Decoding Leaf Hydraulics with a Spatially Explicit Model: Principles of Venation Architecture and Implications for Its Evolution

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Abstract: Leaf venation architecture is tremendously diverse across plant species. Understanding the hydraulic functions of given venation traits can clarify the organization of the vascular system and its adaptation to environment. Using a spatially explicit model (the program K_leaf), we subjected realistic simulated leaves to modifications and calculated the impacts on xylem and leaf hydraulic conductance (K_x and K_leaf, respectively), important traits in determining photosynthesis and growth. We tested the sensitivity of leaves to altered vein order conductivities (1) in the absence or (2) presence of hierarchical vein architecture, (3) to major vein tapering, and (4) to modification of vein densities (length/leaf area). The K_x and K_leaf increased with individual vein order conductivities and densities; for hierarchical venation systems, the greatest impact was from increases in vein conductivity for lower vein orders and increases in density for higher vein orders. Individual vein order conductivities were colimiting of K_x and K_leaf, as were their densities, but the effects of vein conductivities and densities were orthogonal. Both vein hierarchy and vein tapering increased K_x relative to xylem construction cost. These results highlight the important consequences of venation traits for the economics, ecology, and evolution of plant transport capacity.

Keywords: biological networks, hydraulics, leaf size, sectoriality, vein pattern, xylem.

Introduction

Leaf venation architecture is tremendously variable across species and is thus a striking example of diversity in a complex and critical plant feature. Leaf venation functions in hydraulic supply, delivery of nutrients and sugars, and biomechanical support (Niklas 1999; Roth-Nebelsick et al. 2001; Tyree and Zimmermann 2002; Ellis et al. 2009). Vascular traits can impact whole-plant performance be-cause hydraulic supply influences leaf photosynthetic rate and water loss per area and, further, because the mass of venation is an economic “cost” constituting a substantial proportion of leaf mass per area, also an important determinant of plant relative growth rate (Sack and Holbrook 2006; Brodribb et al. 2007; Niinemets et al. 2007a, 2007b). Venation traits can adapt to different environments and may be useful to estimate the function of past plants and environments from fossil leaves (Uhl and Mosbrugger 1999; Roth-Nebelsick et al. 2001; Sack and Holbrook 2006; Boyce et al. 2009). In this study, we present the first detailed examination of the hydraulic consequences and implications of key venation traits.

Leaves are important in determining plant hydraulic capacity because they constitute a bottleneck in the path from roots to leaves, representing at least one-quarter of the whole plant resistance to water movement (Sack and Holbrook 2006). High leaf hydraulic conductance (i.e., flow rate per pressure driving force; K_leaf) is necessary for stomatal opening and for photosynthesis under high evaporative load. Thus, across species sets, K_leaf correlates with stomatal pore area and photosynthetic rate per leaf area (Sack et al. 2003a; Brodribb et al. 2007). Evolution should favor K_leaf that maximizes growth relative to construction costs; a higher K_leaf should be adaptive under high resource supplies (Sack et al. 2005).

The leaf hydraulic system that defines K_leaf can be analyzed as a pipe network or an electronic circuit (Sack and Holbrook 2006). After the petiole, water moves through the xylem conduits within veins arranged in a reticulate hierarchy, with vein conductivity decreasing and vein density increasing from low- to higher-order veins (see table A1 in the online edition of the American Naturalist). Water exits the xylem and moves through bundle sheath, mesophyll, bundle sheath extensions, and/or epidermis (Zwieniecki et al. 2007) and then evaporates and diffuses from the stomata.
The quantity \( K_{\text{leaf}} \) is determined by the conductances of both the xylem (\( K_x \)) and outside-xylem pathways (\( K_{\text{ox}} \)):

\[
\frac{1}{K_{\text{leaf}}} = \frac{1}{K_x} + \frac{1}{K_{\text{ox}}}. \tag{1}
\]

Terms \( K_x \) and \( K_{\text{ox}} \) can be of similar magnitude. Thus, both are important in determining \( K_{\text{leaf}} \), with their proportionality varying across species, and both are subject to dynamics of temperature, leaf water status, and irradiance, which affect \( K_x \) and \( K_{\text{ox}} \) differently (Cochard et al. 2004; Sack et al. 2004, 2005; Sack and Holbrook 2006; Scoffoni et al. 2008; Voicu et al. 2008).

Experimental work has also begun to reveal the structural basis for \( K_x \) and \( K_{\text{ox}} \). Across diverse species, \( K_{\text{leaf}} \) increases with midrib conductivity and minor vein density (Sack and Holbrook 2006; Brodribb et al. 2007). For other analogous systems, flow capacity also increases with channel conductivity and length per area, as in animal vasculature (LaBarbera 1990), stream systems (Gordon et al. 2004), and irrigation systems (Cuenca 1989).

Among plant species, evolutionary changes have resulted in a diversity of leaf venation characteristics that affect \( K_{\text{leaf}} \). To better understand the function of this diversity, we explored the impacts of altering venation traits using a spatially explicit model of the leaf venation system. We tested the responses of \( K_x \) and \( K_{\text{ox}} \) to (1) altered vein conductivities in the absence or (2) presence of hierarchical vein architecture, to (3) tapering of major veins, and to (4) modification of vein densities. We hypothesized that increased vein conductivities and densities would increase \( K_x \) and \( K_{\text{ox}} \). We also estimated changes in \( K_x \) relative to construction costs of xylem. We applied these results toward explaining what is known of the function and evolution of leaf venation architecture and toward guiding further efforts to estimate the function of leaves in extant and past species from vein traits.

