem Anatomy in Herbs, Shrubs and Trees* (Schweingruber *et al.* 2011, 2013). Since this database does not cover all known species, xylem conduit elements types were categorised into groups as (1) ferns, (2) gymnosperms and genera of angiosperms that were (3) composed of tracheid-bearing angiosperm species (Tr), (4) angiosperm species bearing vessels with scalariform perforation plates (Sc), (5) angiosperm species bearing vessels with either scalariform or simple perforation plates (Sc–Si), (6) angiosperm species bearing only vessels with simple perforation plates (Si).

In total, our database comprised 520 land vascular plant species, including 461 angiosperms, 13 gymnosperms and 45 ferns (Table S1). Both ANITA clade (*Amborellaceae*, *Nymphaeales*, *Illiciales*, *Trimeniaceae*, and *Austrobaileaceae*) and magnoliids were defined as basal angiosperms.

### Calculation of $g_{s,max}$ and $g_{s,op}$

For each species, we calculated the theoretical  $g_{s,max}$  according to Franks and Beerling (2009):

$$g_{s,max} = \frac{SDa_{max} \frac{d_{H_2O}}{m_v}}{d_p + \frac{\pi}{2} \sqrt{\frac{a_{max}}{\pi}}}, \quad (1)$$

where  $d_{H_2O}$  is the diffusivity of water in the air ( $0.0000249 \text{ m}^2 \text{ s}^{-1}$ ),  $m_v$  is the molar volume of air normalised to  $25^\circ\text{C}$  ( $0.0224 \text{ m}^3 \text{ mol}^{-1}$ ), SD is in  $\text{mm}^{-2}$ ,  $a_{max}$  is the maximum stomatal pore size and  $d_p$  is the depth of the stomatal pore. Following Franks and Beerling (2009), we defined these as follows:

$$a_{max} = \pi \left( \frac{l_p}{2} \right)^2; \quad (2)$$

$$l_p = \frac{GCL}{2}; \quad (3)$$

$$d_p = 0.36 \times GCL. \quad (4)$$

Furthermore,  $g_{s,op}$  was calculated via the following formula (Brodribb *et al.* 2007):

$$g_{s,op} = \frac{K_{leaf} \Psi}{v}, \quad (5)$$

where:

$$K_{leaf} = 12670 d_m^{-1.27}; \quad (6)$$

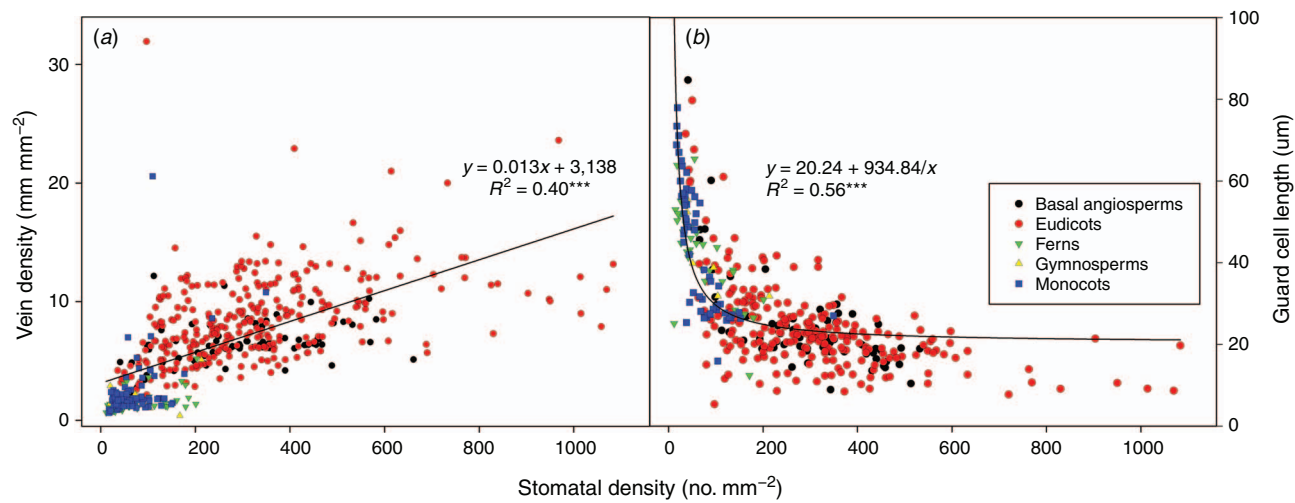
$$d_m = \frac{\pi}{2} \left( d_x^2 + d_y^2 \right)^{\frac{1}{2}}; \quad (7)$$

