Isometric partitioning of hydraulic conductance between leaves and stems: balancing safety and efficiency in different growth forms and habitats

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ABSTRACT

Recent advances in modelling the architecture and function of the plant hydraulic network have led to improvements in predicting and interpreting the consequences of functional trait variation on CO₂ uptake and water loss. We build upon one such model to make novel predictions for scaling of the total specific hydraulic conductance of leaves and shoots (k_L and k_{SH} respectively) and variation in the partitioning of hydraulic conductance. Consistent with theory, we observed isometric (slope=1) scaling between k_L and k_{SH} across several independently collected datasets, and a lower ratio of k_L and k_{SH} , termed the leaf to shoot conductance ratio (C_{LSCR}), in arid environments and in woody species. Isometric scaling of k_L and k_{SH} supports the concept that hydraulic design is coordinated across the plant. We propose that C_{LSCR} is an important adaptive trait that represents the trade-off between efficiency and safety at the scale of the whole plant.

Keywords: Functional trait, hydraulic architecture, leaf conductance, scaling, vascular network

INTRODUCTION

The functional properties of the plant hydraulic system are integral to water and carbon balance (Meinzer et al. 1990; Brodribb et al. 2000; Sperry 2000). This is because water lost through transpiration needs to be replenished via the plant's hydraulic system to ensure that leaves are sufficiently hydrated to allow stomates to open and photosynthesis to proceed. Indeed, hydraulic capacity and photosynthetic capacity are correlated across species (Brodribb et al. 2000). Transpirational flow from soil to leaf (Q_{s-l}) can be described according to the principles of Darcy's law: $Q_{s-l} = k_{\text{plant}} \times \Delta\Psi_{s-l}$, where k_{plant} is the hydraulic conductance of the entire plant and $\Delta\Psi_{s-l}$ is the difference in water potential between the soil and the leaf. For conceptual simplicity, the plant hydraulic system has been described as an analogue of an electrical circuit (van den Honert 1948) typically using resistance, the inverse of conductance, because resistances can be summed in series. Following this convention, one can regard the total plant resistance, the inverse of k_{plant} , as the sum of the root resistance, the stem resistance and the leaf resistance. One can further surmise that as plants increase in size, either within or between species, total root, stem and leaf hydraulic conductance (k_{root} , k_{stem} and k_{leaf} respectively) will increase in a manner that efficiently delivers water to leaves so that Q_{s-l} and CO_2 uptake can be optimised.

Sack et al. (2003) compiled data on the total hydraulic conductance of leaves per unit leaf surface area (leaf-specific conductance, k_L) and the hydraulic conductance of the whole shoot (stems and leaves) per unit of leaf surface area (shoot-specific conductance, k_{SH}) from 34 species and noted that, on average across taxa, k_L was approximately four-fold higher than k_{SH} , irrespective of plant size. Put differently, the ratio of k_L to k_{SH} was 4. The compilation of

Sack et al. (2003) also revealed differences in k_L and k_{SH} on the basis of plant form. Some mature trees, for example, tended to have a lower k_L for a given k_{SH} compared to herbs. In other studies, the ratio of k_L to k_{SH} has been reported as 1.25 in *Cercis siliquastrum* (Nardini et al. 2003), 1.87-2.20 in *Betula pendula* grown over a range of humidities (Sellin et al. 2013) and 2.18 in *Populus x wettsteinii* (Aasamaa, 2014). Combined with the dataset of Sack et al. (2003), these examples illustrate that there is potential for variation in the partitioning of hydraulic conductance to different plant components on the basis of environment, species or functional type.

Despite empirical observations we lack a mechanistic understanding of why the mean ratio of k_L to k_{SH} within a habitat or functional group remains constant across plants of different size.

Moreover, the ecological and morphological factors that influence the partitioning of hydraulic conductance among leaves and stems are not fully understood. It is likely that plant performance during water stress, and in particular to the impact of drought-induced loss of some of the hydraulic capacity, drives differences in the partitioning of hydraulic conductance. As shown by Meinzer (2002), even a 50% loss of stem hydraulic capacity causes a relatively minor decrease in total plant hydraulic conductance, especially if k_{leaf} is low. The ecological benefit of allocating proportionally less hydraulic conductance to leaves than stems becomes apparent when considering the implications of prolonged water stress on survival. In woody perennial plants, the stem hydraulic network is a long term investment (Spicer 2005). Stem xylem conduits produced during one season will affect plant performance in subsequent seasons. Leaves, on the other hand, are a more dispensable component of the plant. By bearing little of the total plant hydraulic conductance, leaves will experience the greatest water potential gradient across the hydraulic network and, if water loss exceeds supply, leaves will be the first organs to experience embolisms, thus buffering

damage to stems (Zimmerman 1978). This has the effect of concentrating the risk of hydraulic failure in the most dispensable above-ground component. For herbaceous annuals, where the turnover of different plant components is similar, it follows that the allocation of hydraulic conductance to leaves will be greater than in co-occurring woody perennial species, although the trends in allocation would hold at the scale of an individual plant.

The patterns of hydraulic conductance observed across the plant broadly reflect the trade-off between efficiency and safety (Tyree et al. 1994; Mencuccini 2002; Sperry et al. 2008). One example of this is the recently described ‘packing rule’ (Sperry et al. 2008) which highlights the trade-off in xylem conduit radius (r_{int}) and the number of xylem conduits per unit of conducting area (N). Thus N is approximately proportional to the inverse of the square of r_{int} , $N \propto r_{\text{int}}^{-2}$. Among different plant species, the concept of efficiency as opposed to safety is illustrated by investment in few wide xylem conduits versus many narrow xylem conduits (Hargrave et al. 1994; McCulloh et al. 2005; Hacke et al. 2006). A few wide conduits will have a greater conductance, but this hydraulic strategy is usually correlated with an increased risk of embolism (Hacke et al. 2006). Many narrow conduits will have a lower risk of embolism, but at the cost of reduced hydraulic conductance.