**Methods**

Parameterization of the Spatially Explicit Model \( K_{\text{leaf}} \)

Leaf simulations were generated using the program \( K_{\text{leaf}} \), version 6 (written by H. Cochard, Institut National de la Recherche Agronomique, Clermont-Ferrand, France; Cochard et al. 2004). \( K_{\text{leaf}} \) creates a spatially explicit model of the leaf with numerous vein orders (fig. 1A) and an outside-xylem pathway, treating the vein system as a square grid of xylem resistors, with “mesophyll” resistors representing the outside-xylem flow branching orthogonally from each junction. The program calculates \( K_x \), \( K_{\text{ox}} \), and \( K_{\text{leaf}} \) after input of parameters including, for the resistors representing each vein order, the vein density and cross-sectional conductivity, and for the mesophyll resistors, the “mesophyll conductance” (table A2 in the online edition of the *American Naturalist*). In the model, water exits veins of all orders through the mesophyll resistors, though mainly from the minor venation that constitutes the majority of grid junctions. Term \( K_x \) depends on vein order conductivities and densities. In contrast, \( K_{\text{ox}} \) depends on both mesophyll conductance and vein density, which determine the number of junctions and parallel mesophyll resistors. In real leaves, as in the model, \( K_{\text{ox}} \) is a “mixed material” affected by the venation density and by outside-xylem tissues and their properties (see “Discussion”).

We tested the effect of manipulating individual parameters while maintaining others at fixed values measured for real leaves (Cochard et al. 2004). When changing vein densities using \( K_{\text{leaf}} \), only the 1\(^{st} \) and 2\(^{nd} \) veins can be treated individually; the 3\(^{rd} \) and higher veins form a mesh, and their densities can be modified only as a group.

We investigated the impact of sectoriality in 1\(^{st} \) and 2\(^{nd} \) veins on \( K_x \) and \( K_{\text{ox}} \). Sectoriality in the leaf venation relates to the way that xylem conduits extend across vein orders within a leaf. In previous models, the leaf venation was considered nonsectorial, with the entire 1\(^{st} \) vein a single conduit that was open to all branching 2\(^{nd} \) veins (Zwieniecki et al. 2002; Cochard et al. 2004; Sack et al. 2004). In real leaves, however, major veins are formed by individual xylem vessels extending from the petiole through the 1\(^{st} \), 2\(^{nd} \), and sometimes 3\(^{rd} \) veins (Larson 1984; Chatelet et al. 2006; Sack and Frolé 2006). \( K_{\text{leaf}} \), version 6, can designate leaves “nonsectoral,” as do previous models (fig. 1B), or as fully sectorial with multiple independent xylem conduits running through the 1\(^{st} \) vein and each conduit branching off to become a 2\(^{nd} \) vein (fig. 1C).

\( K_{\text{leaf}} \) can also simulate leaves with or without vein tapering along the 1\(^{st} \) and 2\(^{nd} \) veins, reflecting diminishing xylem conduit size and number (fig. 1B). In the modeled sectorial leaf, tapering is automatic in the 1\(^{st} \) vein because conductivity is highest at the base and diminishes axially as conduits branch off to form 2\(^{nd} \) veins. The conductivity of these “1\(^{st} + 2\(^{nd} \)” conduits in sectorial leaves can be set in two different ways, depending on whether the 2\(^{nd} \) veins are set to taper. If the 2\(^{nd} \) veins are not set to taper, \( K_{\text{leaf}} \) determines the conductivity of each 1\(^{st} + 2\(^{nd} \) conduit equal to the “1\(^{st} \) vein conductivity” value specified; to specify a desired conductivity for the base of the 1\(^{st} \) vein, one would parameterize the conductivity of each conduit as the desired value divided by the number of 2\(^{nd} \) veins in the leaf. If the 2\(^{nd} \) veins are set to taper, \( K_{\text{leaf}} \) determines the conductivity of the length of the 1\(^{st} \) vein portion of each 1\(^{st} + 2\(^{nd} \) conduit as that of the base of the 2\(^{nd} \) vein; to specify a desired conductivity for the base of the 1\(^{st} \) vein, one would parameterize the 2\(^{nd} \) vein conductivity as the desired value divided by the number of 2\(^{nd} \) veins. In our leaf sim-
Simulations, we implemented 2\textsuperscript{o} vein tapering for realism, except when we explicitly tested the effect of its removal. Simulations were modeled using either the terminal leaflet of a *Juglans regia* compound leaf (Cochard et al. 2004) or, for simulations of alteration of leaf size, an elliptical leaf with realistic proportions (table A2). Our findings should be applicable to other leaves with hierarchical, reticulate venation. Individual vein conductivities were based on estimations from xylem conduit lumen dimensions in *Juglans* vein cross sections using the formula

\[
\sum \frac{ma^4b^4}{64\eta(a^2 + b^2)^4},
\]

where \(a\) and \(b\) are the major and minor axes of ellipses, \(\eta\) is the viscosity of water at 25°C (normalized by path length; units are mmol m \textsuperscript{-1} MPa\textsuperscript{-1}; Lewis and Booce 1995; Cochard et al. 2004; Sack and Frole 2006).

Values of \(K_x\), \(K_{ox}\), and \(K_{leaf}\) were determined in typical units, normalized by leaf area (mmol m \textsuperscript{-1} s\textsuperscript{-1} MPa\textsuperscript{-1}) and plotted using SigmaPlot, version 10.0 (San Jose, CA). The relative responses of \(K_x\), \(K_{ox}\), and \(K_{leaf}\) to alteration of venation features in our simulations are expected to accurately indicate relative trends and principles of leaf venation design. However, the empirical values are not to be taken as meaningful, and units are not presented in our simulation results. For instance, the simulations based on the *Juglans* leaflet data set produced a \(K_x\) of 462 mmol m \textsuperscript{-3} s\textsuperscript{-1} MPa\textsuperscript{-1}, which is very high relative to experimentally measured \(K_x\) and many times greater than measured \(K_{ox}\) (Cochard et al. 2004). Cochard et al. (2004) introduced the “xylem hydraulic efficiency” parameter in \(K_{leaf}\) to calibrate the modeled \(K_x\) (XHE; modeled \(K_x\) divided by measured \(K_x\)) and account for other factors than xylem conduit numbers and diameters that cannot currently be modeled, such as pit membrane resistance (Sperry et al. 2005) or conduit blockage by embolism or tyloses (Salleo et al. 2002; Choat et al. 2005). In our simulations, XHE was set to 1. While not significant for the current study, future work should better reconcile modeled \(K_x\) with experimentally measured values (see “Discussion”).