$$d_x = \frac{650}{VD}. \quad (8)$$

Here,  $K_{leaf}$  is in  $\text{mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$ ,  $d_m$  is the post-vein distance to stomata ( $\mu\text{m}$ ),  $d_x$  is the maximum horizontal distance from the vein to the stomata ( $\mu\text{m}$ ),  $d_y$  is the distance from vein to the epidermis ( $\mu\text{m}$ ),  $\Delta\Psi$  is the water potential difference between the stem and leaf (set to  $0.33 \text{ MPa}$ ) and  $v$  is the vapour pressure deficit (set to  $2 \text{ kPa}$ ). The values of water potential difference and vapour pressure deficit were taken from Simonin and Roddy (2018) to make them comparable. In addition, the distance from vein to the epidermis was set at the median value of each plant group, at  $125 \mu\text{m}$ ,  $292 \mu\text{m}$ ,  $125 \mu\text{m}$ ,  $112 \mu\text{m}$  and  $156 \mu\text{m}$  for ferns ( $n = 30$ ), gymnosperms ( $n = 13$ ), basal angiosperms ( $n = 12$ ), eudicots ( $n = 13$ ) and monocots ( $n = 12$ ) respectively, according to the data of (Zwieniecki and Boyce 2014). Different distances from vein to the epidermis, water potential difference and vapour pressure deficit could influence the modelled value but did change the general trend we tried to assess.

### Data analysis

Pearson's correlation was used to analyse the relationships among leaf traits. We used one-way ANOVA (Tukey's test)



**Fig. 1.** The correlation (a) between leaf stomatal density and vein density and (b) between stomatal density and guard cell length among land vascular plants. Each point represents one species. \*\*\*,  $P < 0.001$ .

to evaluate the differences in each trait among different xylem element groups. All statistical analyses were made in R (version 3.5.1) and figures were drawn with SigmaPlot ver. 12 (Systat Software, Inc.).

Results

We found an overall correlation between leaf SD and VD in vascular land plants (Fig. 1a;  $R^2 = 0.40$ ,  $P < 0.001$ ). Ferns, gymnosperms and monocots had relatively low SD and VD values, with a mean ( $\pm$  s.e.) of  $76 (\pm 57) \text{ mm}^{-2}$  and  $1.8 (\pm 0.91) \text{ mm mm}^{-2}$  in ferns,  $80.47 (\pm 56) \text{ mm}^{-2}$  and  $1.76 (\pm 1.19) \text{ mm mm}^{-2}$  in gymnosperms, and  $69 (\pm 51) \text{ mm}^{-2}$  and  $2.4 (\pm 2.66) \text{ mm mm}^{-2}$  in monocots respectively. In contrast, eudicots had the highest SD and VD, with mean values ( $\pm$  s.e.) of  $333 (\pm 200) \text{ mm}^{-2}$  and  $8.5 (\pm 3.61) \text{ mm mm}^{-2}$  respectively. Basal angiosperms had intermediate values between gymnosperms and eudicots, with a mean SD of  $304 (\pm 143) \text{ mm}^{-2}$  and mean VD of  $6.04 (\pm 1.72) \text{ mm mm}^{-2}$ . Furthermore, a negative correlation between SD and GCL was found in vascular plants (Fig. 1b;  $R^2 = 0.39$ ,  $P < 0.001$ ).

Correlations between VD and SD was found in ferns, angiosperms (including basal angiosperms), and particularly Si species (Table 1), whereas the VD–SD correlation was only marginally significant in Sc and Sc–Si species ( $P = 0.02$  for each group) (Table 1). In contrast, there was no significant correlation between VD and SD within gymnosperms and the Tr species (including some basal angiosperms) ( $P = 0.18$  and  $P = 0.13$  respectively), but the sample sizes of these two groups were small ( $n = 13$  and  $n = 4$  respectively). In addition, all slopes of those correlations were statistically different from each other (Table 1).

