

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 venation 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.

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The quantity K_{leaf} is determined by the conductances of both the xylem (K_x) and outside-xylem pathways (K_{ox}):

$$\frac{1}{K_{\text{leaf}}} = \frac{1}{K_x} + \frac{1}{K_{\text{ox}}}. \quad (1)$$

Terms K_x and K_{ox} can be of similar magnitude. Thus, both are important in determining K_{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_{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_{leaf} . Across diverse species, K_{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_{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_{leaf} 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_{leaf} . 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_{leaf}

Leaf simulations were generated using the program K_{leaf} , version 6 (written by H. Cochard, Institut National de la Recherche Agronomique, Clermont-Ferrand, France; Cochard et al. 2004). K_{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_{ox} , and K_{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_{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_{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_{leaf} , only the 1° and 2° veins can be treated individually; the 3° and higher veins form a mesh, and their densities can be modified only as a group.

We investigated the impact of sectoriality in 1° and 2° veins on K_x and K_{leaf} . 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° vein a single conduit that was open to all branching 2° 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°, 2°, and sometimes 3° veins (Larson 1984; Chatelet et al. 2006; Sack and Frole 2006). K_{leaf} , version 6, can designate leaves “nonsectorial,” as do previous models (fig. 1B), or as fully sectorial with multiple independent xylem conduits running through the 1° vein and each conduit branching off to become a 2° vein (fig. 1C).

K_{leaf} can also simulate leaves with or without vein tapering along the 1° and 2° veins, reflecting diminishing xylem conduit size and number (fig. 1B). In the modeled sectorial leaf, tapering is automatic in the 1° vein because conductivity is highest at the base and diminishes axially as conduits branch off to form 2° veins. The conductivity of these “1° + 2°” conduits in sectorial leaves can be set in two different ways, depending on whether the 2° veins are set to taper. If the 2° veins are not set to taper, K_{leaf} determines the conductivity of each 1° + 2° conduit equal to the “1° vein conductivity” value specified; to specify a desired conductivity for the base of the 1° vein, one would parameterize the conductivity of each conduit as the desired value divided by the number of 2° veins in the leaf. If the 2° veins are set to taper, K_{leaf} determines the conductivity of the length of the 1° vein portion of each 1° + 2° conduit as that of the base of the 2° vein; to specify a desired conductivity for the base of the 1° vein, one would parameterize the 2° vein conductivity as the desired value divided by the number of 2° veins. In our leaf sim-