**Applied Simulations in the Model**

**Modifying Vein Conductivities in Leaves without Hydraulic Hierarchy.** We tested the importance of vein hierarchy by
comparing the Juglans leaflet with "nonhierarchical" leaves that had equal conductivities assigned to all vein orders (fig. 1D, 1E). Chosen vein conductivities spanned the range for Juglans from the base of the 1st vein to that of the 6th veins. We then determined the impacts of increasing individual vein order conductivities in the nonhierarchical system. Beginning with equal conductivity in all veins (5.0 × 10⁻¹¹ mmol m⁻¹s⁻¹ MPa⁻¹, approximately that of the middle of a Juglans 2nd vein), we modified conductivities singly and in combinations (1st and 2nd; 1st, 2nd, and 3rd; etc.; see fig. 1D, 1E) over the same range.

Modifying Vein Conductivities in Leaves with Hydraulic Hierarchy. We determined the impacts of modifying conductivity in a hierarchical system by changing conductivities for each vein order in the Juglans leaflet. Individual vein conductivities were multiplied by 0.5, 1, 2, 3, or 4 singly, in consecutive combinations (1st and 2nd; 1st, 2nd, and 3rd), and by classification as lower-order veins (1st and 2nd) or higher-order veins (3rd and above).

Lower-Order Vein Tapering. To evaluate the effect of tapering of major vein conductivities, we compared the Juglans leaflet, which has tapering 1st and 2nd veins (fig. 1B), to simulated leaves otherwise identical but with uniform conductivities throughout the length of the 1st and/or 2nd veins (fig. 1F).

Modifying Leaf Size and Vein Densities. To test the effects of altering leaf size and vein densities, we conducted five sets of simulations. First, we constructed a series of elliptical leaves ranging 10-fold in area to hold length : width proportions approximately constant, as modifying the size of the more complex Juglans leaflet would have involved changing its shape. The larger elliptical leaves had their major veins spaced proportionally farther apart, and thus a lower major vein density, while minor vein density was held constant. Vein conductivities were set at Juglans values and also at theoretical values as a further test. Second, we tested the impact of altering 2nd density in the Juglans leaflet by fixing leaf size and increasing the number of 2nd veins (fig. 1G, 1H), thereby modifying 2nd density over an eightfold range with other vein densities constant. Third, we evaluated the impact of modifying minor vein density (3rd and higher) in the Juglans leaflet, while maintaining constant 1st and 2nd vein densities (fig. 1I, 1J). Fourth, we tested the impacts of simultaneously altering 2nd and minor vein densities in the Juglans leaflet (four 2nd vein densities × four minor vein densities). Fifth, we tested the impacts of simultaneously altering 2nd vein conductivity and minor vein density (four 2nd vein conductivities × four minor vein densities).

Estimating the Construction Cost of Alternative Vein Designs

We estimated the xylem construction costs of altering venation traits in nonsectorial systems, using a dimensionless index of cell wall volume per leaf area (CC). We assumed xylem conduits of different size to have similar wall thickness, as observed in anatomical studies of leaves and wood of given species and across species (Cochard et al. 2004, 2008; Pittermann et al. 2006; L. Sack, C. Havran, A. McKown, and C. Nakahashi, unpublished data). The CC relates to conduit perimeter:

\[
CC = \sum_{i=1}^{g} \pi \times d_i \times n_i \times D_i,
\]

where \(d_i\) and \(n_i\) are lumen diameter and number of conduits in vein order \(i\) and \(D_i\) is the vein density of that order. Using data for \(d_i\), \(n_i\), and \(D_i\) from Juglans leaflets (Cochard et al. 2004), we determined the percent increase in CC values and the percent change in \(K_c\) relative to construction cost \((K_c/CC)\) for each simulation relative to the control Juglans leaflet. For simulations of increased vein conductivity, we considered that this could arise from increases in \(n\) and/or \(d\). We calculated CC for two bounding scenarios, (A) increased \(d\) for a fixed \(n\) and (B) increased \(n\) of fixed \(d\). Across closely related species, higher vein conductivity tends to arise from both (Coomes et al. 2008; Dunbar-Co et al. 2009), and we did not consider more complex cases of increased conductivity via fewer, larger conduits or via more numerous, smaller conduits. In scenario A, for a given conductivity, we calculated \(d\) for a fixed \(n\) using Poiseuille’s law for round conduits at 20°C. Scenario A leads to a lower increase in CC for a given increase in conductivity than does scenario B: in scenario A, conductivity increases with \(d^4\) and CC increases linearly with \(d\), and thus, CC increases with conductivity; in scenario B, conductivity and CC both increase linearly with \(n\), and thus, CC increases linearly with conductivity. Notably, the range of CC values bounded by scenarios A and B includes the CC value that would occur, contrary to our assumption, the cell wall thickness were to increase linearly with \(d\) (see. Brodribb and Holbrook 2005). In that case, CC would increase with \(d^3\) and conductivity with \(d^4\) and, thus, CC with conductivity.

Determining the Relative Sensitivity of \(K_c\), to Venation Characters

To compare the sensitivity of \(K_c\) to different aspects of venation, for each character manipulated in our study we calculated a response index, the slope of log \(K_c\) plotted against the log value of the character across the range of simulations tested. This index reduced the scale depen-
idence of responses, thereby allowing comparisons of $K_s$ sensitivity to characters that varied over different absolute ranges. The response curves showed a range of structural forms, including linear, power law, or saturating (see “Results”). Thus, the response index would reduce but not completely remove the scale dependences of some responses (i.e., responses that showed a saturating behavior).