In agreement with the evolutionary trend from ferns and gymnosperms to angiosperms, and from Tr to Si species as described above, VD and SD increased significantly, whereas GCL decreased significantly (Fig. 2). The GCL values were significantly lower in the Si, Sc–Si and Sc angiosperm species than in ferns, gymnosperms and Tr angiosperm species,

**Table 1.** The correlation between stomatal density and vein density among plant groups with different xylem conduit elements

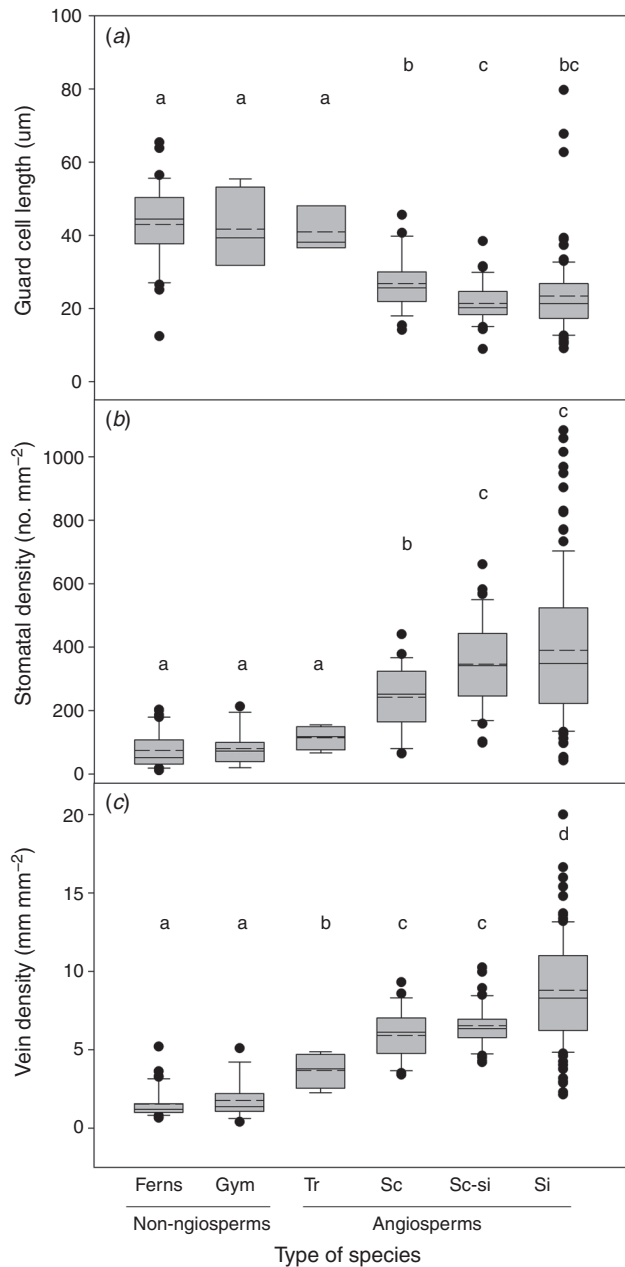
Tr, tracheid-bearing angiosperm species; Sc, angiosperm species bearing vessels with scalariform perforation plates; Sc–Si, angiosperm species bearing vessels with either scalariform or simple perforation plates; Si, angiosperm species bearing vessels with simple perforation plates. Bold  $R^2$  values indicate statistical significance ( $P < 0.05$ )

