Leaf Maximum Photosynthetic Rate and Venation Are Linked by Hydraulics1[W][OA]

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Leaf veins are almost ubiquitous across the range of terrestrial plant diversity, yet their influence on leaf photosynthetic performance remains uncertain. We show here that specific physical attributes of the vascular plumbing network are key limiters of the hydraulic and photosynthetic proficiency of any leaf. Following the logic that leaf veins evolved to bypass inefficient water transport through living mesophyll tissue, we examined the hydraulic pathway beyond the distal ends of the vein system as a possible limiter of water transport in leaves. We tested a mechanistic hypothesis that the length of this final traverse, as water moves from veins across the mesophyll to where it evaporates from the leaf, governs the hydraulic efficiency and photosynthetic carbon assimilation of any leaf. Sampling 43 species across the breadth of plant diversity from mosses to flowering plants, we found that the post-vein traverse as determined by characters such as vein density, leaf thickness, and cell shape, was strongly correlated with the hydraulic conductivity and maximum photosynthetic rate of foliage. The shape of this correlation provided clear support for the a priori hypothesis that vein positioning limits photosynthesis via its influence on leaf hydraulic efficiency.

Leaves have sustained virtually all terrestrial ecosystems over the last 400 million years (Kenrick and Crane, 1991; Boyce et al., 2002; Boyce, 2005). Over this period land plants have evolved an enormous diversity of leaf structure; diversity that marks phylogenetic relations among clades and constrains the functional performance and climatic associations of plant species (Givnish, 1987; Wing and Greenwood, 1993; Smith et al., 1997; Osborne et al., 2004; Sack and Holbrook, 2006). Leaf veins are at the core of this structural diversity, forming the transport network for water, nutrients, and carbon for nearly all plants. While our knowledge of the molecular and developmental processes that guide leaf vein development and patterning grows at an ever-increasing rate (Kang and Dengler, 2004; Fleming, 2005; Scarpella et al., 2006; Sieburth and Deyholos, 2006), the underlying physical principles that connect vein pattern with leaf productivity remain unresolved. Discovering the specific mechanisms that link leaf venation to fundamental global processes such as photosynthetic rate and evapotranspiration is a prerequisite for understanding how leaf form has responded to, or perhaps driven, changes in atmospheric conditions through geological time (Volk, 1989; Osborne et al., 2004; Beerling, 2005).

The adaptive significance of efficient water transport (i.e. the mass of water transported per unit pressure gradient) is evident throughout the history of vascular plants. Evolutionary innovations in xylem structure that increase hydraulic efficiency while maintaining the continuity of the water column have been crucial to the success of the vascular plants (Carlquist, 1975; Niklas, 1985; Sperry, 2003). What remains unclear is how leaf structural evolution has influenced the hydraulic efficiency and subsequent photosynthetic performance of land plants. This is a significant gap in our knowledge considering the enormous potential of leaves as ecological and climatic indicators through time (Wing and Greenwood, 1993; Givnish, 2003; Royer et al., 2005; Sack and Froel, 2006). The potential role of leaf characters such as vein density (Sack and Froel, 2006) and mesophyll thickness (Aasamaa et al., 2001) as determinants of the hydraulic efficiency of the leaf have been recently recognized. Uncovering the mechanistic foundations that underpin correlations between leaf anatomy and hydraulic function will enable leaf hydraulic traits to be used as broad indices of plant function in ecological and evolutionary contexts.