**Results**

**Impact of Sectoriality versus Nonsectoriality**

There was no impact of sectoriality per se on $K_s$ and $K_{ref}$. In our comparison of simulated sectorial and nonsectorial leaves with matched conductivity at the base of the 1° vein (see “Methods”), leaves had equivalent $K_s$ and $K_{ref}$ (table 1).

**Impact of Vein Hierarchy and Modifying Vein Conductivities in Nonhierarchical Systems**

Hierarchy of vein orders provided a benefit relative to cost. In simulations of nonhierarchical leaves (i.e., with equal vein conductivities assigned across orders; see fig. 1D, 1E), the $K_s$ of the control *Juglans* leaflet (horizontal line in fig. 2A) was achieved when vein conductivity was $5.0 \times 10^{-3}$ mmol m s$^{-1}$ MPa$^{-1}$, or approximately that of the tip of the 1° or the middle of a 2° vein in *Juglans*. The hierarchical *Juglans* leaflet venation had a 15-fold higher $K_s$/CC than the nonhierarchical leaf of equivalent $K_s$.

Increasing vein conductivities had a strong effect in leaves lacking vein order hierarchy. Modifying individual vein order conductivities resulted in diminishing returns in $K_s$ with the effect size depending on vein order (fig. 2C, 2D). Different patterns arose for nonsectorial and sectorial leaves. In the nonsectorial leaf, increasing 5° vein conductivity caused a dramatic increase in $K_s$ (fig. 2C). This pattern evidently arose because the higher-order veins branch off to become 1° vein in high densities, representing a larger number of parallel exit pathways. In the sectorial leaf, however, increasing the 1° + 2° conductivity had greatest impact (fig. 2D). In both cases, modifying other vein conductivities also increased $K_s$, but shallowly and with rapid saturation. Increasing the conductivity of vein orders in sequential groups demonstrated that the effect of changing conductivity in multiple vein orders on $K_s$ was additive (fig. 2E, 2F), and the increase was linear when all vein orders were included (fig. 1D, 1E; fig. 2A). Notably, $K_s$ was higher for the sectorial than for the nonsectorial leaf, reflecting these leaves' different vascular construction, as the sectorial leaf had multiple conduits along most of the 1° length, each with the same conductivity as the 1° vein in the nonsectorial leaf (see “Methods”).

Although increasing all vein conductivities led to a linear increase of $K_s$, it had a saturating impact on $K_{ref}$ (fig. 2B). This occurred because $K_{ref}$ in the model was set much lower than $K_s$ originally, and as $K_s$ increased, $K_{ref}$ became limiting for $K_{ref}$ (see eq. [1]). Increasing $K_s$ or $K_{ref}$ alone thus produced a bottleneck in $K_{ref}$. The diminishing impact of venation traits on $K_{ref}$, due to the declining role of $K_s$, was found in all our trait manipulations, with the notable exception of minor vein density, which also affected $K_{ref}$ (see following sections).

**Modifying Vein Conductivities in Hierarchical Systems**

In leaves with hydraulic hierarchy (based on the *Juglans* leaflet; fig. 1A, 1D), whether nonsectorial or sectorial, increasing the conductivities of individual vein orders led to qualitative impacts on $K_s$ similar to those for sectorial, nonhierarchical venation (fig. 3A, 3B). The greatest effect on $K_s$ was caused by increasing the 1° and 2° conductivities, followed by those of higher-order veins in sequence (fig. 3A, 3B). Higher-order veins showed a more rapid saturation than the 1° and 2° vein orders. In the sectorial leaf, the conductivity of 1° and 2° veins increased together because the conduits were continuous across those vein orders. In a comparable test with the nonsectorial leaf, increasing the 1° and 2° conductivities together achieved the same effect relative to other vein orders (diamonds, fig. 3A).

As observed in the nonhierarchical vein system, increasing the conductivity of individual vein orders in combinations had an additive impact on $K_s$. Modifying the conductivity of all vein orders led to a linear increase in $K_s$ for both nonsectorial and sectorial leaves (fig. 3C, 3D). When veins were grouped as “major” or “minor” veins, increasing the conductivity of the major veins (1° and 2°) had a disproportionate impact on $K_s$ (fig. 3E, 3F).

The CC increased with increasing vein conductivity, more strongly for the lower-order veins, due to their a priori larger conduits, than for minor veins (table 2). The

| Table 1: Modeled $K_s$ and $K_{ref}$ values comparing nonsectorial and sectorial in *Juglans regia* leaflet simulations |
|---|---|---|
| Leaf vein design | $K_s$ | $K_{ref}$ |
| *Juglans* leaflet (nonsectorial)$^a$ | 58 | 8.33 |
| Test leaf, sectorial$^b$ | 295 | 12.74 |
| Test leaf, nonsectorial$^c$ | 298 | 12.75 |

$^a$ Values from simulated *Juglans* leaflet calibrated from anatomical measurements (Cochard et al. 2004).

$^b$ The sectorial test leaf was parameterized in the $K_s$ leaf program as for the *Juglans* leaflet but with sectoriality implemented in the 1° and 2° veins. The $K_s$ is higher because the parameterization of 1° and 2° conductivity is different; one inputs as the 2° conductivity the conductivity of the "1° + 2°" conduits, which run along the 1° vein and branch off to become 2° veins, rather than the conductivity of the whole 1° vein.