The packing rule has recently been incorporated into a plant network model that couples internal vascular anatomy to external branching structure (Savage et al. 2010). Savage et al. (2010) built upon the foundational work of West et al. (1999) to predict scaling relationships for numerous aspects of plant form and function in idealized “fractal-like” plants. A core prediction of this model is that hydraulic design is coordinated across different branching orders and within the primary stem. As plants increase in size, for example, so too will the hydraulic conductance of the whole shoot (k_{shoot}), and this increase in overall hydraulic

performance will be matched by proportional increases in the hydraulic conductance of both the primary stem and the terminal twigs. The Savage et al. (2010) model considers the hydraulic flow path from the basal stem to terminal twigs, but does not explicitly consider leaves, beyond predicting that their number is proportional to the number of terminal stems. While its conceptual predecessor, the WBE plant model (West et al. 1999), has been subject to considerable scrutiny, for example, Savage et al. (2008); Price et al. (2010); Price et al. (2012), the Savage et al. (2010) derivative model has received far less attention (Sperry et al. 2012; von Allmen et al. 2012).

THEORY

Here we expand the scope of the Savage et al. (2010) hydraulic model by explicitly considering leaf hydraulic conductance, and by combining and rearranging several relationships from Savage et al. (2010) to arrive at novel predictions. For simplicity, we consider scaling relationships as proportionalities, only explicitly considering proportionality constants (y-intercepts) in our last equation below. Formally, the Savage et al. model predicts that k_{shoot} scales as:

$$k_{\text{shoot}} \propto r_{\text{ext},0}^q \propto M^{\frac{3q}{8}} \quad (1)$$

Where, $r_{\text{ext},0}$ is the radius of the basal stem, M is plant mass and q is an exponent describing the scaling of hydraulic conductance with stem radius (Savage et al. 2010). The model also predicts that the number of leaves (N_{leaf}) scales with plant mass as

$$N_{\text{leaf}} \propto A_L \propto M^{\frac{3}{4}} \quad (2)$$

Total leaf conductance (k_{leaf}) should be equal to the number of leaves multiplied by the hydraulic conductance of the average leaf ($\overline{k_{\text{leaf}}}$),

$$\overline{k_{\text{leaf}}} \times N_{\text{leaf}} = k_{\text{leaf}} \quad (3)$$

Assuming that $\overline{k_{\text{leaf}}}$ does not change as a function of plant size, it can be treated as a constant, and therefore

$$k_{\text{leaf}} \propto M^{\frac{3}{4}} \quad (4)$$

Combining Eq. 1 and Eq. 4 we have:

$$\frac{k_{\text{leaf}}}{k_{\text{shoot}}} \propto \frac{M^{\frac{3}{4}}}{M^{\frac{3q}{8}}} \quad (5)$$

Savage et al. (2010) predict an upper limit of $q=2$ (which means that k_{stem} scales in an isometric fashion with stem cross-sectional area) leading to

$$\frac{k_{\text{leaf}}}{k_{\text{shoot}}} \propto M^0 \quad (6)$$

or alternatively:

$$k_{\text{leaf}} \propto c_{\text{LSCR}} k_{\text{shoot}}^1 \quad (7)$$

which implies independence between the partitioning of hydraulic conductance and plant size and thus isometric scaling between k_{leaf} , k_{stem} and k_{shoot} (refer to Figure 1). The leaf to shoot conductance ratio (C_{LSCR}) is a dimensionless proportionality constant which reflects the relative partitioning of stem and leaf hydraulic conductance as we discuss below. The derivation described above assumes that $q = 2$, the upper bound for this exponent. For trees of a realistic size, q will be less than 2 (Savage et al. 2010). Under these conditions, isometry between k_{leaf} and k_{plant} will require $\overline{k_{\text{leaf}}}$ or C_{LSCR} to vary with plant size (refer to Supporting Information Figure S1). Our empirical investigations do not discount this possibility but for simplicity we compare observed scaling exponents with predictions based on an assumed value of $q = 2$.

HYPOTHESES

The recurring observation in comparative studies of a correlation between xylem function and leaf gas exchange suggests that these traits coevolved (Sperry 2004) and this implies that hydraulic function is at least partially under genetic control (Maherali et al. 2008). Many other studies have demonstrated links between hydraulic performance and environmental variables, both within and across species, for example, Feild et al. (2001); Brodribb et al. (2003); Sack et al. (2005). Thus the partitioning of hydraulic conductance, as reflected in C_{LSCR} , may be under strong selection in different environments, consistent with the definition of an adaptive trait. However, in a common environment, and in similar growth forms/functional types, C_{LSCR} may converge to a similar value across taxa if, by doing so, an optimal solution to surviving water stress while maintaining an adequate k_{shoot} is obtained (refer to Figure 1). Indeed, a recent synthesis by Nardini & Luglio (2014) has suggested that

the trade-off between leaf hydraulic capacity and resistance to drought stress shifts systematically across biomes. In this study we use a collection of species from the *Eucalyptus* genus, and a published dataset on 34 additional species to test three main hypotheses related to the scaling of plant form and hydraulic function: 1) that leaf and plant hydraulic conductance scales in an isometric fashion, consistent with the theory we present in Eqs 1-7; 2) that allometric relationships between leaf area, shoot mass and basal stem radius are also consistent with this theory, and 3) that species from more arid environments will partition less hydraulic conductance to leaves and thus exhibit a lower C_{LSCR} . Although our study design lends itself to an analysis of hydraulic traits at the species or genus level, our focus here is to explore interspecific patterns as a means of testing the potential for general hydraulic scaling relationships across taxa.