Here we evaluate a new hypothesis that explicitly links the structural properties of the leaf vein system to functional processes of water transport and photosynthetic capacity across the entire range of terrestrial plant diversity. Our a priori hypothesis was that the length of mesophyll tissue that must be traversed as the transpiration stream passes from a vein ending to...
Anatomical Determinants of Leaf Hydraulics

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Hydraulic and Photosynthetic Capacity

RESULTS

Hydraulic and Photosynthetic Capacity

Across the diverse range of land plant diversity sam-
ples, an intimate association between light-saturated net CO$_2$ assimilation rate (A$_{max}$) and the hydraulic conductance (K$_{leaf}$) of whole leaves was found (Fig. 1A). A single regression accurately described the dependence of A$_{max}$ upon K$_{leaf}$ in all 43 species, including mosses, ferns, lycopsids, gymnosperms, and angiosperms. At values of K$_{leaf}$ < 10 mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$ mean A$_{max}$ was highly sensitive to increasing K$_{leaf}$ but this sensitivity decreased as A$_{max}$ in the tropical angiosperm species approached the upper limits for C3 photosynthesis in woody plants (Larcher, 1995; Santiago et al., 2004). It should be pointed out that according to protocol, K$_{leaf}$ was normalized at a temperature of 20°C, however, the mean leaf temperature at A$_{max}$ was 26°C. Due to the effects of viscosity, K$_{leaf}$ would be 15% higher at the mean temperature at which A$_{max}$ was measured.

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Vein Density, Leaf Width, and Photosynthesis

Combining the observed relationships between D$_{m}$/K$_{leaf}$ and A$_{max}$ enabled us to model how the width of single-veined leaves influences leaf photosynthetic capacity (Fig. 3). Among single-veined species, only those with narrow scale or needle leaves were able to attain high photosynthetic rates characteristic of sun-adapted species (Fig. 3A), while broader leaves were associated with low photosynthetic rates (and typically shady, low evaporative demand environments). A similar analysis on multiveined leaves illustrates the impact of vein density on K$_{leaf}$ and A$_{max}$ (Fig. 3B). With leaf thickness held constant it would be expected that A$_{max}$ should respond positively to increasing vein density, with a gradual plateau in slope as vein density reached the upper limits for our angiosperm sample (15.8 mm mm$^{-2}$). It should be noted that vein density was not found to be strongly correlated with either K$_{leaf}$ or A$_{max}$ in our sample of multivein leaves presumably due to the large range of vein-epidermal
thickness in this morphologically diverse species sample (T.J. Brodribb, unpublished data). This is understandable viewed in the context of Figure 3B showing that the modeled effect of increasing leaf thickness was to reduce the impact of vein density upon $A_{\text{max}}$. In our sample of multiveined leaves, we found little overlap between the vein density of ferns and the higher vein density in angiosperms (Fig. 3B).

**DISCUSSION**

We found that the photosynthetic capacity of leaves in terrestrial plants is strongly correlated with proximity of veins to the evaporative surfaces of the leaf. The close relationship between these two parameters supports our a priori hypothesis that the length of hydraulic pathway through the mesophyll should exert a controlling influence over $K_{\text{leaf}}$ and secondarily over $A_{\text{max}}$. This influence of vein placement over leaf physiology was found to transcend the enormous phylogenetic, ecological, and functional variation represented in our species sample. The data presented here suggest that throughout the evolutionary history of terrestrial plants, the hydraulic properties of the leaf tissue have played a fundamental role in linking leaf construction with photosynthetic capacity (Fig. 1).

**Mechanisms of Coordination between Vein Location, Hydraulics, and Productivity**

Previously it has been suggested that vein density was related to photosynthetic function because the vein surface area is thought to limit photosynthate transport away from the leaf (Amiard et al., 2005). Here we demonstrate strong evidence that the relationship between vein placement and leaf productivity is determined by the flow of water through the mesophyll. The mechanisms that culminate in leaf vein arrangement functioning as a governor of both water flow and leaf productivity are 2-fold. Primarily there is the physical control of leaf hydraulic conductance by the length of post-venous hydraulic pathway (from the vein end to the site of evaporation) traversed by the transpiration stream. This links secondarily to leaf