$^c$ Conductivity was matched with that of the sectorial leaf at the base of the 1° vein.
The American Naturalist

Figure 2: Response of leaf xylem hydraulic conductance ($K_x$) and whole-leaf hydraulic conductance ($K_{\text{leaf}}$) for simulations of leaves without vein order hierarchy. A, B. Responses of $K_x$ and $K_{\text{leaf}}$, respectively, to increases in conductivities of all vein orders in a nonsectorial leaf and a sectorial leaf. Horizontal lines represent the modeled $K_x$ of the hierarchical Juglans leaflet for comparison. C, D. Responses of $K_x$ to increases in conductivity of individual vein orders in a nonsectorial leaf and a sectorial leaf, respectively. E, F. Responses of $K_x$ to increases in conductivity of sequential groups of vein orders in a nonsectorial leaf and a sectorial leaf, respectively.

$K_x/CC$ was also sensitive, typically declining when higher conductivity was achieved with more numerous conduits of fixed size but increasing when it was achieved by widening a fixed number of conduits (table 2). The $K_x/CC$ increased most strongly when conductivity was increased in lower-order veins, and $K_x/CC$ increased by 68% when all vein conductivities were modified together.

**Tapering in Major Veins**

In general, tapering of the major veins imposed a marginal limitation of $K_x$. A small increase in $K_x$ was achieved by increasing the conductivity of 1$^o$ and 2$^o$ veins along their lengths to their maximum conductivity (i.e., that of their bases; fig. 1B, 1F). Removing tapering in this way for the 1$^o$ veins, 2$^o$ veins, and both orders increased $K_x$ by 10%, 3%, and 14%, respectively (fig. A1 in the online edition of the American Naturalist). By contrast, $K_x$ decreased substantially when the conductivity along the 1$^o$ and/or 2$^o$ veins was reduced to that of the middle or apical portion of the 1$^o$ and 2$^o$ veins. Using data available for conduit numbers and diameters at four points along the length of the 1$^o$ vein in the Juglans leaflet (H. Cochard, unpublished data), the leaflet with a tapering 1$^o$ vein had a 15% lower...
Figure 3: Response of leaf xylem hydraulic conductance ($K_x$) for simulations of leaves with hierarchy of vein order conductivities based on the *Juglans* leaflet. A, B, Responses of $K_x$ to increases in conductivity of individual vein orders (plus 1° and 2° order veins together) in a nonsectorial leaf and a sectorial leaf, respectively. C, D, Responses of $K_x$ to increases in conductivity of sequential groups of vein orders in a nonsectorial leaf and a sectorial leaf, respectively. E, F, Responses of $K_x$ to increases in conductivity of categorical groups of vein orders in a nonsectorial leaf and a sectorial leaf, respectively.

CC, resulting in a 6% higher $K_x$/CC than a leaf with the conductivity along the length of the 1° vein increased to that of its base.

**Modifying Vein Densities: Major Veins, 2° Order Veins, and Minor Veins**

Increasing vein densities led to an increase of $K_x$ in leaves with fixed vein conductivities, whether the higher vein density was achieved by modifying leaf size or by increasing vein length. Increasing leaf area, such that major veins were spaced farther apart, while holding minor vein densities fixed led to a decline of 1° and 2° densities (fig. 4A inset; slopes of log vein density vs. log leaf area were $-0.51$ and $-0.48$, respectively, lines fitted as standard major axes; Sokal and Rohlf 1995). This decline was geometric, as vein density (length per area) should decrease with the square root of leaf area, as typically found for 1° and 2° vein densities across species with leaves varying in size (L. Sack, unpublished data). Decreasing major vein density led to a strong decrease of $K_x$ with leaf area (fig. 4A, inset). In accordance with these patterns, $K_x$ increased strongly with vein density across leaves varying in size (fig. 4B). Similar results were obtained for sectorial leaves and for leaves
Table 2: Estimated increase in xylem construction costs (CC, dimensionless) and change in vein xylem hydraulic conductance relative to CC ($K_x/CC$) when doubling values for given vein traits in *Juglans regia* leaflet simulations (nonsectorial)

<table>
<thead>
<tr>
<th>Venation trait</th>
<th>Increase in CC with doubling of trait (%)</th>
<th>Change in $K_x/CC$ with doubling of trait (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doubling conductivity in:</td>
<td>Scenario A</td>
<td>Scenario B</td>
</tr>
<tr>
<td>All vein orders</td>
<td>19</td>
<td>100</td>
</tr>
<tr>
<td>1° vein</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>2° veins</td>
<td>5</td>
<td>28</td>
</tr>
<tr>
<td>3° veins</td>
<td>2</td>
<td>11</td>
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<tr>
<td>4° veins</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>5° veins</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>6° veins</td>
<td>.004</td>
<td>2</td>
</tr>
<tr>
<td>Major veins</td>
<td>11</td>
<td>59</td>
</tr>
<tr>
<td>Minor veins</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>Doubling 2° vein density by increasing 2° vein number</td>
<td>24</td>
<td>+30</td>
</tr>
<tr>
<td>Doubling minor vein density</td>
<td>28</td>
<td>+7</td>
</tr>
<tr>
<td>Keeping major vein density constant</td>
<td>26</td>
<td>+109</td>
</tr>
</tbody>
</table>

* For simulations of increases in vein conductivity, we present two bounding scenarios, A and B, for the impacts on CC and $K_x/CC$ values (see "Methods"). In scenario A, higher vein conductivity was achieved by increasing the diameter of conduits for a fixed number of conduits. In scenario B, higher vein conductivity was achieved by increasing the number of conduits of fixed diameter. Scenario A leads to an increase of $K_x/CC$, while scenario B often leads to a reduction of $K_x/CC$, because CC actually increases more than $K_x$ and therefore the percent change in $K_x/CC$ is a negative number.

with different conductivities (data not shown). Halving the size of the leaf, thereby increasing the 1° and 2° vein densities, while keeping minor vein density and vein conductivities fixed led to a 24% increase in the (leaf area-specific) CC but to a 30% increase in $K_x/CC$ (table 2).