MATERIAL AND METHODS

Plant material

Twelve eucalypt species ($n = 5$ for each) were grown from seed in pots (diameter = 12 cm) filled with an organic potting mix. After 3 months the seedlings were transferred to larger pots (diameter = 20 cm) with the void filled with coarse river sand. The seedlings were grown in a shade house and were well watered throughout development. The species were as follows: *Eucalyptus accedens* W.Fitzg., *Eucalyptus albida* Maiden & Blakely, *Eucalyptus caesia* Benth., *Eucalyptus diversicolor* F.Muell., *Eucalyptus gardneri* Maiden, *Eucalyptus gomphocephala* DC., *Eucalyptus incrassata* Labill., *Eucalyptus longicornis* (F.Muell.) Maiden, *Eucalyptus marginata* Sm., *Eucalyptus salicola* Brooker, *Eucalyptus salubris* F.Muell. and *Eucalyptus wandoo* Blakely. The species were deliberately chosen to originate

from a variety of natural habitats characterised by a range in annual average rainfall of approximately 300 mm for *E. salicola* to 960 mm for *E. diversicolor* (Nix 1986). This range in rainfall extends the dataset for the hydraulic conductance of leaves and shoots to plants originating from arid conditions.

Hydraulic properties

Hydraulic conductance (k) was determined with a high pressure flow meter (HPFM, Dynamax Incorporated, Houston, USA, (Tyree et al. 1995)) filled with a solution of degassed 0.01 M KCl in ultra-pure water. Measurements were taken on two to three plants per day between approximately 08:00 and 13:00. Plants were kept well-hydrated prior to measurements. For each plant, the shoot was excised from the root system approximately 20 mm from the soil surface and re-cut while immersed in water. After removing a cylinder of bark approximately 10 mm long, the shoot was fitted to the HPFM and positioned under a sodium lamp which illuminated the plant with a quantum flux density of approximately 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Sack et al. 2002; Sack et al. 2003). A clear Perspex shield was positioned below the lamp which reduced the radiant heat by around 50%. Throughout measurements, the temperatures of the shoot and ambient air were monitored with a thermocouple. The shoot temperature remained within 3°C of ambient air temperature during measurements. All measurements were made on well-hydrated plants and since water exuded from stomates in all cases, we assume that water potentials were near zero. Despite illuminating leaves, it is unlikely that evaporation from the lamina surface would have decreased leaf water potentials, and thus the pressure drop across the plant equalled the HPFM pressure. As in Sack et al. (2002), we allowed each shoot to reach equilibrium, defined as the variation in k being $\pm 5\%$ for 5 minutes.

The hydraulic conductance of the whole shoot, stems + leaves, (k_{shoot} , kg water s⁻¹ MPa⁻¹) was determined in the steady-state mode of the HPFM. Stable readings were obtained after logging data for between 10 and 40 minutes at 8 second intervals. Leaf laminae were then excised from the shoot and the hydraulic conductance of the entire stem network, including petioles if present, (k_{stem} , kg water s⁻¹ MPa⁻¹) was measured in the same manner within 5 - 30 minutes. After removing the shoot from the HPFM, stem radius ($r_{\text{ext},0}$, mm) at the base (bark removed) was measured using digital callipers.

Measurements of k were corrected for shoot temperature following Tyree et al. (1995) to account for changes in the viscosity of water. A measure of hydraulic conductance of all leaves, leaf blades minus petioles, (k_{leaf} , kg water s⁻¹ MPa⁻¹) integrated over the entire shoot was then derived as

$$k_{\text{leaf}} = 1 \div \left(\frac{1}{k_{\text{shoot}}} - \frac{1}{k_{\text{stem}}} \right) \quad (8)$$

For each plant, 5-15 leaf blades were collected and their images taken at a resolution of 300 dpi using a dual-lens scanner (Model V700, Seiko Epson Corporation, Nagano, Japan) to determine the leaf surface area of the subsample (A_1 , cm²) and an average area per leaf ($\bar{A}$, m²) using Image J (National Institute of Health, USA). The subsample of leaves was then oven-dried at 70°C and the dry weight (DW) obtained. The remaining leaves were also oven-dried at 70°C and their DW measured. Leaf mass per unit surface area (LMA, g cm⁻²) of the leaf subsample was calculated, and used to estimate the area of leaves for the entire plant (A_L , m²). The total number of leaves present on each plant (N_{leaf}) was estimated as $A_L/\bar{A}$.

The total leaf-specific hydraulic conductance (k_L , $\text{kg s}^{-1} \text{MPa}^{-1} \text{m}^{-2} \text{leaf}$) was calculated as

$$k_L = \frac{k_{\text{leaf}}}{A_L} \quad (9)$$

Similarly k_{shoot} was scaled to A_L to yield the total shoot-specific conductance (k_{SH} , $\text{kg s}^{-1} \text{MPa}^{-1} \text{m}^{-2} \text{leaf}$)

$$k_{\text{SH}} = \frac{k_{\text{shoot}}}{A_L} \quad (10)$$

and the average leaf hydraulic conductance per leaf ($\overline{k_{\text{leaf}}}$, $\text{kg s}^{-1} \text{MPa}^{-1} \text{leaf}^{-1}$) estimated as

$$\overline{k_{\text{leaf}}} = \frac{k_{\text{leaf}}}{N_{\text{leaf}}} \quad (11)$$

Terminology and statistical analyses

A central goal of our study was to compare elements of the Savage et al. (2010) model, and of our model expansion, with empirical observations. Some clarification of terminology is required to accomplish this. Firstly, while Savage et al. (2010) refers to k_{plant} for the stem component of plant conductance, we use the terms k_{shoot} and k_{leaf} so as to distinguish shoot and leaf conductance components. Additionally, although k_{leaf} in our study did not include the hydraulic contribution of petioles, we assume that it is a close approximation of the leaf hydraulic conductance with intact petioles (Nardini et al. 2003). Similarly plant mass (M) is

presented here as shoot DW. Furthermore, to enable direct comparison with hydraulic data presented in the literature, for example Sack et al. (2003), we explore the potential for isometric scaling and variation in C_{LSCR} in k_L and k_{SH} rather than k_{leaf} and k_{shoot} . Given that both k_L and k_{SH} are derived by dividing by A_L , the scaling relationships would not differ from those obtained from analysis of k_{leaf} and k_{shoot} .