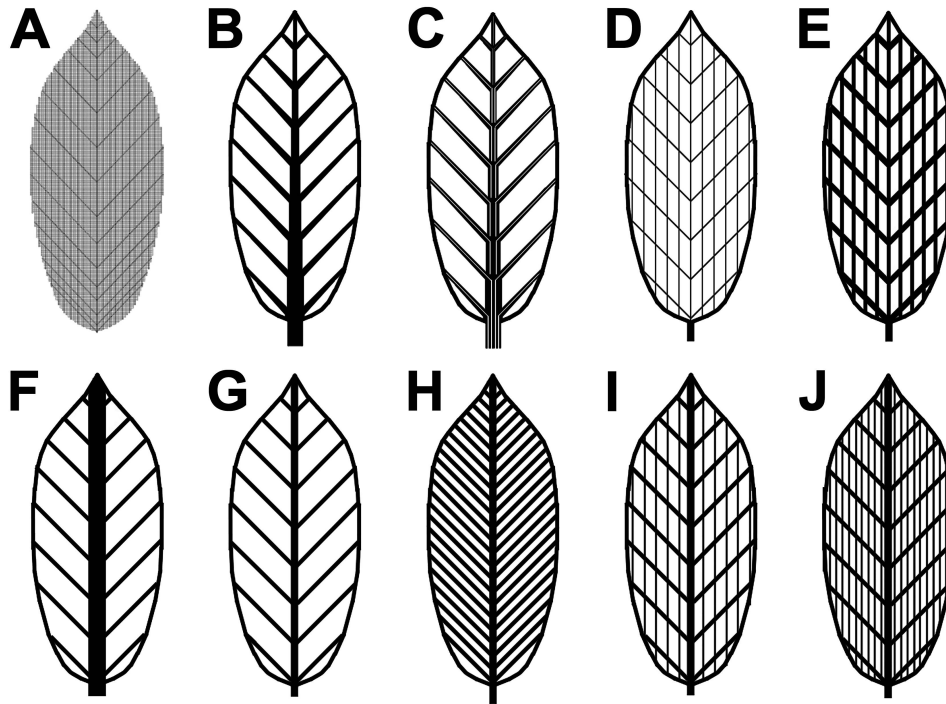


Figure 1: Schematics of simulated leaves. *A*, *Juglans* leaflet simulated by the program *K_leaf* showing all six vein orders; *B*, leaf with 1° and 2° order veins tapering; *C*, leaf with sectoriality in the 1° and 2° veins; *D*, no vein order hierarchy with low vein conductivity; *E*, no vein order hierarchy with high conductivity; *F*, no 1° or 2° vein tapering; *G*, low 2° vein density; *H*, high 2° vein density; *I*, low 3° vein density; *J*, high 3° vein density. Note: for simulations shown in *I* and *J*, the higher-order veins (4° and above) also increased in density along with the 3° veins but are not illustrated.

ulations, we implemented 2° 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

$$\sum \frac{\pi a^3 b^3}{64\eta(a^2 + b^2)}, \quad (2)$$

where a and b are the major and minor axes of ellipses, and η is the viscosity of water at 25°C (normalized by path length; units are $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$; Lewis and Boose 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 ($\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) 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 accu-

rately 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 $462 \text{ mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$, 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 1° vein to that of the 6° veins. We then determined the impacts of increasing individual vein order conductivities in the nonhierarchical system. Beginning with equal conductivity in all veins ($5.0 \times 10^{-3} \text{ mmol m s}^{-1} \text{ MPa}^{-1}$, approximately that of the middle of a *Juglans* 2° vein), we modified conductivities singly and in combinations (1° and 2°; 1°, 2°, and 3°; 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 (1° and 2°; 1°, 2°, and 3°), and by classification as lower-order veins (1° and 2°) or higher-order veins (3° and above).

Lower-Order Vein Tapering. To evaluate the effect of tapering of major vein conductivities, we compared the *Juglans* leaflet, which has tapering 1° and 2° veins (fig. 1B), to simulated leaves otherwise identical but with uniform conductivities throughout the length of the 1° and/or 2° 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 2° density in the *Juglans* leaflet by fixing leaf size and increasing the number of 2° veins (fig. 1G, 1H), thereby modifying 2° density over an eightfold range with other vein densities constant. Third, we evaluated the impact of modifying minor vein density (3° and higher) in the *Juglans* leaflet, while maintaining constant 1° and 2° vein densities (fig. 1I, 1J). Fourth, we tested the impacts of simultaneously altering 2° and minor vein densities in the *Juglans* leaflet (four 2° vein densities \times four minor vein densities). Fifth, we tested the impacts of simultaneously altering 2° vein conductivity and minor vein density (four 2° vein conductivities \times 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:

$$\text{CC} = \sum_{i=1}^6 \pi \times d_i \times n_i \times D_i, \quad (3)$$

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_p , n_p , and D_i from *Juglans* leaflets (Cochard et al. 2004), we determined the percent increase in CC values and the percent change in K_x relative to construction cost (K_x/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^{1/4}; 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 if, 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^2 and conductivity with d^4 and, thus, CC with conductivity^{1/2}.

Determining the Relative Sensitivity of K_x to Venation Characters

To compare the sensitivity of K_x to different aspects of venation, for each character manipulated in our study we calculated a response index, the slope of $\log K_x$ plotted against the log value of the character across the range of simulations tested. This index reduced the scale depen-

dence of responses, thereby allowing comparisons of K_x 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_x and K_{leaf} . 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_x and K_{leaf} (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_x of the control *Juglans* leaflet (horizontal line in fig. 2A) was achieved when vein conductivity was $5.0 \times 10^{-3} \text{ mmol m s}^{-1} \text{ MPa}^{-1}$, 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_x/CC than the nonhierarchical leaf of equivalent K_x .