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**Figure 1.** A, $K_{\text{leaf}}$ versus $A_{\text{max}}$ in leaves of bryophytes (black), lycopods (white), ferns (green), conifers (red), angiosperms (blue), and gymnosperms with lignified sclereids in the mesophyll (brown). A highly significant quadratic regression ($y = 0.259x^2 + 1.41x - 0.60; r^2 = 0.94; P < 0.001$) indicates a strong linkage between $A_{\text{max}}$ and efficiency of hydraulic supply (data for 16 of the 43 species are from Brodribb and Holbrook, 2006). Symbols denote means with s.e.s ($n = 12$). B, $D_m$ from the end of the vein xylem to the gas-exchange epidermis plotted against $1/K_{\text{leaf}}$. Means for both parameters are shown for single-vein (white circles) and multivein (red triangles) leaves plotted together, excluding the species with lignified sclereids in the mesophyll (brown). A linear regression ($r^2 = 0.95; P < 0.001$) is fitted to all data ($y = 0.0005x - 0.031$). To improve resolution, the two species with $D_m > 1,400 \mu$m (Hymenophyton and Tmesipteris) are offscale, but these species did not deviate from the overall trend (see Fig. 1C). C, Raw data showing $D_m$ from the end of the vein xylem to the gas-exchange epidermis plotted against $K_{\text{leaf}}$ (color legend identical to Fig. 1A). Both axes are on a log scale to compress the enormous range in $D_m$ observed in our species sample. Two regression equations are shown, first the same function as fitted in Figure 1B (dotted line), and second an exponential function ($y = 12.674x^{-1.26}; r^2 = 0.97$). Species with lignified mesophyll sclereids are circled and again not included in the regressions.

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1/$K_{\text{leaf}}$. Means for both parameters are shown for single-vein (white circles) and multivein (red triangles) leaves plotted together, excluding the species with lignified sclereids in the mesophyll. A linear regression ($r^2 = 0.95; P < 0.001$) is fitted to all data ($y = 0.0005x - 0.031$). To improve resolution, the two species with $D_m > 1,400 \mu$m (Hymenophyton and Tmesipteris) are offscale, but these species did not deviate from the overall trend (see Fig. 1C). C, Raw data showing $D_m$ from the end of the vein xylem to the gas-exchange epidermis plotted against $K_{\text{leaf}}$ (color legend identical to Fig. 1A). Both axes are on a log scale to compress the enormous range in $D_m$ observed in our species sample. Two regression equations are shown, first the same function as fitted in Figure 1B (dotted line), and second an exponential function ($y = 12.674x^{-1.26}; r^2 = 0.97$). Species with lignified mesophyll sclereids are circled and again not included in the regressions.
productivity because of the strong coordination between $K_{leaf}$ and $A_{max}$ (Fig. 1A).

The first of these two processes is explained by the fact that water flow through the leaf is analogous to current flow in an electrical circuit (Cowan, 1972; Sack and Holbrook, 2006). In its passage through the leaf, water must move from the highly conductive medium of the leaf veins into the highly resistive medium of the living mesophyll before it escapes the leaf via stomata. According to the electrical analog, the hydraulic conductance of this low-conductivity mesophyll pathway should be inversely proportional to the path length (Fig. 1B). The length of this pathway is likely to strongly influence the total hydraulic conductance of the leaf because the hydraulic conductivity of living cells in the plant is very low relative to xylem cells (Boyer, 1985; Passioura, 1988; Frensch and Steudle, 1989), leading to a high proportion of extravascular resistance in the leaf (Nardini and Salleo, 2005; Sack et al., 2005). Indeed, we found that the length of this ultimate hydraulic traverse represents a unifying limit to the hydraulic efficiency of leaves (Fig. 1B). The range of $K_{leaf}$ expressed by these species varied over 20-fold.

The second mechanism that coordinates leaf vein arrangement and leaf photosynthetic potential is the tendency for the leaves of terrestrial C₃ plants to produce a conservative CO₂/water exchange ratio (Cowan and Farquhar, 1977; Franks and Farquhar, 1999). As a result, the capacity for water delivery to the photosynthetic tissue corresponds with the photosynthetic capacity of the leaf (Brodribb et al., 2005). Here we established that hydraulic mediation of $A_{max}$ appears to be a uniform feature of all major terrestrial plant groups (Fig. 1A and B).