Increasing the 2° vein density of the *Juglans* leaflet by adding 2° veins (fig. 1G, 1H) led to a rapidly saturating increase of $K_x$ and $K_{x\text{tot}}$ (fig. 5A, 5B). This same response was observed for nonsectorial and sectorial leaves with different parameterizations (data not shown). The saturation response of $K_x$ with increasing 2° vein number appeared similar to that obtained by increasing 2° vein conductivity (see above). Increasing 2° vein density involved a substantial cost, as doubling the 2° vein density in the *Juglans* leaflet led to a 28% increase in CC and only a 7% increase in $K_x/CC$ (table 2).

Increasing minor vein density led to higher $K_s$ and $K_{s\text{tot}}$ in simulations using *Juglans* leaflet data (fig. 5C, 5D) and alternative parameterizations (data not shown). The $K_s$ increased linearly over a wide range of minor vein densities. By contrast, $K_{s\text{rel}}$ showed an accelerating increase with minor vein density (fig. 5C, inset) because the number of mesophyll resistors increased with the grid junctions per area and thus with the second power of vein length per area. These responses in both $K_s$ and $K_{s\text{rel}}$ resulted in an overall accelerating response of $K_{s\text{tot}}$ (fig. 5D), which contrasted with the saturating response in $K_{s\text{rel}}$ typically observed when altering characters that influenced $K_s$ (see above). Notably, the shape of the $K_{s\text{tot}}$ response would depend on whether $K_s$ or $K_{s\text{rel}}$ was a greater limitation to $K_{s\text{tot}}$; in our simulations, $K_{s\text{rel}}$ was much lower than $K_s$ and thus $K_{s\text{rel}}$ and $K_{s\text{tot}}$ showed identical responses (fig. 5C, inset, 5D). Increasing minor vein density also carried a substantial cost, and doubling the *Juglans* leaflet minor vein density increased its CC by 26%, but $K_x/CC$ increased by 109% (table 2). Further, because venation density affects both $K_s$ and $K_{s\text{rel}}$, the increase in $K_{s\text{rel}}$ relative to CC would be greater than that of other venation traits, which affect $K_s$ alone.

Increasing both 2° and minor vein densities together led to a synergistic effect on $K_s$ (fig. 5E, 5F). The same pattern appeared in similar tests increasing 2° vein conductivity over the range of 1.0 $\times$ 10^{-7} to 1.0 $\times$ 10^{-1} mmol m s^{-1} MPa^{-1} together with minor vein density while keeping 2° vein density fixed (data not shown). As demonstrated above, increasing either 2° vein density or conductivity led to diminishing returns for $K_s$ and a saturating response for $K_{s\text{rel}}$ while increasing minor vein density led to a linear increase of $K_s$ and an accelerating increase of $K_{s\text{rel}}$. Increasing both together produced an accelerating impact on $K_s$ and $K_{s\text{rel}}$.

**Summarizing the Relative Sensitivity of $K_s$ to Different Venation Traits**

We determined the relative sensitivity of $K_s$ to changes in each venation trait (fig. 6). As discussed above, $K_s$ was
Figure 4: Response of xylem hydraulic conductance ($K_x$) to modifying leaf area for elliptical leaves with fixed vein xylem conductivities based on the Juglans leaflet (nonsectorial); larger leaves have 1° and 2° order veins spaced farther apart. A, $K_x$ versus leaf area; schematic shows vein density declining in larger leaves. Inset shows 1° and 2° vein density versus leaf area. B, $K_x$ versus 1° and 2° vein density; schematic shows that greater major vein densities correspond to smaller leaves.

more strongly affected by increasing the conductivity of major than minor veins, and increasing the conductivity of all vein orders together had an additive impact. The $K_x$ was very sensitive to leaf size reductions, which modified 1° and 2° vein densities. An increase of $K_x$ of comparable magnitude could be achieved with increases in conductivity across all vein orders, in 2° vein density, or in minor vein density. In order of $K/CC$ increase, from highest to lowest, traits ranked (1) minor vein density, (2) all vein order conductivities, (3) altering 1° and 2° density (by reducing leaf size), (4) low-order individual vein conductivities, (5) 2° vein density, and (6) high-order individual vein conductivities (table 2).

Discussion

Using a spatially explicit model, we isolated impacts of altering venation architecture traits, individually and combined, in simulated leaves. Our modeled results correspond with previously reported findings for variation of venation traits across diverse species and can be used to generate further evolutionary and ecological hypotheses. In general, modifications leading to higher $K_x$ and $K_{leaf}$ can contribute to greater photosynthetic rates in leaves and to faster growth for a given leaf area allocation and, thus, should be adaptive in environments with higher resource supplies when there is a high return for vascular construction costs (Sack et al. 2005). By contrast, modifications leading to lower $K_x$ and $K_{leaf}$ should be beneficial for carbon balance in lower resource conditions by reducing construction costs. We assessed impacts on $K_x$ and on $K/CC$, indices respectively of venation hydraulic capacity and of capacity relative to cost. Which of the two would be more important may depend on the context; we assume here that any trait modification that benefits either one should be adaptive in higher resource environments, especially if it benefits both.

Hydraulic Importance of Vein Order Hierarchy and Sectoriality

Vein hierarchy may constitute a key innovation in the evolution of high $K_{leaf}$. We found that the $K_x$ of the Juglans leaflet could be matched by that of a leaf without hierarchy, but the hierarchy conferred a 15-fold higher $K/CC$ due to reduction of lignified tissues. The advantage of vein order hierarchy would extend to further evolution of the system, as increasing vein conductivity is cheaper for hierarchical leaves, with smaller higher-order veins (see below). Notably, hierarchical vein systems evolved mainly in the angiosperms (Roth-Nebelsick et al. 2001), where they diversified strongly, including species with high $K_{leaf}$ and rapid photosynthetic rates under high-resource conditions.