Increasing vein conductivities had a strong effect in leaves lacking vein order hierarchy. Modifying individual vein order conductivities resulted in diminishing returns in K_x , 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_x (fig. 2C). This pattern evidently arose because the higher-order veins branch off the 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_x , 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_x was additive (fig. 2E, 2F), and the increase was linear when all vein orders were included (fig. 1D, 1E; fig. 2A). Notably, K_x 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

Table 1: Modeled K_x and K_{leaf} values comparing nonsectoriality and sectoriality in *Juglans regia* leaflet simulations

Leaf vein design	K_x	K_{leaf}
<i>Juglans</i> 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_{leaf} program as for the *Juglans* leaflet but with sectoriality implemented in the 1° and 2° veins. The K_x 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.

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

Modifying Vein Conductivities in Hierarchical Systems

In leaves with hydraulic hierarchy (based on the *Juglans* leaflet; fig. 1A, 1I), whether nonsectorial or sectorial, increasing the conductivities of individual vein orders led to qualitative impacts on K_x similar to those for sectorial, nonhierarchical venation (fig. 3A, 3B). The greatest effect on K_x 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_x . Modifying the conductivity of all vein orders led to a linear increase in K_x 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_x (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

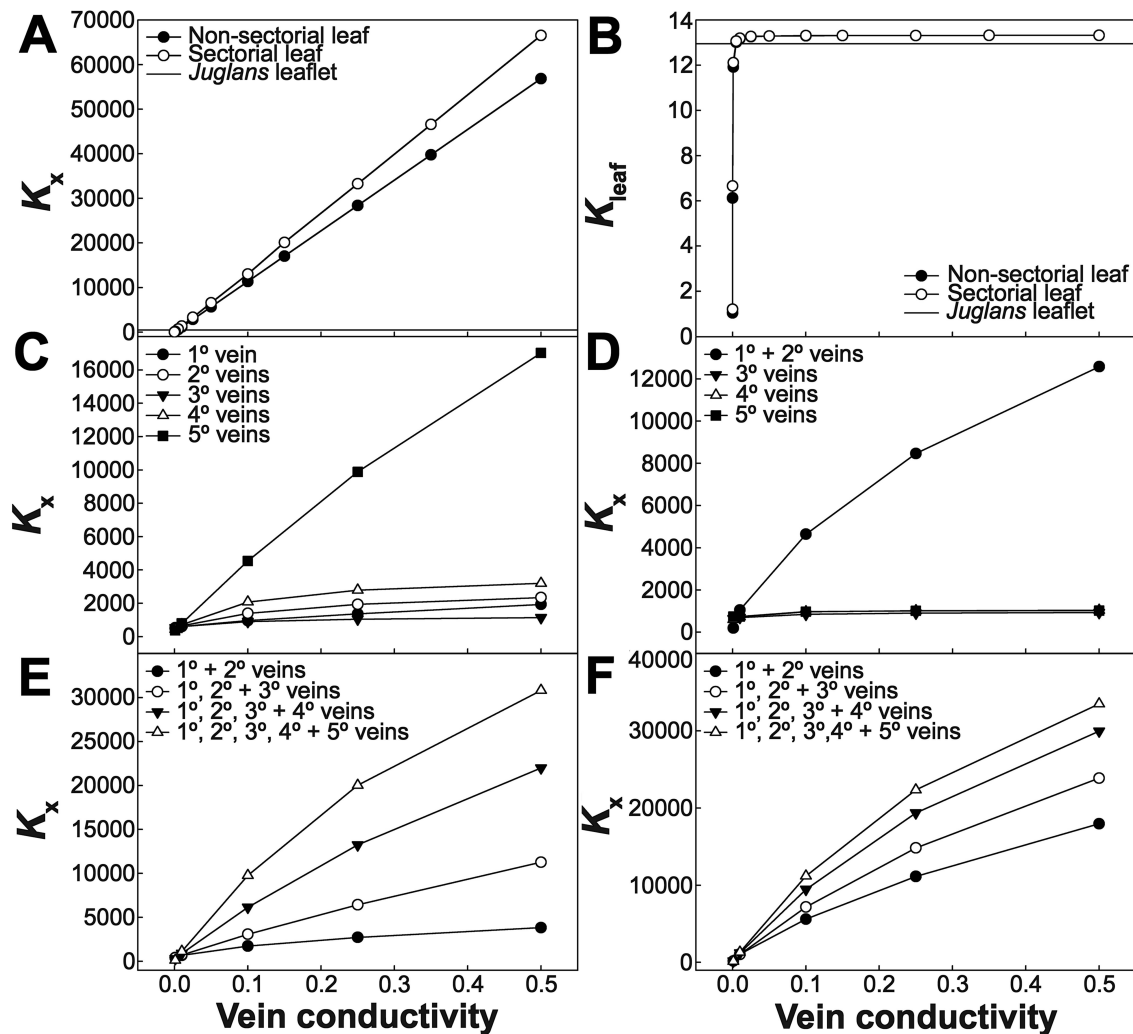


Figure 2: Response of leaf xylem hydraulic conductance (K_x) and whole-leaf hydraulic conductance (K_{leaf}) for simulations of leaves without vein order hierarchy. A, B, Responses of K_x and K_{leaf} , respectively, to increases in conductivities of all vein orders in a nonsectorial leaf and a sectorial 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° and 2° 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° veins, 2° 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° and/or 2° veins was reduced to that of the middle or apical portion of the 1° and 2° veins. Using data available for conduit numbers and diameters at four points along the length of the 1° vein in the *Juglans* leaflet (H. Cochard, unpublished data), the leaflet with a tapering 1° vein had a 15% lower