**Leaf Dimensions, Vein Density, and CO₂ Assimilation**

High CO₂ assimilation rates in plants where water is supplied to leaves through internal conducting cells should, according to our data, be associated with either very narrow leaves (in the case of single-vein leaves), or high vein densities (Fig. 3). However, the impact of leaf thickness on $K_{leaf}$ and $A_{max}$ is likely to be more complicated due to the interaction of palisade thickness and the proximity of the vein to the stomatal surface of the leaf. We found here that increasing the vertical distance from the vein to the stomatal epidermis should reduce hydraulic and photosynthetic...
performance (Fig. 3B), but other studies have shown positive correlations between leaf and palisade thickness and $K_{\text{leaf}}$ (Aasamaa et al., 2001; Sack et al., 2003; Sack and Frole, 2006). These observations are entirely compatible because the palisade tissue is rather hydraulically isolated from the bulk of the transpiration stream (Zwieniecki et al., 2007). As a result, relationships between palisade mesophyll thickness, $A_{\text{max}}$ (Terashima et al., 2001), and $K_{\text{leaf}}$ can exist while $K_{\text{leaf}}$ remains fundamentally controlled by the position of the vein relative to the stomatal epidermis (the vein-to-epidermal thickness shown in Fig. 3B). The distinction between leaf thickness and vein-to-epidermal thickness is important, only the latter is expected to unequivocally influence $K_{\text{leaf}}$ according to our hypothesis.

**An Alternative to Vein Branching**

A fundamental implication of the relationship shown here between $K_{\text{leaf}}$ and vein proximity (Fig. 1B) is that the hydraulic conductivity of the cell matrix comprising the mesophyll tissue of the leaf is consistently low among terrestrial plants. While it is likely that there is variation between species in the hydraulic conductivity of leaf tissue due to cell anatomy and packing (possibly contributing to the variability in the angiosperm data and the fact that $K_{\text{leaf}}$ was not exactly related to $D_{m}^{-1}$), this was outweighed by the influence of path length on the hydraulic conductance of the mesophyll pathway. However, many gymnosperms possess obvious modifications to the mesophyll tissue apparently used to increase the post-venous hydraulic conductivity of their leaves. These plants have water-filled lignified cells (Fig. 2, E and F) that are highly pitted and generally elongated in the plane of water movement from the vein to the leaf margin (Brodribb and Holbrook, 2005). The function of these cells is analogous to the tracheids of the xylem, effectively providing a large apoplastic volume and pits by which water moving toward to stomata can bypass the much slower passage through living mesophyll cells. The effectiveness of this adaptation is clearly evident in enabling single-vein leaves to achieve leaf widths that are orders of magnitude wider than predicted if they possessed unmodified mesophyll (Fig. 1B).

**Implications for Leaf Evolution**

The presence of highly pitted lignified tissue in the mesophyll of gymnosperms leaves has been formally noted in the conifer family Podocarpaceae and the cycad genus *Cycas* (Griffith, 1957; Hu and Yao, 1981). We found that similar tissues are present in conifers from all families including *Sciadopitys* and *Amentotaxus* (T. Brodribb, unpublished data) and in each case they were associated with leaf widths in excess of those achievable with unmodified mesophyll tissue (Fig. 1B). Indeed it appears that terrestrial plants have adopted two different systems to overcome the intrinsically low hydraulic conductivity of the leaf mesophyll. The most common of these is to branch the vein system such that a high volume apoplastic flow pathway (the xylem) is allowed to approach very close to the sites of evaporation. This is the means of water distribution in most angiosperm leaves. The second system, identified here, is to modify the mesophyll by directing the lignification and apoptosis (Griffith, 1957) of a proportion of mesophyll cells, thereby greatly increasing its conductivity to water. This fascinating divergence in leaf water distribution systems appears to have arisen in the gymnosperm clade, as there have been no reports...
of lignified mesophyll cells in any plant groups basal to gymnosperms.