Sectoriality within the major veins might also act as a means to evolve high transport capacity relative to cost. Sectoriality by itself did not affect $K_x$; however, sectoriality did impose a “hierarchical behavior” on nonhierarchical venation. In a nonhierarchical venation system with sec-
toriality, increasing the conductivity of low-density major veins allowed a rapid response in $K_x$, cheaply, compared with increasing the conductivity of high-density minor veins. Sectoriality may also confer tolerance to leaf damage or limit the spread of embolism during damage or drought (Orians et al. 2005; Schenk et al. 2008).

**Hydraulic Importance of Vein Order Conductivities**

We demonstrated that $K_\text{x}$ and $K_\text{x}/CC$ respond strongly to modifying conductivities of vein orders, with different impacts across vein orders. In nonhierarchical, nonsectorial leaves, $K_\text{x}$ was most strongly affected by increasing con-
Decoding Leaf Venation Architecture

Figure 6: Response index of $K_c$ to simulations of modifications of vein traits in *Juglans* leaves. Index calculated as the log-log slope of $K_c$ versus the trait value; positive values indicate a positive response of $K_c$ to an increase in the trait values. *White* = nonsectorial leaf; *gray* = sectorial leaf.

ductivity of the highest-density vein order. In hierarchical leaves with or without sectoriality and nonhierarchical leaves with sectoriality, $K_c$ was most strongly affected by increasing lower-order vein conductivity. Hierarchical reticulate venation has components arranged in series and in parallel, leading to a division of labor akin to "supply" and "distribution" lines in irrigation systems (Cuenca 1989), whereby high-conductivity lower-order veins take the role of supply veins, and less conductive, redundant higher-order veins serve as parallel distribution pathways. The finding that $K_c$ is most affected by increasing lower-order vein conductivity is noteworthy because it contrasts with the expected behavior of circuits with components in series. In such systems, the most resistant component is most limiting, and reducing its resistance by a given factor has the greatest impact in reducing overall resistance (Meinzer 2002). In leaves, lower-order veins are the least resistant component, but reducing their resistance (i.e., increasing their conductance) has the greatest effect on $K_c$. The dramatic increase of $K_c$ and relatively high increase in $K_c/CC$ conferred by increasing the 1$^o$ vein conductivity suggest a powerful evolutionary mechanism and explain why $K_c$ correlates with 1$^o$ vein conductivity in sets of trees and grasses (Sack and Frolo 2006; Maherali et al. 2008). A second implication of these findings is that damage or blockage of the 1$^o$ vein in a pinnately veined leaf should dramatically reduce $K_c$ and $K_{cat}$, as has been observed experimentally for several species (Nardini and Salleo 2003; Sack et al. 2003b, 2008).

In our simulations, increasing conductivity of a single vein order led to diminishing returns in $K_c$ due to other emerging bottlenecks in the system. A linear increase in $K_c$ was accomplished only by increasing the conductivity of all vein orders simultaneously, which also strongly increased $K_c/CC$. Proportional modification of conductivity in all vein orders may be common in the evolution of higher $K_c$ and warrants further attention. Across 10 species of *Quercus*, the conduit hydraulic diameters in the petiole and in the 1$^o$ and 2$^o$ veins scaled linearly (Coomes et al. 2008), indicating coordinated evolutionary changes in conductivities of multiple vein orders.

**Hydraulic Importance of Major Vein Tapering**

Our simulations showed that major vein tapering significantly increased $K_c/CC$, reducing CC more than $K_c$ and thus providing benefit relative to cost. These results would explain widespread tapering of 1$^o$ and 2$^o$ veins in leaves (Jeje 1985; Canny 1990), and they correspond with analytical studies that also showed tapering improved hydraulic capacity relative to cost (McCulloh et al. 2003, 2004; McCulloh and Sperry 2005).

**Hydraulic Importance of Leaf Size, 2$^o$ Vein Number, and Major Vein Density**

Reducing leaf size, thereby increasing major vein density, drove a nearly linear increase of $K_c$ and $K_{cat}$ and a greatly increased $K_c/CC$. Thus, $K_c$ and $K_{cat}$ should decline with increasing leaf size, given major vein density declines simultaneously, if other traits are constant. Such a scenario may arise in some evolutionary radiations; for eight Hawaiian *Viola* taxa, $K_{cat}$ correlated negatively with leaf size and positively with major vein density (L. Sack, C. Havran, A. McKown, and C. Nakahashi, unpublished data). However, leaf size is a key trait affecting many other aspects of leaf and canopy function besides $K_c$ and manifests strong plasticity and adaptation (Givnish 1987); thus, solely altering leaf size is unlikely to be a general mechanism for evolving higher $K_c$ or $K_{cat}$. In many cases, the relationship is not found. The $K_{cat}$ correlated negatively with leaf size for exposed leaves in only three of eight diverse woody species tested (Sack et al. 2004; Scoffoni et al. 2008; L. Sack, unpublished data), and no correlation was found for two grass species (Meinzer and Grantz 1990;
Martre et al. 2001) or across 10 diverse tropical rainforest tree species (Sack and Frole 2006). The general independence of $K_c$ and $K_{leaf}$ from leaf size allows high $K_{leaf}$ and high rates of gas exchange per area to occur in small as well as large leaves and probably arises from compensatory changes to other traits. Such compensation may be common: across 10 Quercus species and seven Hawaiian Plantago taxa, larger-leaved species had higher vein conductivities (Coomes et al. 2008; Dunbar-Co et al. 2009).

Increasing $2^\circ$ vein numbers and thereby $2^\circ$ vein density also caused an increase in $K_c$. However, this response showed saturation, in contrast to the linear response found when increasing major vein density via reducing leaf size. Increasing $2^\circ$ vein numbers would lead other major veins to become increasingly limiting to $K_c$. The saturating effect on $K_c$ and low increase in $K_c/CC$ suggest that increasing $2^\circ$ vein numbers would be a weak evolutionary mechanism to achieve higher $K_c$. This prediction concurs with findings for sets of temperate and tropical woody species, where $K_c$ and $K_{leaf}$ did not correlate with $2^\circ$ vein number or density (Sack and Frole 2006; Sack et al. 2008). We note that a high density of $2^\circ$ veins represents redundancy that may confer tolerance of hydraulic disruption by damage or blockage (Sack et al. 2008).