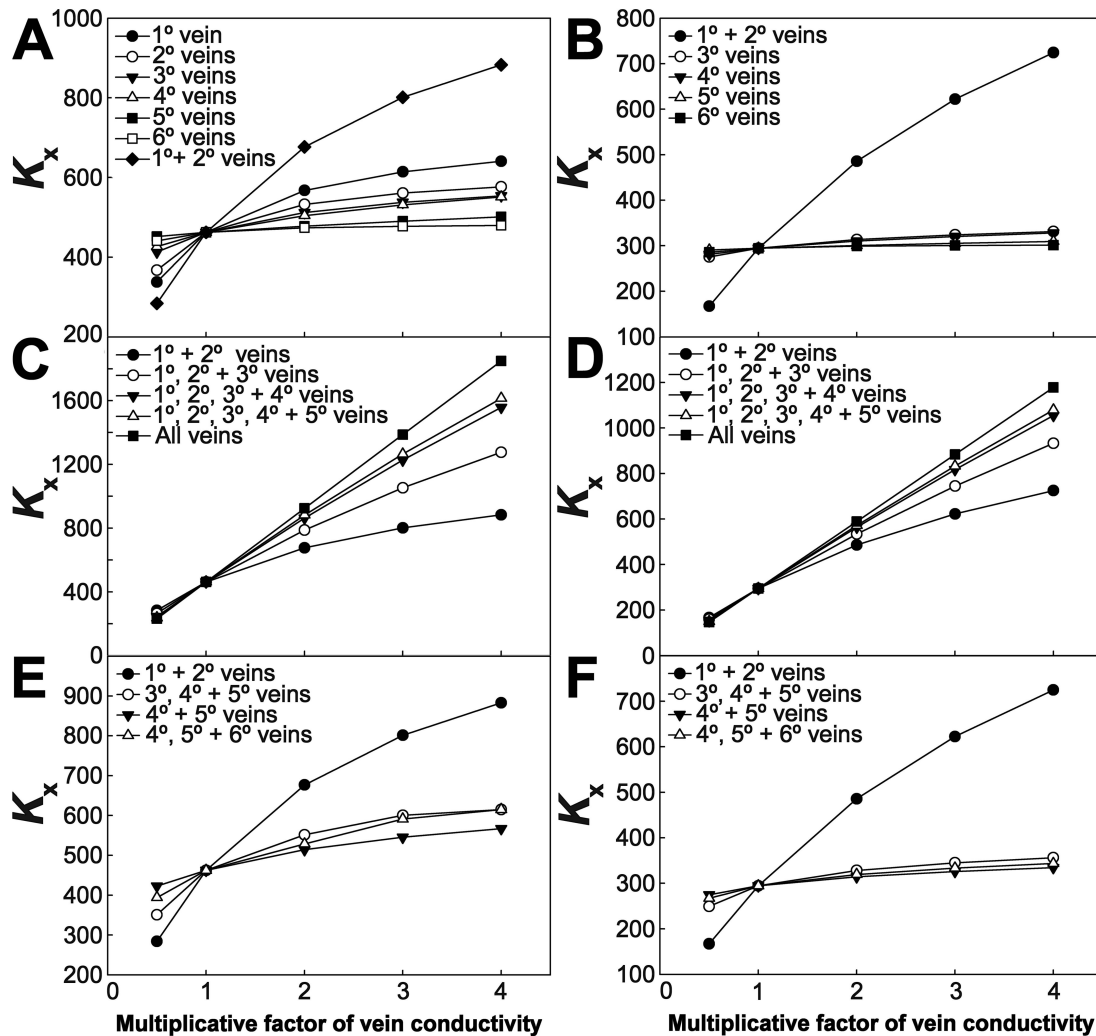


Figure 3: Response of leaf xylem hydraulic conductance (K_x) for simulations of leaves with hierarchy of vein order conductivities based on the *Juglans* leaflet. A, B, Responses of K_x to increases in conductivity of individual vein orders (plus 1° and 2° order veins together) in a nonsectorial leaf 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 den-

sities 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)

Venation trait	Increase in CC with doubling of trait (%) ^a		Change in K_x/CC with doubling of trait (%) ^a	
	Scenario A	Scenario B	Scenario A	Scenario B
Doubling conductivity in:				
All vein orders	19	100	+68	+0
1° vein	6	30	+16	-6
2° veins	5	28	+9	-10
3° veins	2	11	+8	-.003
4° veins	3	19	+5	-8
5° veins	2	8	+2	-5
6° veins	.004	2	+2	+0.001
Major veins	11	59	+32	-8
Minor veins	8	40	+11	-15
Increasing major vein density by halving leaf size, keeping minor vein density constant				
		24		+30
Doubling 2° vein density by increasing 2° vein number				
		28		+7
Doubling minor vein density				
		26		+109

^a 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_{leaf} (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_x and K_{leaf} in simulations using *Juglans* leaflet data (fig. 5C, 5D) and alternative parameterizations (data not shown). The K_x increased linearly over a wide range of minor vein densities. By contrast, K_{ox} 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_x and K_{ox} resulted in an overall accelerating response of K_{leaf} (fig. 5D), which contrasted with the saturating response in K_{leaf} typically observed when altering characters that influenced K_x (see

above). Notably, the shape of the K_{leaf} response would depend on whether K_x or K_{ox} was a greater limitation to K_{leaf} ; in our simulations, K_{ox} was much lower than K_x , and thus K_{ox} and K_{leaf} 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_{ox} and K_{leaf} , the increase in K_{leaf} relative to CC would be greater than that of other venation traits, which affect K_x alone.

Increasing both 2° and minor vein densities together led to a synergistic effect on K_x (fig. 5E, 5F). The same pattern appeared in similar tests increasing 2° vein conductivity over the range of 1.0×10^{-4} to 1.0×10^{-1} mmol $m^{-1} MPa^{-1}$ 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_x and a saturating response for K_{leaf} , while increasing minor vein density led to a linear increase of K_x and an accelerating increase of K_{leaf} . Increasing both together produced an accelerating impact on K_x and K_{leaf} .