CONCLUSION

Considering that the total length of the transpiration pathway can exceed 100 m, it is remarkable that the last few tens of microns should reveal fundamental physiological information about the photosynthetic and hydraulic performance of the individual plant. Despite all the myriad of variation and adaptation in xylem anatomy to improve long-distance transport, the hydraulic conductivity of the mesophyll appears as a unifying limitation to maximum leaf photosynthesis. This result indicates that leaf structure, as it relates to the hydraulic performance, contains important information about how plants have evolved higher yielding

Table 1. Species sample showing vein morphology

| Species Family | Vein Type | A\textsubscript{max} (mmol m\textsuperscript{-2} s\textsuperscript{-1}) | K\textsubscript{leaf} (mmol m\textsuperscript{-2} s\textsuperscript{-1} MPa\textsuperscript{-1}) | D\textsubscript{m} (mm) |
|----------------|----------|---------------------------|--------------------------------|-----------|
| Bryophytes     |          |                           |                                |           |
| Davsonia superpa Polytrichaceae | SV | 2.332 | 2.79 | 577.2 |
| Hymenophyton flabellatum Hymenophytaceae | MV | 0.891 | 1.00 | 1,498 |
| Polycladum commutatum Polycladaceae | SV | 7.109 | 8.50 | 373.6 |
| Polycladum juniperinum Polycladaceae | SV | 8.648 | 7.42 | 359.9 |
| Lycopodites     |          |                           |                                |           |
| Lycopodium annotinum Lycopodiaceae | SV | 2.955 | 2.93 | 663.8 |
| Lycopodium clavatum Lycopodiaceae | SV | 2.893 | 3.70 | 551.1 |
| Lycopodium obscurum Lycopodiaceae | SV | 4.555 | 3.42 | 590.2 |
| Selaginella longipinnae Selaginellaceae | SV | 6.15  | 2.03 | 1,048 |
| Selaginella pallescens Selaginellaceae | SV | 1.689 | 4.83 | 532.5 |
| Lycophytes     |          |                           |                                |           |
| Lycopodium annotinum Lycopodiaceae | SV | 2.955 | 2.93 | 663.8 |
| Lycopodium clavatum Lycopodiaceae | SV | 2.893 | 3.70 | 551.1 |
| Lycopodium obscurum Lycopodiaceae | SV | 4.555 | 3.42 | 590.2 |
| Selaginella longipinnae Selaginellaceae | SV | 6.15  | 2.03 | 1,048 |
| Selaginella pallescens Selaginellaceae | SV | 1.689 | 4.83 | 532.5 |
| Ferns           |          |                           |                                |           |
| Bolbitis portoricensis Dryopteridaceae | MV | 2.78  | 2.40 | 947.9 |
| Lygodium venustum Lygodiaceae | MV | 5.378 | 5.23 | 396.2 |