Linear regression analysis was performed using Origin Pro version 9.1 (OriginLab Corporation, Northampton, USA) and R version 3.0.2 (R Development Core Team 2013). Data were log-transformed prior to analysis to meet standard regression assumptions. We used type 2 (standard major axis, SMA) regression analysis to define linear trends between variables. Differences in the slope and intercept of various datasets were assessed via likelihood ratio tests (LRTs) and Wald tests respectively using the SMARTR package in R version 3.0.2 (Warton et al. 2006). For comparison to our study, published data were obtained using the plot digitizer function of Origin Pro version 9.1.

RESULTS

Hydraulic scaling with plant size

The hydraulic conductance of the whole shoot (k_{shoot}) was positively correlated with the radius of the stem base ($r_{ext,0}$) (Figure 2) and the slope of the linear model describing the correlation, equivalent to q in Eq 1, was 1.58 which conforms to the prediction of the model presented by Savage *et al.* (2010) (Table 1). The raw data for this and subsequent correlations can be found in Supporting Information Table S1.

[Insert Table 1 near here]

Scaling of total leaf surface area (A_L), $r_{\text{ext},0}$, k_{shoot} and the leaf hydraulic conductance (k_{leaf}) with shoot dry weight (Shoot DW) (Figure 3a-d) was broadly consistent with the Savage et al. model (Table 1). We observed scaling exponents of 1.02 and 0.46 for A_L versus Shoot DW and $r_{\text{ext},0}$ versus Shoot DW respectively, and exponents of approximately 0.75 for the correlations between k_{leaf} and k_{shoot} with Shoot DW. The average leaf hydraulic conductance per leaf ($\overline{k_{\text{leaf}}}$) was invariant with Shoot DW (Figure 3e). Similarly, we found that the ratio of k_{leaf} to k_{shoot} was statistically invariant with Shoot DW (data omitted).

Partitioning of hydraulic capacity in the plant

Bigger plants (greater shoot DW) had a higher total hydraulic conductance of leaves (k_{leaf}) and hydraulic conductance of the whole stem (k_{stem}) (Figure 4a). The slope of the model presented in Figure 4a is close to 1. k_{leaf} and A_L were also strongly correlated (Figure 4b, Table 1). The slope describing this relationship (0.75) differs significantly from the results of Simonin et al. (2012) (LRT, $P < 0.05$). In the latter study, the slope between k_{leaf} and A_L was not different from 1 (refer to Figure 4b for a visual comparison) which is consistent with the implicit assumption of Savage et al. (2010).

A frequency histogram of the ratio of k_{leaf} to k_{stem} showed a skewed distribution that could be described by a lognormal model (Figure 4c). The median value indicated by the model distribution was 0.5. In other words, k_{leaf} in these eucalypts was most commonly 50% of k_{stem} .

The specific conductance of leaves (k_L) was positively correlated with k_{SH} ($r^2 = 0.92$, $P < 0.001$, Figure 5, Table 1). As expected, all data populated the geometric space above the 1:1 line such that k_L was larger than k_{SH} across the entire range of measurements. The correlation between k_L and k_{SH} exhibited a scaling exponent of 1 which is consistent with isometry (refer to Table 1). Overlaid on Figure 5 are the data compiled by Sack et al. (2003) for herbs, woody seedlings and saplings and mature trees from wetter environments in the northern hemisphere. A statistically common slope of one was observed in both data sets (LRT, $P > 0.05$) although the y-intercept of the data presented by Sack et al. (2003) is significantly greater than our data (Wald test, $P < 0.05$), indicating that, for a given k_{SH} , k_L was lower in our study. Our data resemble those of mature trees rather than herbs or woody seedlings and saplings. For comparison, we have included the regression analysis of each plant form presented in Figure 5 (refer to the legend in the figure) and note that, with the exception of the comparison between our dataset and the published values for woody saplings and seedlings, the slope of each model was not significantly different (LRT, $P > 0.05$). The y-intercept of log-transformed data varied from $0.19 \text{ kg s}^{-1} \text{ MPa}^{-1} \text{ m}^{-2}$ in our study to $1.63 \text{ kg s}^{-1} \text{ MPa}^{-1} \text{ m}^{-2}$ for Sack et al.'s woody seedlings and saplings. Wald tests revealed that the y-intercept differed significantly between our data and both herbs and woody seedlings and saplings ($P < 0.05$). Although there was no statistical difference in the y-intercept of our data and trees, the P -value for this comparison was 0.08 and variation in the tree data was such that the linear model describing the relationship between k_L and k_{SH} was not significant. Within the Sack et al. (2003) dataset, Wald tests revealed a significant difference in the y-intercept of mature trees and woody seedlings and saplings ($P < 0.05$). The variation in the y-intercept of Figure 5 represents differences in C_{LSCR} (Eq. 7), a measure of the relative partitioning of hydraulic conductance between leaves and the whole plant.

Within the eucalypt dataset, the value of C_{LSCR} observed at the species level showed a slight, but insignificant, positive trend with the average annual rainfall associated with the natural distribution of each species (data not shown, $P > 0.05$).

DISCUSSION

Consistent with our expectations, k_L scaled isometrically with k_{SH} . This relationship has been shown before empirically (Sack et al. 2003), but has not been linked with a mechanistic model of conductance partitioning as we have done here. Also consistent with expectations, the y-intercept for our data was lower than that of Sack et al. (2003) (Figure 5), suggesting the potential for variation in the allocation of hydraulic conductance in different habitats as reflected in C_{LSCR} . We also observed positive scaling of k_{leaf} with A_L but in this instance the slope of the model describing the relationship was less than one, suggesting a diminishing return on leaf hydraulic conductance for an increased investment in leaf surface area. We discuss the underlying principles of isometric scaling of hydraulic properties and put forward a rationale for variation in the allocation of hydraulic resistance on the basis of plant functional diversity.