Summarizing the Relative Sensitivity of K_x to Different Venation Traits

We determined the relative sensitivity of K_x to changes in each venation trait (fig. 6). As discussed above, K_x was

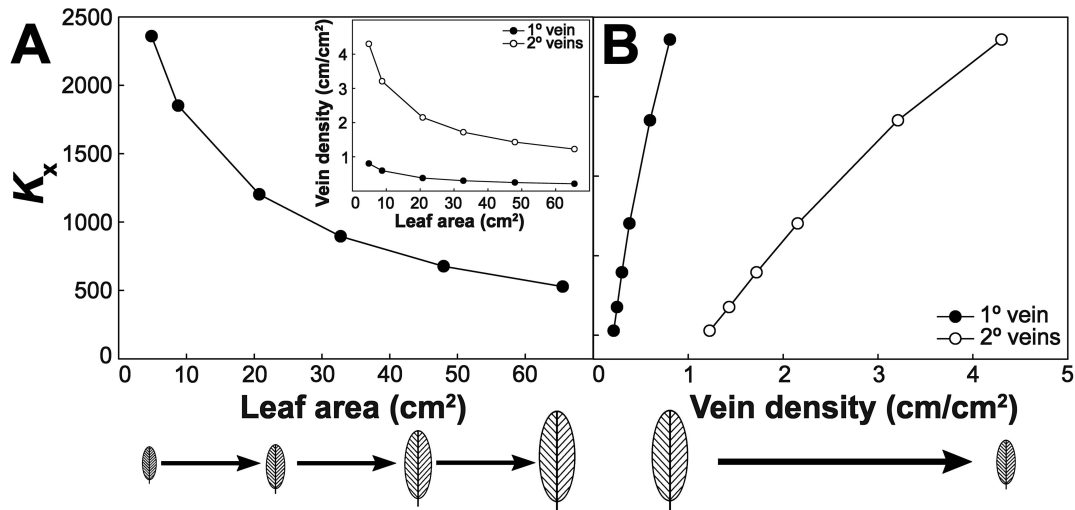


Figure 4: Response of xylem hydraulic conductance (K_x) to modifying leaf area for elliptical leaves with fixed vein xylem conductivities based on the *Juglans* leaflet (nonsectorial); larger leaves have 1° and 2° order veins spaced farther apart. A, K_x versus leaf area; schematic shows vein density 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_x/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_x/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_x/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-