| Polypodium trisernale Polypodiaceae | MV | 3.176 | 2.36 | 907.8 |
| Pteris altissima Pteridaceae | MV | 5.414 | 3.91 | 562.9 |
| Tectaria confluae Aspleniaceae | MV | 2.128 | 3.19 | 794.4 |
| Tmesipteris obliqua Psilotaceae | SV | 1.1   | 0.50 | 3,218 |
| Gymnosperms     |          |                           |                                |           |
| Acmopyle sahnianna Podocarpaceae | SV | 6.165 | 4.71 | 1,547 |
| Callitris rhomboidea Cupressaceae | SV | 12.31 | 11.30 | 202.4 |
| Cytisus caerulina Cupressaceae | SV | 11.08 | 10.80 | 1,416 |
| Cylxus media Cipadaceae | SV | 9.649 | 7.65 | 2,758 |
| Delsema archeri Cupressaceae | SV | 5.446 | 4.62 | 516.8 |
| Fitzroya cupressoides Cupressaceae | SV | 10.56 | 10.59 | 310.9 |
| Lagarostrobos franklinii Podocarpaceae | SV | 9.784 | 8.09 | 322.2 |
| Metasequoia glyptostroboides Cupressaceae | SV | 4.821 | 4.53 | 544.3 |
| Nagoea nagi Podocarpaceae | MV | 6.496 | 5.44 | 443.4 |
| Pinus radiata Pinaceae | SV | 9.802 | 10.80 | 287.5 |
| Podocarpus dispermis Podocarpaceae | SV | 3.518 | 3.05 | 11,410 |
| Podocarpus drunianus Podocarpaceae | SV | 5.627 | 5.59 | 1,107 |
| Retrophyllum comptonii Podocarpaceae | SV | 5.722 | 4.58 | 2,684 |
| Sciadopitys verticillata Cupressaceae | SV | 7.26  | 7.20  | 699.1 |
| Sundacarpus amara Podocarpaceae | SV | 5.537 | 5.00 | 4,716 |
| Torrey a taxifolia Taxaceae | SV | 5.796 | 3.56 | 699.9 |
| Tsuga canadensis Pinaceae | SV | 9.514 | 8.40 | 347.5 |
| Angiosperms     |          |                           |                                |           |
| Amborella trichopoda Amborellaceae | MV | 4.848 | 4.66 | 427.6 |
| Beddorha salicina Asteraceae | MV | 14.66 | 12.25 | 249.4 |
| Byrsonima crassifolia Malpighiaceae | MV | 15.59 | 17.19 | 180.0 |
| Curatella americana Dilleniaceae | MV | 15.83 | 21.10 | 128.8 |
| Dalbergia retusa Fabaceae | MV | 19.02 | 20.00 | 186.8 |
| Eucalyptus globulus Myrtaceae | MV | 16.85 | 13.20 | 203.4 |
| Genipa americana Rubiaceae | MV | 14.29 | 17.00 | 167.7 |
| Gymnostoma australis Casuarinaceae | SV | 14.67 | 14.20 | 262.1 |
| Oplismenus hirtellus Poaceae | MV | 16.92 | 20.30 | 154.9 |
| Pharus lappulaceus Poaceae | MV | 5.97  | 5.66 | 404.4 |
| Rehdera trinervus Verbenaceae | MV | 17.03 | 20.60 | 193.9 |
leaves. Considering the substantial cost to plants of leaf vein differentiation, the data presented here provide a basis for evaluating the structural costs associated with increasing leaf photosynthetic rate, and the resultant impact on plant productivity.