Scaling relationships

In accordance with empirical observations (Cannell 1982; Price et al. 2007) and theory (West et al. 1999; Enquist et al. 2002; Price et al. 2007), both $r_{ext,0}$ and A_L scaled allometrically with Shoot DW (Table 1). The mass allometry component of the Savage et al. (2010) model is based in part on the ‘elastic similarity’ model (McMahon 1973), which recognizes the necessity for an architectural safety margin to prevent trees from buckling under their own

weight. Our estimated scaling exponent for the relationship between $r_{\text{ext},0}$ and Shoot DW is 0.46, which is higher, but close to the expectation for an elastically similar column (0.375) as predicted by McMahon (1973) and incorporated into the Savage et al. (2010) model. We also observed a scaling exponent for k_{shoot} versus $r_{\text{ext},0}$ (exponent q) of 1.58 which is less than the theoretical maximum of 2 predicted by the Savage et al. (2010) model.

The scaling exponent for k_{shoot} and Shoot DW was 0.72. For an infinite tree size the Savage et al. 2010 model predicts a scaling exponent of 0.75 for these variables. For finite size trees, the exponent will depend on the species-specific relationship between k_{shoot} and Shoot DW, which has been observed to range from 0.62 to 0.72 (Savage et al. 2010). Since k_{shoot} will potentially limit CO_2 uptake, the scaling exponent observed here is a likely reflection of the constraints on plant growth rate imposed by metabolic rate, which is limited by hydraulic efficiency (West et al. 1999; Savage et al. 2010). Although our study is comprised of related species, it appears that coordinated design of the plant hydraulic network to match metabolic demand is a hallmark for plant selection in different species across a range of environments (West et al. 1999; Price et al. 2007; Savage et al. 2010).

An increase in k_{SH} , the total specific hydraulic conductance of the above-ground plant, resulted in a proportional increase in the total specific hydraulic conductance of leaves, k_{L} , thus conforming to the prediction of an approximately isometric scaling relationship. Our model shows how isometric scaling between k_{L} and k_{SH} will manifest given the assumptions of the Savage et al. model (refer to Eqs 1 to 7), which are largely consistent with our experimental data. One key assumption of our model (to demonstrate isometric scaling) is that k_{leaf} is independent or “invariant” of Shoot DW. Recent analysis of a global compilation of leaf functional trait data has shown that most leaf functional traits do not vary

systematically as a function of interspecific plant size (Price et al. in press). Indeed, 95% confidence intervals for this relationship in our data include the assumed value of 0 (Table 1), although to achieve isometry when $q < 2$, $\overline{k_{\text{leaf}}}$ may need to vary with Shoot DW (see below).

Our observed value of 1.58 for q (from the relationship between k_{shoot} and $r_{\text{ext},0}$) is less than our predicted value of 2 for isometry in a tree of infinite size. Within the context of our theoretical treatment, for isometry to persist when q is less than 2 either $\overline{k_{\text{leaf}}}$ or C_{LSCR} must change with tree mass (refer to Supporting Information Figure S1). There was a slight negative trend in the relationship between $\overline{k_{\text{leaf}}}$ and Shoot DW, but not statistically different than zero. It is possible that larger trees have proportionally more leaves that experience suboptimal conditions (see below) and this would have the effect of lowering $\overline{k_{\text{leaf}}}$. The fact that our empirical value of q is lower than that assumed, yet the scaling of k_L with k_{SH} remains isometric suggests that either the underlying theory or empirical data are flawed or incomplete. While we have pointed to several ways in which this issue might be reconciled (i.e. if $\overline{k_{\text{leaf}}}$ or C_{LSCR} vary with mass), future work is needed to determine the source of this inconsistency.

Although our dataset covers a relatively narrow range in k_{leaf} and A_L , it is intriguing that, unlike the study of Simonin et al. (2012), the cost of building more leaf area did not lead to an isometric increase in total leaf hydraulic conductance. The network of veins within a leaf, comprised of vessel bundles with xylem conduits, represents the distal component of the plant transpiration stream as well as a mechanical framework for leaf structure: this network provides water to tissue resulting in the formation of a hydrostatic scaffold through the maintenance of turgor (Niklas 1999; Sack et al. 2003; Sack & Frole 2006). A diminishing

hydraulic return with increased investment in leaf area may be associated with a reduction in photosynthetic carbon fixation per unit leaf area and per unit of carbon invested. This trend matches the well-known decrease in relative growth rate with increasing plant size (Lambers and Poorter 1992). As plants increase in size and their canopies become denser, an increasing proportion of the leaf population experiences suboptimal conditions for photosynthesis and transpiration. Such leaves tend to be older (Reich et al. 2009) and have lower photosynthetic capacities due to biochemical and structural acclimation (Niinemets 2007). As these leaves experience lower irradiation, lower vapour pressure deficits and lower temperatures they presumably do not require the same hydraulic support capacity (Sack et al. 2005; Brodribb et al. 2008; Markesteijn et al. 2011).

A rationale for the partitioning of hydraulic capacity across the plant

When viewed in conjunction with the data of Sack et al. (2003), it is apparent that the C_{LSCR} can differ on the basis of plant functional grouping. In other words, the partitioning of hydraulic conductance to leaves is a potentially adaptive trait that underscores the performance of species in a given environment. In our study a frequency histogram of the ratio of k_{leaf} to k_{stem} indicated that k_{leaf} was frequently 50% of k_{stem} . By allocating little of the total shoot hydraulic conductance to leaves, plants may ensure that stems are spared from potentially damaging water potentials arising from excessive water loss during drought (Zimmerman 1978).

From the perspective of hydraulic segmentation, the benefits of allocating less of the total shoot hydraulic conductance to leaves would depend on the relative vulnerability of different regions of the plant to embolisms. Take, for example, a plant where 80% of k_{shoot} was

allocated to stems with the remaining 20% allocated to leaves. If vulnerability to embolism was much greater in stems than in leaves, drought stress would reduce k_{stem} prior to a drop in k_{leaf} , meaning that the allocation of less hydraulic conductance to leaves would do little to spare stems from hydraulic failure. However, empirical observations suggest that leaves are usually more vulnerable to embolism than stems (Hao et al. 2008; Johnson et al. 2011).