Type	Slope	Intercept	$R^2$	$N$	$P$
Global	30.78	58.70	<b>0.40</b>	520	<0.001
Ferns	31.88	26.06	<b>0.26</b>	45	<0.001
Gymnosperms	18.52	65.92	0.15	13	0.18
Angiosperms	29.40	72.45	<b>0.11</b>	462	<0.001
Groups of angiosperms					
Basal angiosperms	31.48	99.41	<b>0.14</b>	76	<0.001
Eudicots	24.46	124.32	<b>0.19</b>	305	<0.001
Monocots	9.37	46.62	<b>0.24</b>	81	<0.001
Types of xylem conduit elements in angiosperms					
Tr	29.20	7.27	0.74	4	0.13
Sc	28.40	74.10	<b>0.18</b>	29	0.02
Sc–Si	34.24	122.45	<b>0.11</b>	44	0.02
Si	32.43	103.84	<b>0.26</b>	121	<0.001

although there was no significant variation in GCL among the Sc, Sc–Si and Si species. The SD and VD values were significantly higher in Si, Sc–Si and Sc species than in ferns, gymnosperms and Tr angiosperm species. In addition, the SD values were significantly higher in Si and Sc–Si species than in Sc species but there was no significant difference in SD between Si and Sc–Si species. The Si species had a significantly higher VD value than the Sc and Sc–Si species, though there no significant differences in VD between the Sc and Sc–Si species.

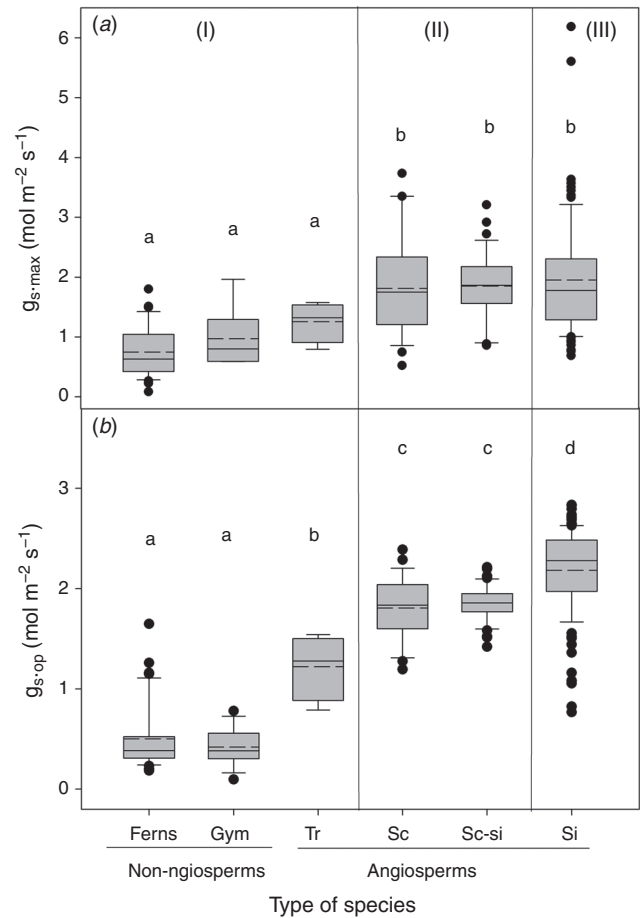
We also found that  $g_{s,max}$  and  $g_{s,op}$  increased along with the evolutionary trend (Fig. 3). Specifically, there was a significant difference in  $g_{s,max}$  between the lineages with vessels and those without vessels but no difference among angiosperms with different perforation plates. Moreover, there were significant





**Fig. 2.** The evolution of xylem conduit elements is associated with a decrease in stomatal size and an increase in stomatal and vein density. Gym, gymnosperms; Tr, tracheid-bearing angiosperm species; Sc, angiosperm species bearing vessels with scalariform perforation plates; Sc-Si, angiosperm species bearing vessels with either scalariform or simple perforation plates; Si, angiosperm species bearing vessels with simple perforation plates. Box plots depict the variation within each lineage, with the bottom and top of the box indicating the 25th and 75th percentiles respectively, the two whiskers representing the 10th and 90th percentiles, the black points represent the outliers, the horizontal line within the box represents the median value, and the dotted line within the box represents the mean value. Different lowercase letters in each panel indicate significant differences among lineages ( $P < 0.05$ ).

differences in  $g_{s,op}$  between lineages with and those without vessels, and also among angiosperms with different