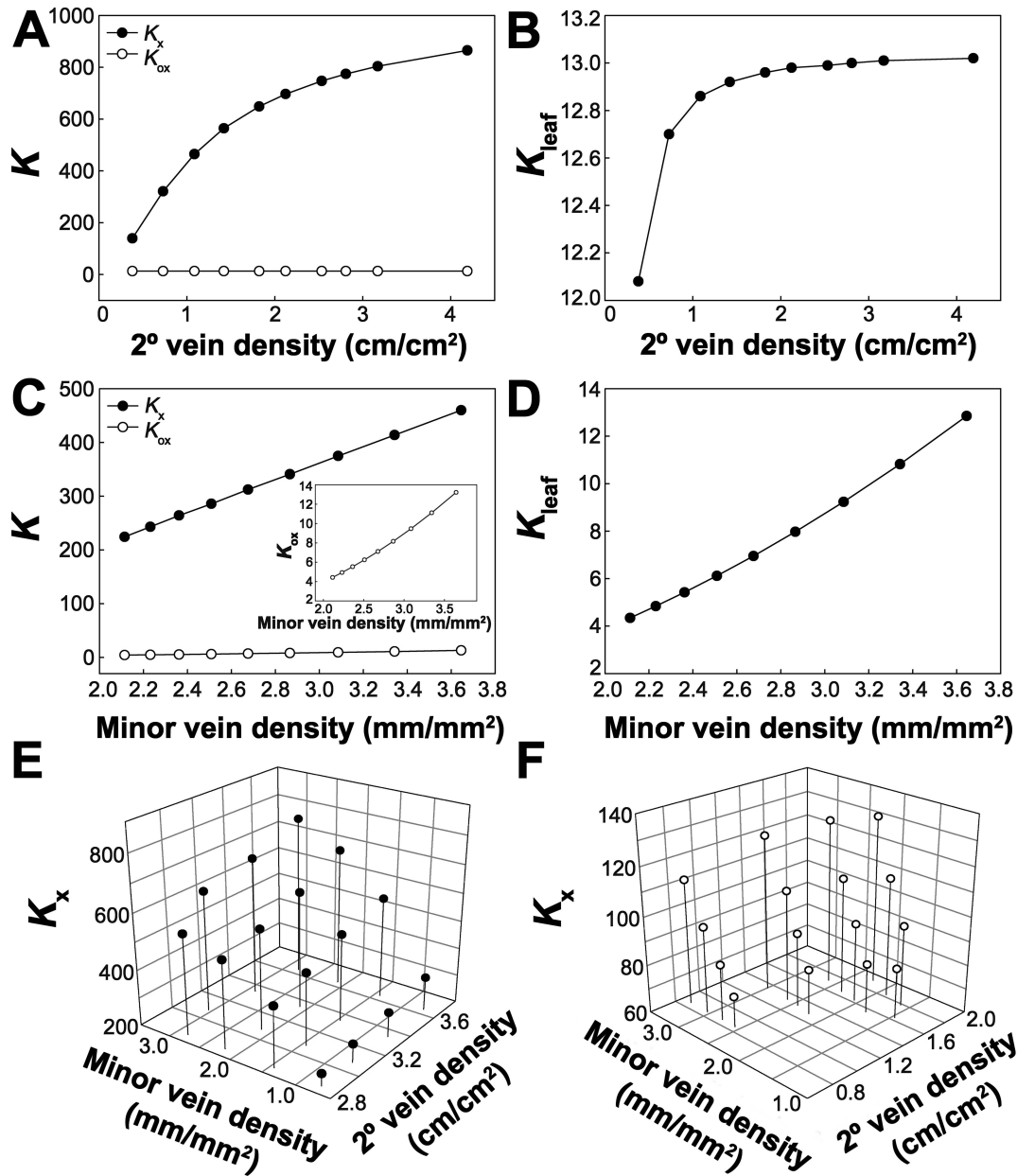


Figure 5: Response of xylem (K_x), outside-xylem (K_{ox}), and whole-leaf hydraulic conductance (K_{leaf}) for leaf simulations based on the *Juglans* leaflet (no sectoriality, with tapering in 1° and 2° veins), modifying either 2° vein density or minor vein density or both. *A, B*, Simulated leaves with greater 2° density (increased numbers of 2° veins). *C, D*, Simulated leaves with higher minor vein densities. *Inset*, K_{ox} versus vein density at smaller scale. *E, F*, Response of K_x to modifying 2° and minor vein density together in nonsectorial and sectorial leaves, respectively.

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_x and K_x/CC respond strongly to modifying conductivities of vein orders, with different impacts across vein orders. In nonhierarchical, nonsectorial leaves, K_x was most strongly affected by increasing con-