We observed that plants from the eucalypt genus, originating from dry environments, partitioned more resistance to leaves than plants from wetter northern hemisphere habitats (Figure 5). Most of the hydraulic flow path of plants is characterised by bulk flow through xylem conduits which allows for a relatively high hydraulic conductance. Upon reaching the distal component of the xylem network in leaves, in the mesophyll, the rate of water flow diminishes (Cowan 1977). Leaf structural traits such as the distance between mesophyll cells and stomata, mesophyll porosity and cell wall properties influence both the resistance to water flow from xylem to evaporative surfaces and the resistance to CO_2 diffusion from stomates to mesophyll cells (Flexas et al. 2013). Because of the shared pathway of transpirational water loss and photosynthetic carbon gain, any increase in leaf hydraulic resistance will potentially incur a penalty of lower CO_2 uptake. Selection for greater hydraulic resistance in leaves must therefore reflect an adaptation to surviving arid environments, and evidence for this has recently emerged (Nardini & Luglio 2014; Pivovarov et al. 2014). The ratio of k_L and k_{SH} , i.e. C_{LSCR} , observed in this study and elsewhere may be an important trait that represents the trade-off between hydraulic efficiency for increased CO_2 uptake versus hydraulic safety to ensure survival during water stress. Although we did not detect a significant correlation between C_{LSCR} and rainfall in the eucalypts studied here, evidence of a weak trend between these parameters warrants future research using a larger dataset.

Within a habitat, plant form may influence the partitioning of hydraulic conductance. In herbaceous annuals, for example, one would predict that rules governing hydraulic segmentation would be relaxed and more of the total shoot hydraulic conductance would be allocated to leaves, and this is consistent with our empirical observations. This is because the term of investment in both leaves and stems is similar in this growth form, and these short-lived species are less subject to seasonal changes in water availability. Hydraulic segmentation would also suggest that the stem network of large woody plants requires more buffering from water stress than small woody plants, and this conforms to our observation of a lower C_{LSCR} in woody trees compared to herbs and woody seedlings and saplings.

Conclusions

After rearranging the model of Savage et al. (2010) we successfully demonstrate its utility in predicting interspecific hydraulic scaling relationships across our combined datasets. We also highlight the potential for variation in the allocation of hydraulic conductance to leaves as a function of total plant hydraulic conductance, represented by C_{LSCR} , which is predicted to vary systematically with aridity. We argue that this important adaptive trait reflects the trade-off between hydraulic efficiency and safety between the stems and leaves of plants, within and across habitats.

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REFERENCES

Aasamaa K., Koivik K., Kupper P. & Sober A. (2014). Growth environment determines light sensitivity of shoot hydraulic conductance. *Ecological Research*, 29, 143-151.

Brodribb T.J. & Feild T.S. (2000) Stem hydraulic supply is linked to leaf photosynthetic capacity: evidence from New Caledonian and Tasmanian rainforests. *Plant, Cell and Environment* 23, 1381-1388.

Brodribb T.J. & Holbrook N.M. (2003) Changes in leaf hydraulic conductance during leaf shedding in seasonally dry tropical forest. *New Phytologist* 158, 295-303.

Brodribb T.J. & Jordan G.J. (2008) Internal coordination between hydraulics and stomatal control in leaves. *Plant, Cell and Environment* 31, 1557-1564.

Cannell M.G.R. (1982) *World Forest Biomass and Primary Production Data*. Academic Press, London.

Cowan I.R. (1977) Stomatal behaviour and environment. *Advances in Botanical Research* 4, 117-228.

Enquist B.J. & Niklas K.J. (2002) Global allocation rules for patterns of biomass partitioning in seed plants. *Science* 295, 1517-1520.

Feild T.S., Brodribb T., Jaffré T. & Holbrook M.N. (2001) Acclimation of leaf anatomy, photosynthetic light use, and xylem hydraulics to light in *Amborella trichopoda* (Amborellaceae). *International Journal of Plant Sciences* 162, 999-1008.

Flexas J., Scoffoni C., Gago J. & Sack L. (2013) Leaf mesophyll conductance and leaf hydraulic conductance: an introduction to their measurement and coordination. *Journal of Experimental Botany* 64, 3965-3981.

Hacke U.G., Sperry J.S., Wheeler J.K. & Castro L. (2006) Scaling of angiosperm xylem structure with safety and efficiency. *Tree Physiology* 26, 689-701.

Hao G.Y., Hoffmann W.A., Scholz F.G., Bucci S.J., Meinzer F.C., Franco A.C., Cao K.F. & Goldstein G. (2008) Stem and leaf hydraulics of congeneric tree species from adjacent tropical savanna and forest ecosystems. *Oecologia* 155, 405-415.

Hargrave K.R., Kolb K.J., Ewers F.W. & Davis S.D. (1994) Conduit diameter and drought-induced embolism in *Salvia mellifera* Greene (Labiatae). *New Phytologist* 126, 695-705.

Johnson D.M., McCulloh K.A., Meinzer F.C., Woodruff D.R. & Eissenstat D.M. (2011) Hydraulic patterns and safety margins, from stem to stomata, in three eastern US tree species. *Tree Physiology* 31, 659-668.

Lambers H. and Poorter H. (1992) Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Advances in Ecological Research* 23, 187-261.

Accepted Article
Maherali H., Sherrard M.E., Clifford M.H. & Latta R.G. (2008) Leaf hydraulic conductivity and photosynthesis are genetically correlated in an annual grass. *New Phytologist* 180, 240-247.

Markestijn L., Poorter L., Bongers F., Paz H. & Sack L. (2011) Hydraulics and life history of tropical dry forest tree species: coordination of species' drought and shade tolerance. *New Phytologist* 191, 480-495.

McCulloh K.A. & Sperry J.S. (2005) Patterns in hydraulic architecture and their implications for transport efficiency. *Tree Physiology* 25, 257-267.

McMahon T.A. (1973) Size and shape in biology. *Science* 179, 1201-1204.

Meinzer F.C. (2002) Co-ordination of vapour and liquid phase water transport properties in plants. *Plant, Cell and Environment* 25, 265-274.