**Fig. 3.** The evolution of xylem conduit elements is associated with an increase in (a) maximum stomatal conductance ( $g_{s,max}$ ) and (b) operational stomatal conductance ( $g_{s,op}$ ). Gym, gymnosperms; Tr, tracheid-bearing angiosperm species; Sc, angiosperm species bearing vessels with scalariform perforation plates; Sc-Si, angiosperm species bearing vessels with either scalariform or simple perforation plates; Si, angiosperm species bearing vessels with simple perforation plates. I, II, and III represent three stages in the evolution of xylem elements. Box plots depict the variation within each lineage, with the bottom and top of the box indicating the 25th and 75th percentiles respectively, the two whiskers representing the 10th and 90th percentiles, the black points indicating the outliers, the horizontal line within the box indicating the median value, and the dotted line within the box indicating the mean value. Different lowercase letters in each panel indicate significant differences among lineages ( $P < 0.05$ ).

perforation plates. Our data suggest three distinct levels in  $g_{s,max}$  and  $g_{s,op}$ : Level I: ferns, gymnosperms and Tr angiosperms with the lowest  $g_{s,max}$  and  $g_{s,op}$ ; Level II, Sc and Sc-Si angiosperms with high  $g_{s,max}$  but medium  $g_{s,op}$ ; Level III, Si angiosperms with high  $g_{s,max}$  and  $g_{s,op}$ .

## Discussion

We found an overall correlation between leaf SD and VD across all groups of vascular land plants, suggesting a coordinated leaf design during plant evolution and providing further evidence that coordination of VD and SD

is not a unique feature of highly productive modern angiosperms (Fig. 1; Table 1). Thus it appears that balancing water supply with demand in leaves to maximise photosynthetic returns for the minimal C cost is broadly selected for in vascular land plants. However, SD and the complexity of vein networks increased in association with evolutionary changes in the xylem conduit elements, with the leaves of derived angiosperms achieving the highest levels of theoretical  $g_s$ . This suggests that less individually resistive xylem conduit elements, along with densely packed leaf veins and stomata, are part of the suite of characteristics that contribute to the high productivity of derived angiosperms.

Our results indicated that two events are associated with enhanced SD and VD in angiosperms, and thus increased gas exchange capacity. The first event (between Levels I and II) was the emergence of vessels in angiosperms. This evolutionary innovation diminished the physical limitation on SD to maintain  $g_{s,max}$ . Specifically, SD,  $g_{s,max}$  and  $g_{s,op}$  are significantly higher in angiosperms with vessels (Sc, Sc–Si and Si) than in nonangiosperms (ferns and gymnosperms) and vessel-less angiosperms (Tr). Although vessels also emerged in some ferns and gymnosperms, their impact on hydraulic performance appears to be limited. For instance, vessel distribution in ferns is often limited to the roots and thus would not necessarily enhance whole-plant hydraulic performance (Carlquist and Schneider 2007). Furthermore,  $K_{leaf}$  is lower in cycads than in vessel-bearing angiosperms (Zhang *et al.* 2015b). The second event (between Levels II and III) was the emergence of vessels with simple perforation plates. This enabled higher VD, which would reduce any potential hydraulic limitation on  $g_{s,op}$  (Simonin and Roddy 2018). The VD and  $g_{s,op}$  are higher in Si species than in the Sc and Sc–Si lineages, indicating that plants with derived xylem conduit elements have a higher potential range of  $g_{s,op}$  by adjusting the stomatal aperture to adapt to short-term environmental changes (McElwain *et al.* 2016). Our result that SD and  $g_{s,max}$  were maximal at Level II and VD and  $g_{s,op}$  were maximal at Level III is consistent with the previous suggestion that increases in VD and SD may not always be synchronised during evolution (e.g. in some basal angiosperms, VD seems to be more evolutionary conservative than SD) (Zhang *et al.* 2014b). Leaf VD and SD of the vessel-bearing gymnosperm *Gnetum montanum* Markgr. (5.1 mm mm<sup>−2</sup> and 212 mm<sup>−2</sup>, respectively) are intermediate between those of the Tr and Sc lineages, identifying the xylem as a transitional feature. This provides further support for our hypothesis that the evolution of xylem conduit elements is associated with changes in leaf VD and SD.