**Hydraulic Importance of Minor Vein Density**

Increasing minor vein density had strong hydraulic effects, driving a linear increase in $K_c$ an accelerating increase in $K_{leaf}$ and the greatest increase in $K_c/CC$ of all vein traits. In our model, as in real leaves, the xylem and outside-xylem pathways are in series (Cochard et al. 2004), and increasing minor vein density not only increased $K_c$ by adding additional xylem flow routes in parallel but also influenced $K_m$ by increasing parallel exit routes from the xylem. Because minor vein density affected both $K_m$ and $K_{leaf}$ it also should have the highest cost-effectiveness for increasing $K_{leaf}$. This impact is consistent with the observed correlations of $K_{min}$ and $K_{leaf}$ with minor vein density across diverse species (Sack and Frole 2005).

In real leaves, a higher minor vein density may also increase $K_{min}$ by shortening the mesophyll water paths. Across a diverse species set, $K_{leaf}$ correlated negatively with the “mesophyll distance” ($D_m$), a proxy for the mesophyll water path length, calculated as the hypotenuse of the “horizontal distance” between veins (a negative correlate of minor vein density), and the “vertical distance” between vein and stoma (Brodribb et al. 2007). The potential importance of both measures in determining $K_{leaf}$ was supported by a physical leaf model (Noblin et al. 2008). Future work is necessary to determine the relative importance of the various contributions of high minor vein density to $K_{leaf}$ in real leaves (i.e., the greater number of parallel xylem and/or outside-xylem flow pathways, greater permeable xylem surface area, and/or shorter outside-xylem pathways). Other traits would also affect “mesophyll conductance” (thereby modifying $K_m$ and thus $K_{leaf}$), including more conductive flow pathways through bundle sheath, mesophyll, or epidermis and the development of bundle sheath extensions in heteroblastic leaves, which may be important in conducting water from veins to epidermis (Sack and Holbrook 2006; Kenzo et al. 2007; Zwieniecki et al. 2007).

**Linking Venation Architecture with Leaf and Plant Performance**

Any of the traits shown in this study to increase $K_c$ or $K_{leaf}$ have potential for predicting how venation traits should influence hydraulic capacity and photosynthesis per leaf area. Thus, higher vein densities and conductivities may be expected to evolve in higher resource supply environments, and vein hierarchy and tapering in angiosperms indicate selection for efficient hydraulic design. Further empirical work can also determine the precise degree to which individual venation traits influence photosynthetic rates per leaf area in given lineages, as well as whole-plant-level traits such as growth and water use.

The importance of a given trait in “driving” differences in $K_c$ or $K_{leaf}$ should depend on its relative variability in a lineage, and we note that species, lineages, and/or communities will differ in the importance of particular venation traits in determining $K_c$ and $K_{leaf}$. However, unlike the simulation approach used here, in which individual venation traits were manipulated while others were held constant, evolution can generate variation in many traits simultaneously. In this study, traits varied in the range of sensitivity of their response, with some combinations of traits affecting $K_c$ in series, leading to colimitation, and others affecting $K_c$ in parallel, with orthogonal, additive effects. Venation traits thus determine $K_c$ through a complex combination of factors, and some may have functional equivalence (i.e., high vein conductivities compensating to some degree for low vein densities). Similarly, real leaves may achieve high or low $K_{leaf}$ through alternative vein trait combinations, just as models have shown that equivalent function in whole organisms can be achieved by multiple trait combinations or alternative designs (Marks and Lechowicz 2006; Wainwright 2007). We expect that sustained selection may produce coordinated changes in multiple features that influence $K_c$ and $K_{leaf}$ in the same direction. Optimizing higher capacity in a network of resistance components in series produces relatively even colimitation by components, as found for distribution of resistances between stem xylem lumen and endwall resistances (Sperry et al. 2005) and between $R_c$ and $R_m$ in leaves (Sack et al. 2005; Noblin et al. 2008). Such selection on
multiple components simultaneously would also be effective for components with impacts in parallel (i.e., vein conductivities and vein densities).

There are numerous future avenues for investigation of the role of leaf venation in determining plant function and its potential for estimating from fossils the physiology and ecology of past vegetation and environments (Uhl and Mosbrugger 1999; Royer et al. 2007; Boyce et al. 2009). Our study shows that variation in these features confers responses in $K_s$, $K_{on}$, and $K_{off}$ that probably influence photosynthetic rate and water use. Further work is needed to clarify the additional, biomechanical functions of venation and to refine the measurement of vascular construction costs. Another exciting area for future research is the determination of the constraints on the evolution of vascular architecture, given that changes must occur within stable genetic and developmental programs for vein formation during leaf expansion (Prusinkiewicz 2004; Runions et al. 2005; Rolland-Lagan et al. 2009).

The $K_{leaf}$ model constitutes a first-step hypothesis, and further work is necessary to model additional venation scenarios not covered here. For example, work is needed to elucidate the importance of reticulate relative to nonreticulate (open-branching) venation. Reticulate venation has evolved many times and potentially improves local water distribution at the cell scale and/or tolerance of mechanical or insect damage (Roth-Nebelsick et al. 2001; Sack et al. 2008), but nonreticulate venation still exists in many ferns and in Ginkgo. We note that further modeling is also needed of other arrangements of the outside-xylem pathways. Our study did not consider the possibility of variation in mesophyll conductance across the leaf lamina, that is, among tissues or vein orders, or of multiple reticulate flow paths through the mesophyll. With further experimental data, modeling scenarios can help resolve the functional consequences of variation in all elements, inside and outside the xylem, that contribute to the hydraulic capacity of the leaf.

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