Meinzer F.C. & Grantz, D.A. (1990) Stomatal and hydraulic conductance in growing sugarcane: stomatal adjustments to water transport capacity. *Plant, Cell and Environment* 13, 383-388.

Mencuccini, M. (2002) Hydraulic constraints in the functional scaling of trees. *Tree Physiology* 22, 553-565.

Nardini A. & Luglio J. (2014) Leaf hydraulic capacity and drought vulnerability: possible trade-offs and correlations with climate across three major biomes. *Functional Ecology* 28, 810-818.

Nardini A., Salleo S. & Raimondo F. (2003) Changes in leaf hydraulic conductance correlate with leaf vein embolism in *Cercis siliquastrum* L. *Trees-Structure and Function* 17, 529-534.

Niinemets Ü. (2007) Photosynthesis and resource distribution through plant canopies. *Plant, Cell and Environment* 30, 1052–1071.

Niklas K.J. (1999) A mechanical perspective on foliage leaf form and function. *New Phytologist* 143, 19-31.

Nix H. (1986) A biogeographic analysis of Australian Elapid snakes. In *Snakes: Atlas of Elapid Snakes of Australia* (ed R. Longmore), pp. 4-10, Bureau of Flora and Fauna, Canberra.

Pivovarovoff A.L., Sack L. & Santiago L.S. (2014) Coordination of stem and leaf hydraulic conductance in southern Californian shrubs: a test of the hydraulic segmentation hypothesis. *New Phytologist* 203, 842-850.

Price C.A., Enquist B.J. & Savage V.M. (2007) A general model for allometric covariation in botanical form and function. *Proceedings of the National Academy of Sciences USA* 104, 13204-13209.

Price C.A., Gilooly J.F., Allen A.P., Weitz J.S. & Niklas K.J. (2010) The metabolic theory of ecology: prospects and challenges for plant biology. *New Phytologist* 188, 696-710.

Price C.A., Wright I.J., Ackerly D.D., Niinemets Ü., Reich P.B. & Veneklaas E.J. (2014) Are leaf functional traits 'invariant' with plant size and what is 'invariance' anyway? *Functional Ecology* in press. doi: 10.1111/1365-2435.12298.

Price C.A., Weitz J.S., Savage V.M., Stegen J., Clarke A., Coomes D.A.,..., Swenson N.G. (2012) Testing the metabolic theory of ecology. *Ecology Letters* 15, 1465-1474.

R Development Core Team (2013) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.

Reich P.B., Falster D.S., Ellsworth D.S., Wright I.J., Westoby M., Oleksyn J., Lee T.D. (2009) Controls on declining carbon balance with leaf age among 10 woody species in Australian woodland: do leaves have zero daily net carbon balances when they die? *New Phytologist* 183, 153–166.

Sack L., Cowan P.D., Jaikumar N. & Holbrook N.M. (2003) The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species. *Plant, Cell and Environment* 26, 1343-1356.

Sack L. & Frole K. (2006) Leaf structural diversity is related to hydraulic capacity in tropical rain forest trees. *Ecology* 87, 483-491.

Sack L., Melcher P.J., Zwieniecki M.A. & Holbrook N.M. (2002) The hydraulic conductance of the angiosperm leaf lamina: a comparison of three measurement methods. *Journal of Experimental Botany* 53, 2177-2184.

Sack L., Tyree M.T. & Holbrook N.M. (2005) Leaf hydraulic architecture correlates with regeneration irradiance in tropical rainforest trees. *New Phytologist* 167, 403-413.

Savage V.M., Bentley L.P., Enquist B.J., Sperry J.S., Smith D.D., Reich P.B. & von Allmen E. I. (2010) Hydraulic trade-offs and space filling enable better predictions of vascular structure and function in plants. *Proceedings of the National Academy of Sciences USA* 107, 22722-22727.

Savage V.M., Deeds E.J. & Fontana W. (2008) Sizing up allometric scaling theory. *PLOS Computational Biology*, 4, 1-17.

Sellin A., Tullus A., Niglas A., Ounapuu E., Karusion A. & Lohmus K. (2013). Humidity-driven changes in growth rate, photosynthetic capacity, hydraulic properties and other functional traits in silver birch (*Betula pendula*). *Ecological Research*, 28, 523-535.

Simonin K.A., Limm E.B. & Dawson T.E. (2012) Hydraulic conductance of leaves correlates with leaf lifespan: implications for lifetime carbon gain. *New Phytologist* 193, 939-947.

Sperry J.S. (2000) Hydraulic constraints on plant gas exchange. *Agricultural and Forest Meteorology* 104, 13-23.

Sperry, J.S. (2004) Coordinating stomatal and xylem functioning - an evolutionary perspective. *New Phytologist* 162, 568-570.

Sperry J.S., Meinzer F.C. & McCulloh K.A. (2008). Safety and efficiency conflicts in hydraulic architecture: scaling from tissues to trees. *Plant, Cell and Environment* 31, 632-645.

Sperry J.S., Smith D.D., Savage V., Enquist B.J., McCulloh K.A., Reich P.B.,..., von Allmen E.I. (2012) A species-level model for metabolic scaling in trees I. Exploring boundaries to scaling space within and across species. *Functional Ecology* 26, 1054-1065.

Spicer R.A. (2005) Senescence in Secondary Xylem: Heartwood Formation as an Active Developmental Program. In: *Vascular Transport in Plants* (eds M.N. Holbrook, & M.A. Zwieniecki), pp. 457-475, Elsevier, Philadelphia.

Tyree M.T., Davis S.D. & Cochard H. (1994) Biophysical perspectives of xylem evolution: Is there a trade-off of hydraulic efficiency for vulnerability to xylem dysfunction? *International Association of Wood Anatomists Journal* 15, 355-360.