Basal angiosperms underwent the transitional evolution from the first event to the second event described above. Because they include all the angiosperm xylem element groups (Tr, Sc, Sc–Si and Si), transitional characteristics can be detected among them. For instance, they have a relatively high SD combined with a low VD, as described above, which weakens the correlation between the traits (Table 1). Moreover, although the Tr lineages (all basal angiosperms and basal eudicots) have broad reticular leaf vein networks with relatively high VD, their SD is similar

to that of needle-leaf gymnosperms, suggesting that the broad reticular leaf shape may preferentially affect VD (Sack and Scoffoni 2013) or that stomatal porosity increased independently of SD and GCL. Our VD values for basal angiosperms were higher than those published previously (in a study that did not include magnoliids) (Sack and Scoffoni 2013), on average 6.44 vs 3.72 mm mm<sup>−2</sup> respectively; this discrepancy in VD can be explained by the high value in magnoliids. Although the sample size of the ANITA basal angiosperms in this study was relatively limited, the significant difference between ANITA and magnoliids provided additional evidence for assessing the evolution of angiosperms. In gymnosperms, typically conifers, the leaf structure differs from the broad leaves of angiosperms; therefore, leaf water supply–demand balance within this group may be independent of the SD–VD correlation. Similarly, with accessory transfusion tissue extending from the low-resistance pathway-like vein network, the VD of cycads is relatively low despite the more evolved vessel-bearing xylem (Brodribb *et al.* 2010; Sack and Scoffoni 2013).

It has been suggested that decreases in genome size enabled increased VD and SD because limitations to the packing of veins and stomata were relieved by the minimisation of cell size (Simonin and Roddy 2018). Because the stomata size is determined by GCL and correlates with the epidermal size, the GCL represents the approximate cell size (Simonin and Roddy 2018). By using GCL to represent the cell size, we found that the decrease in the cell size only happened in vessel-bearing angiosperms, which have a significantly smaller GCL. In contrast, there was no difference in GCL among vessel-less angiosperms, ferns and gymnosperms. However, to our knowledge, no empirical evidence or theory suggests that decreasing cell size is linked to vessel emergence in angiosperms. It appears more likely that the event that decreased GCL and increase SD depended on the decreasing genome size (Simonin and Roddy 2018). However, without the evolution of xylem conducting elements with lower intraconduit resistance in angiosperms, small conduits such as tracheids or small vessels with scalariform perforation plates could not support very high water demand (Christman and Sperry 2010; Feild and Brodribb 2013). In agreement with this view, Trueba *et al.* (2019) suggested that angiosperms with tracheids and those bearing vessels with scalariform perforation plates have similar xylem-specific hydraulic conductivity. Thus both the decrease in genome size and the evolution of xylem conduit elements in angiosperms could be the structural basis for increasing the VD and SD. Thus, we found that evolutionary changes in stem hydraulic structure (xylem conducting elements) were associated with changes in leaf hydraulic structure (VD and SD), indicating that the evolution of stem and leaf hydraulics was structurally correlated, enabling derived angiosperms to achieve higher  $g_s$  to compensate for declines in CO<sub>2</sub> concentration since the Cretaceous.

In conclusion, global convergence in the coordination between leaf VD and SD was found across vascular land plant lineages, although the relationships were not significant within gymnosperms and vessel-less angiosperm

species. We also found that evolutionary changes in stem hydraulic structure (xylem conducting elements) were associated with changes to leaf hydraulic structure (VD and SD). This suggests that VD–SD coordination is important in all plant groups, though only derived angiosperms have xylem conduits with low individual resistance and small cells that allow very high densities of veins and stomata to be packed into leaves. Thus the evolution of xylem with low intraconduit resistance in angiosperms (and the miniaturisation of leaf cells) is linked with increases in leaf SD and VD and consequently, increases in both  $g_{s,max}$  and  $g_{s,op}$ . Our results indicate there were two major events in angiosperm evolution associated with the surge in plant hydraulic capacity: (1) the origins of vessels and (2) the emergence of vessels with simple perforation plates, which diminished the physical limitation on  $g_s$ . These evolutionary innovations enabled the derived angiosperms to be more productive and adaptive to the changing climate.

### Conflicts of interest

The authors declare no conflicts of interest.

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