**MATERIALS AND METHODS**

**Plant Material**

Measurements of maximum photosynthetic rate, leaf hydraulic conductance, and leaf anatomy were carried out on 43 C₃ species carefully selected to represent the breadth of land plant evolutionary diversity (Table I). A mixture of tropical and temperate species were sampled primarily from their natural habitats and included representatives from three major phylogenetic categories (angiosperms, gymnosperms, and ferns), while mosses and lycopods were collected only from temperate regions. Species were chosen to represent the range of vein architecture within major groups. Our sampling included eight species of gymnosperms known to possess lignified transition tissue or sclerids in the mesophyll (Griffith, 1957; Hu and Yao, 1981). These structures greatly increase the apoplastic volume of the leaf mesophyll and were expected to result in different leaf hydraulic properties as compared with those species that lack such modifications. Where possible, measurements were made on leaves in open habitats or forest gaps so as to avoid complications due to variable light induction of hydraulic conductance and photosynthesis (Pearsé, 1990; Cochard et al., 2007).

**Leaf Photosynthetic and Hydraulic Capacity**

$K_{m}$ was measured on excised leaves allowed to reach a transpirational steady state while attached to a flowmeter measuring the transpiration flux (Sack et al., 2002; Brodribb and Holbrook, 2006). Leaves were excised under water at 9 AM to 10 AM and measurements were made between 9 AM and 12 PM when transpiration rates were maximal with leaves maintained under natural sun or a halide lamp delivering 1,500 μmol quanta m⁻² s⁻¹. After 3 to 5 min at steady state (less than 10% variation over 180 s), leaves were removed and water potential measured with a pressure chamber (PMS). Leaf temperature was monitored by two thermocouples pressed against the abaxial surface of the leaf, and leaf temperature was maintained between 20°C and 24°C by directing a stream of air (heated if necessary) uniformly across the leaf blade. This small temperature range was sufficient to produce steady-state leaf water potentials spanning the range −0.2 to −0.8 MPa. The leaf hydraulic conductance was calculated as the ratio of transpirational flux to leaf water potential, and standardized to the viscosity of water at 20°C using an empirical function based on data from Korson et al. (1969). Between 10 and 30 leaves from five individuals of each species were measured and the maximum hydraulic conductance for each species calculated as the $y$-intercept of a plot of water potential versus hydraulic conductance (Brodribb and Holbrook, 2006).

$A_{max}$ was measured on 10 healthy, mature leaves of each species using a portable gas analyzer (Li-COR 6400) to quantify CO₂ uptake under conditions of saturating light and water availability. All species were measured on plants in the field under conditions of high soil water availability. During all measurements a high flow rate (500 mL min⁻¹) through the cuvette was used depending on the saturating photosynthetic photon flux density of the species (as determined by a light response curve made on one individual per species); the lower photosynthetic photon flux density was used in species with low $A_{max}$ to avoid oversaturation with light.

**Leaf Anatomy**

The xylem pathway through the leaf mesophyll was quantified by measuring the linear path length from the minor veins to stomata and then calculating the hydraulic path length assuming water moved in the cell apoplast, and that mesophyll cells were uniform capsules of known aspect ratio (measured for each species). Multiple measurements were made on each leaf by dividing leaves into grids of either 200 × 200 μm or 500 × 500 μm depending on leaf thickness and vein density. For each grid cell the maximum distance between neighboring veins and between the vein and the epidermis was recorded. Maximum rather than mean distance was used because it was easily measured and scales with average distance if stomata are distributed relatively homogeneously across the leaf. Calculation of a mean (maximum) linear mesophyll path length for each leaf required measurements to be made along two axes; first, the lateral distances between veins were measured (termed the $x$ axis; Fig. 2B) and second, the perpendicular distance from the vein to the epidermis (termed the $y$ axis; Fig. 2A). A mean value for maximum $x$ and $y$ distance was calculated from 30 to 40 subsamples per leaf in each of three to four leaves previously sampled for $K_{m}$. Paradermal sections were used to visualize the $x$ axis distance between adjacent minor veins, or to the most distant adjacent stomata for single-vein leaves (Fig. 2, B and D), and cross sections allowed determination of the $y$ axis distance from the vein endings to the stomatal gas exchange epidermis (Fig. 2A). Randomly spaced windows in the epidermis were cut with a razor, and then cleared in 1 M KOH. After clearing, veins were stained with toluidine blue to highlight xylem lignin. In the dicots of bryophytes and angiosperms, sylloporous-B was infused into the xylem of transpiring leaves and leaves viewed using a fluorescence microscope (Axiophot, Carl Zeiss) to clearly visualize the water-conducting tissue. Thirty images of vein architecture under 10X magnification were captured from three leaves of each species using a digital camera attached to a compound microscope and the largest distance between adjacent veins measured for all veins. In the case of single-vein leaves or parathonal veins, measurements were made using a digital ruler in Image-J (National Institutes of Health, available online). In leaves with reticulate venation, the center of the areole was found by tracing the veins in each field of view in Image-J and increasing the pen size until no white space remained in the areole. The last point of white space remaining was the center of the areole, and this maximum pen size was converted back to a linear distance.