Tyree, M.T., Patino, S. & Alexander, J. (1995) Dynamic measurements of root hydraulic conductance using a high-pressure flow meter in the laboratory and field. *Journal of Experimental Botany* 46, 83-94.

van den Honert T.H. (1948) Water transport as a catenary process. *Discussions of the Faraday Society* 3, 146-153.

von Allmen E.I., Sperry J.S., Smith D.D., Savage V.M, Enquist B.J., Reich P.B. & Bentley

LP. (2012) A species-level model for metabolic scaling of trees II. Testing in a ring- and diffuse-porous species. *Functional Ecology* 26, 1066-1076.

Warton D.I., Wright I.J., Falster D.S. & Westoby M. (2006). Bivariate line-fitting methods for allometry. *Biological Reviews* 81, 259-291.

West G.B., Brown J.H. & Enquist B.J. (1999) A general model for the structure and allometry of plant vascular systems. *Nature* 400, 664-667.

Zimmerman M.H. (1978) Hydraulic architecture of some diffuse porous trees. *Canadian Journal of Botany* 56, 2286-2295.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Theoretical relationships between the ratio of leaf and shoot hydraulic conductance.

Table S1. Raw data for the 12 eucalypts of the study.

TABLE

Table 1 Comparison of modelled and observed hydraulic scaling exponents. The predicted values are the exponents based on the model derivation of Savage et al. (2010). Observed values are the slopes obtained from standard major axis regression analyses of log-transformed empirical data from this study. The variables are as follows: the hydraulic conductance of the entire shoot (k_{shoot}), the radius of the stem base ($r_{\text{ext},0}$), the total leaf surface area (A_L), the shoot dry weight (Shoot DW), the hydraulic conductance of the shoot leaves (k_{leaf}), the average leaf hydraulic conductance per leaf ($\overline{k_{\text{leaf}}}$), the specific hydraulic conductance of the shoot leaves (k_L) and the specific hydraulic conductance of the entire shoot (k_{SH}). Observations that are statistically consistent with model predictions are underlined and in bold type, and a reference to the relevant equation for each prediction is given.

Y variable	X variable	Prediction	Observation	Lower CI	Upper CI	Figure	Equation #
k_{shoot}	r	<2.00	<u>1.58</u>	1.34	1.84	2	1
A_L	Shoot DW	0.75	1.02	0.93	1.12	3a	2
$r_{\text{ext},0}$	Shoot DW	0.375	0.46	0.42	0.50	3b	1
k_{shoot}	Shoot DW	<0.75	<u>0.72</u>	0.61	0.85	3c	1
k_{leaf}	Shoot DW	<0.75	<u>0.77</u>	0.65	0.90	3d	4
$\overline{k_{\text{leaf}}}$	Shoot DW	0.00	<u>-0.18</u>	-0.51	0.16	3e	3 & 4
k_L	k_{SH}	1.00	<u>1.00</u>	0.93	1.09	5	7
k_{leaf}	A_L	1.00	0.75	0.65	0.88	4b	na

FIGURE LEGENDS

Figure 1. Theoretical relationships between leaf hydraulic conductance (k_{leaf}) and shoot hydraulic conductance (k_{shoot}). Based on our rearrangement and expansion of the Savage et al. 2010 model, k_{leaf} will scale in an isometric fashion with k_{plant} to reflect independence between the allocation of hydraulic capacity and plant size. Across different plant forms (herbaceous plants, small woody plants and large woody plants) and habitats, the intercept of the relationship between k_{leaf} and k_{plant} will change to reflect the differing investment in the hydraulic conductance of leaves as a function of the total plant hydraulic conductance. The y-intercept represents the dimensionless proportionality constant, C_{LSCR} (Eq. 7). In general accordance with the concepts of safety and efficiency (Tyree et al. 1994; Mencuccini 2002; Sperry et al. 2008), plants with a lower k_{leaf} for a given k_{plant} will convey water across their hydraulic flow path less efficiently. Given that this will result in a relatively lower water potential gradient across stems and branches, we contend that this strategy will incur a lower risk of drought-induced xylem dysfunction in branches and the primary stem. Although these theoretical relationships represent leaf and shoot hydraulic conductance, the same slope and intercept would occur for specific leaf and shoot hydraulic conductance (conductance scaled to leaf area) except that individual plants would migrate along the lines depending on their leaf area.

Figure 2. Plot of the hydraulic conductance of the whole shoot (k_{shoot}) vs. the stem (sapwood) radius at the base of the plant ($r_{\text{ext},0}$) for 12 eucalypt species ($n = 5$ for each). The fitted line is a SMA regression across all species.

Figure 3. Total leaf surface area (A_L , panel a), stem radius at the base of the plant ($r_{\text{ext},0}$, panel b), hydraulic conductance of the whole shoot (k_{shoot} , panel c), total leaf hydraulic conductance (k_{leaf} , panel d) and the average leaf hydraulic conductance per leaf ($\overline{k_{\text{leaf}}}$, panel e) as a function of shoot dry weight (Shoot DW). In each panel the fitted line is a SMA regression across 12 eucalypt species ($n = 5$ for each).

Figure 4. Correlation between total leaf hydraulic conductance (k_{leaf}) and stem hydraulic conductance (k_{stem}) (Panel a), k_{leaf} and leaf surface area (A_L) for this study and for the compilation of Simonin et al. (2012) (Panel b) and a frequency histogram of the ratio of k_{leaf} and k_{stem} ($k_{\text{leaf}}/k_{\text{stem}}$) (Panel c). In Panels a and b, the fitted lines are SMA regression models. The diameters of the bubbles in Panel a are representative of shoot dry weight. The distribution in Panel c is represented by a lognormal model.

Figure 5. Correlations between total specific leaf hydraulic conductance (k_L) and specific shoot hydraulic conductance (k_{SH}). Data for herbs, woody seedlings and saplings and mature trees from the study of Sack et al. (2003) are included with our data. Each fitted line is a SMA regression. The inset table shows the slope and intercept of each line. This intercept is representative of C_{LSCR} , the leaf-shoot conductance ratio that we define in Eq 7.

PCE_12511_f1

PCE_12511_f2

PCE_12511_f3

PCE_12511_f4

PCE_12511_f5