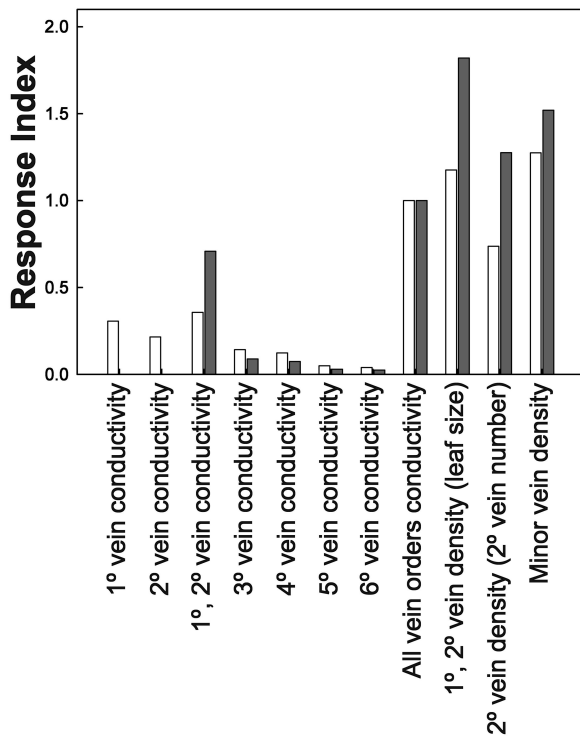


Figure 6: Response index of K_x to simulations of modifications of vein traits in *Juglans* leaves. Index calculated as the log-log slope of K_x versus the trait value; positive values indicate a positive response of K_x to an increase in the trait values. White = nonsectorial leaf; gray = sectorial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

ductivity of the highest-density vein order. In hierarchical leaves with or without sectoriality and nonhierarchical leaves with sectoriality, K_x 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_x 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_x . The dramatic increase of K_x and relatively high increase in K_x/CC conferred by increasing the 1° vein conductivity suggest a powerful evolutionary mechanism and explain

why K_x correlates with 1° vein conductivity in sets of trees and grasses (Sack and Frole 2006; Maherali et al. 2008). A second implication of these findings is that damage or blockage of the 1° vein in a pinnately veined leaf should dramatically reduce K_x and K_{leaf} , 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_x due to other emerging bottlenecks in the system. A linear increase in K_x was accomplished only by increasing the conductivity of all vein orders simultaneously, which also strongly increased K_x/CC . Proportional modification of conductivity in all vein orders may be common in the evolution of higher K_x and warrants further attention. Across 10 species of *Quercus*, the conduit hydraulic diameters in the petiole and in the 1° and 2° 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_x/CC , reducing CC more than K_x and thus providing benefit relative to cost. These results would explain widespread tapering of 1° and 2° 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° Vein Number, and Major Vein Density

Reducing leaf size, thereby increasing major vein density, drove a nearly linear increase of K_x and K_{leaf} and a greatly increased K_x/CC . Thus, K_x and K_{leaf} 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_{leaf} 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_x and manifests strong plasticity and adaptation (Givnish 1987); thus, solely altering leaf size is unlikely to be a general mechanism for evolving higher K_x or K_{leaf} . In many cases, the relationship is not found. The K_{leaf} 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_x 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 *Platago* taxa, larger-leaved species had higher vein conductivities (Coomes et al. 2008; Dunbar-Co et al. 2009).

Increasing 2° vein numbers and thereby 2° vein density also caused an increase in K_x . However, this response showed saturation, in contrast to the linear response found when increasing major vein density via reducing leaf size. Increasing 2° vein numbers would lead other major veins to become increasingly limiting to K_x . The saturating effect on K_x and low increase in K_x/CC suggest that increasing 2° vein numbers would be a weak evolutionary mechanism to achieve higher K_x . This prediction concurs with findings for sets of temperate and tropical woody species, where K_x and K_{leaf} did not correlate with 2° vein number or density (Sack and Frole 2006; Sack et al. 2008). We note that a high density of 2° 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_x , an accelerating increase in K_{ox} , and the greatest increase in K_x/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_x by adding additional xylem flow routes in parallel but also influenced K_{ox} by increasing parallel exit routes from the xylem. Because minor vein density affected both K_x and K_{ox} , it also should have the highest cost-effectiveness for increasing K_{leaf} . This impact is consistent with the observed correlations of K_{ox} and K_{leaf} with minor vein density across diverse species (Sack and Frole 2006).

In real leaves, a higher minor vein density may also increase K_{ox} 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_{ox} and thus K_{leaf}), including more conductive flow pathways through bundle sheath, mesophyll, or epidermis and the development of bundle sheath extensions in heterobaric 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_x 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_x 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_x 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_x in series, leading to colimitation, and others affecting K_x in parallel, with orthogonal, additive effects. Venation traits thus determine K_x 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_x 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_x and R_{ox} 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_x , K_{ox} , and K_{leaf} 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 non-reticulate (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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