The vein to the epidermis was measured from leaf sections cut from the same leaves used for leaf clearing. Ten sections from three to four leaves per species were cut and stained with toluidine blue. Minor veins or vein endings were identified by their distinctive morphology (paradermal sections allowed clear identification of the types of tracheids that characterized vein endings, typically thin-walled large lumen cells) and these were photographed. Ten images of leaf cross sections were used to measure the distance from the vein xylem to the gas-exchange epidermis (the stomata-bearing surface in tracheophytes and the abaxial leaf surface in bryophytes). Only one amphistomatic leaf was included in our sample (Eucalyptus globulus) and in this species leaves were isobilateral, so measurements were made equally on both sides of the leaf. Cross-section images were also used to measure the dimensions of mesophyll cells along the same two axes described above. A sample of 100 cells located between the vein and the site of evaporation were measured for $x$ and $y$ dimensions for each species.

We assumed that the majority of water flowed apoplastically in the mesophyll (Steedle, 1994), and that there was good contact between neighboring cells. Hence the hydraulic pathway from the vein to epidermis was simplified to the shortest route around the perimeter of rounded mesophyll cells. Mesophyll cells were approximated as cylindrical capsules packed closely together such that water could flow directly from cell wall to cell wall. Because cells were typically elongated in one dimension, the distance through the mesophyll corresponding to the longer cell axis was calculated from the number of cells traversed multiplied by the cell length, plus the distance around the minor axis of the cells. The mean distance traversed along the minor axis of cells was calculated from the number of cells traversed multiplied by the distance around half the cylindrical cell perimeter. Hence, if cells are elongated so $C_{m}$ (mean cell $x$ dimension) > $C_{y}$ (mean cell $y$ dimension) then $X = v/C_{m} ([C_{m} - C_{y}] + \pi C_{y}/2)$ and $Y = 1/C_{y}(\pi C_{y}/2)$, where $X$ is the horizontal apoplastic path length from the vein to stomata, $Y$ is the mean longest distance from veins to stomata, $Y$ is the vertical apoplastic path length from the vein to stomata, and $l$ is the mean leaf thickness from vein to stomata.

Finally, $D_{v}$ was taken as the hypotenuse of a right-angled triangle produced by these $x$ and $y$ dimensions: $D_{v} = (X^2 + Y^2)^{0.5}$. In leaves where cells were elongated such that $C_{m}$ > $C_{y}$ (such as E. globulus), $X$ and $Y$ distances were calculated as follows: $X = v/C_{m}(C_{m}/2)$ and $Y = (1/C_{m}[C_{m} - C_{y}] + \pi C_{y}/2)$.
Modeling the Impact of Leaf Morphology on $A_{\text{max}}$

The effects of leaf width and vein density on $A_{\text{max}}$ were modeled by combining empirical relationships between $K_w$ and $A_{\text{max}}$ and $D_{\text{vein}}$ and $K_w$ (regression equations from Fig. 1, A and C). Combining these two equations allowed $A_{\text{max}}$ to be solved empirically as a function of $D_{\text{vein}}$. In single-vein species the $x$ dimension in leaves is equivalent to the leaf width (from vein to furthest adjacent stomata in single-vein leaves; Fig. 2D), hence the effect of leaf width on $A_{\text{max}}$ was modeled while holding vein-epidermis thickness at a constant value of 70 $\mu$m and using a uniform cell aspect ratio of 1:1.4 (the mean value for all species). In multivein leaves it is the vein density that influences the $x$ dimension in leaves (Fig. 2B); hence, we were able to model the effect of increasing vein density on $A_{\text{max}}$. Vein density was simplified to a square network and vein-epidermal thicknesses of 70, 100, and 130 $\mu$m were imposed. This range corresponded to the observed range in the angiosperm sample.

Statistical Analysis

Our a priori hypothesis was that $K_w$ should be related to $1/D_{\text{vein}}$, so the logarithm of $K_w$ was regressed on the logarithm of $D_{\text{vein}}$, then tested to see if the slope was equal to $-1$. Regression analysis was used to test for variation in slopes and intercepts between single- and multivein leaves, among major phylegynous groups, and between species with and without sclereids in the mesophyll. All analyses except the latter excluded species with sclereids in the mesophyll. Model fitting for the regression between mesophyll. All analyses except the latter excluded species with sclereids in the angiosperm sample.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Table S1. Leaf anatomical parameters.

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