Tansley review

Leaf venation: structure, function, development, evolution, ecology and applications in the past, present and future

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Contents

Summary

The design and function of leaf venation are important to plant performance, with key implications for the distribution and productivity of ecosystems, and applications in paleobiology, agriculture and technology. We synthesize classical concepts and the recent literature on a wide range of aspects of leaf venation. We describe 10 major structural features that contribute to multiple key functions, and scale up to leaf and plant performance. We describe the development and plasticity of leaf venation and its adaptation across environments globally, and a new global data compilation indicating trends relating vein length per unit area to climate, growth form and habitat worldwide. We synthesize the evolution of vein traits in the major plant lineages throughout paleohistory, highlighting the multiple origins of individual traits. We summarize the strikingly diverse current applications of leaf vein research in multiple fields of science and industry. A unified core understanding will enable an increasing range of plant biologists to incorporate leaf venation into their research.

I. Introduction

Within and across species, leaves are enormously diverse in venation architecture. Ever since a seminal review introduced a new integrative science of leaf venation (Roth-Nebelsick *et al.*, 2001), there has been increasing recognition across plant biology and ecology of the importance of leaf venation. We aimed to initiate new researchers into the breadth of classical and recent research, and to highlight principles that operate across levels of organization.

II. Overall structure of the leaf venation

Veins are composed of xylem and phloem cells embedded in parenchyma, sometimes sclerenchyma, and surrounded by bundle

sheath cells. The vein xylem transports water from the petiole throughout the lamina mesophyll, and the phloem transports sugars out of the leaf to the rest of the plant. Leaf venation systems vary strongly across major plant lineages, with many early groups having dichotomously branching, open systems, but reticulation evolved frequently. Angiosperms have greatest diversity in vein structure but share key architectural elements, that is, a hierarchy of vein orders forming a reticulate mesh (Hickey, 1973; Ellis *et al.*, 2009; McKown *et al.*, 2010). Typically there are three orders of lower-order veins, known as 'major veins', often ribbed with sclerenchyma (Esau, 1977). One or more first-order veins run from the petiole to the leaf apex, with second-order veins branching at intervals, and third-order veins branching between. The major veins can be distinguished from minor veins, typically present only in angiosperms, which include up to four additional orders of smaller, reticulate higher-order veins. Major and minor veins can be distinguished by their distinct timing of formation, and differences in gene expression during development, sizes and branching in the mature leaf, and in cross-sectional anatomy (Esau, 1977; Haritatos *et al.*, 2000a). In several lineages, particularly the monocotyledons, a grid-like 'striate' venation is typical, including several orders of longitudinal veins of different sizes, with small transverse veins connecting them (Ueno *et al.*, 2006).

III. Function of leaf venation traits: a 'Rosetta Stone'

As a guide to its function, we focused on 10 aspects of the venation and associated traits (Table 1; Figs 1,a–h, 2a–s; Supporting Information, Notes S1). For each aspect of the venation we describe the influence on hydraulic and gas exchange physiology, and then on sugar transport, biomechanics, plant–animal interactions and construction costs. We also highlight how vein traits vary with leaf size within and across species.

We begin by describing the hydraulic role of the veins in each case, as the leaf is a central component in the plant hydraulic system, which is a key determinant of maximum rates of photosynthetic gas exchange and growth, and their responses to changing environmental conditions (Tyree & Zimmermann, 2002; Brodribb, 2009). The leaf hydraulic conductance (K_{leaf} = flow rate across the leaf divided by the water potential driving force; Sack *et al.*, 2002; Sack & Tyree, 2005; Blackman & Brodribb, 2011) quantifies the efficiency of water movement from petiole to the air spaces where it evaporates and diffuses from the stomata. Because the plant hydraulic conductance (K_{plant}) tends to scale with K_{leaf}, the venation has a strong influence on the degree to which the stomata may remain open for photosynthesis without desiccating the leaf (Sack & Holbrook, 2006). Within and across species, K_{leaf} correlates strongly with stomatal pore area per leaf area, stomatal conductance, light-saturated photosynthetic rate per leaf area, and also with species' growth form and ecology, being higher for species adapted to high vs low irradiance, and higher for crop herbs than for angiosperm trees, and, in turn, higher than for conifers and ferns (Brodribb & Holbrook, 2003; Sack *et al.*, 2003; Sack & Holbrook, 2006; Brodribb *et al.*, 2010). K_{leaf} is influenced by vein traits that affect the hydraulic conductance of the xylem (K_x) and of the pathways outside of the xylem, that is, through the bundle sheath

and the mesophyll (K_{ox}; Cochard *et al.*, 2004; Sack *et al.*, 2004; McKown *et al.*, 2010) (Fig. 3):

$$K_{leaf} = (K_x^{-1} + K_{ox}^{-1})^{-1} \qquad \text{Eqn 1}$$

1. Dimensions of vascular cells within veins and of whole veins

Plant species vary enormously in the cross-sectional dimensions of xylem, phloem and sclerenchyma cells within leaf veins of given orders (Fig. 2p–s) and in the external diameters of the whole veins (i.e. vascular bundles). This variation contributes to multiple functional differences (Notes S2). All else being equal, larger conduit lumens provide greater xylem flow conductivity and K_{leaf}, and greater sensitivity to xylem embolism, whereas larger xylem conduit wall thickness relative to lumen diameter provides greater biomechanical strength and resistance to implosion, and longer xylem conduits can contribute to the spread of pathogens across the leaf. Finally, greater phloem conduit diameters can contribute to higher phloem flow conductivities. These differences can drive impacts on leaf gas exchange and its responses to external conditions.

The arrangement of tissues within veins may also influence transport. In the large veins of most angiosperms, the phloem is abaxial to the xylem (Esau, 1977), but species in at least 27 families also have adaxial phloem (Metcalfe & Chalk, 1950). In some species, within the lower-order veins, the vascular cells are separated in discrete bundles, a type of 'sectoriality' which may protect the leaf from the spread of embolism within veins (Orians *et al.*, 2005; Brodersen *et al.*, 2012).

The diameters of whole veins can also reflect differences in transport capacity when they contain greater sizes and numbers of xylem and phloem cells (Fisher & Evert, 1982; Russin & Evert, 1985a; Coomes *et al.*, 2008; Taneda & Terashima, 2012). A larger vein diameter provides greater mechanical support and protection for a given vein cross-sectional shape and composition and proportion of lignified cells (Niklas, 1992; Onoda *et al.*, 2011; Méndez-Alonzo *et al.*, 2013), and can better resist animal damage (Read & Stokes, 2006). Investment of mass and volume in petiole and midrib can compensate for weaker lamina to provide whole leaf strength (Méndez-Alonzo *et al.*, 2013). Thicker veins are typical in larger leaves (Sack *et al.*, 2012). In some species large midveins can provide a frame for the lamina to curl, or to allow flexural bending along the midrib, to reduce transpiration and mechanical load (Givnish, 1979; King *et al.*, 1996; Cooley *et al.*, 2004).

The veins entail substantial cost. Across species, the major veins can contribute a substantial amount to the leaf mass per unit area, and their carbon and nitrogen concentrations are correlated with those of the lamina (Niinemets *et al.*, 2007a,b).

2. Vein hierarchy

In the hierarchical leaf vein system of angiosperms, veins of higher branching orders in a given leaf have smaller diameters but greater branching frequencies and lengths (Figs 1a–h, 2j). The bulk of vein length is in the minor veins, whereas substantial vein cross-sectional

Table 1 Vein features and quantifiable traits discussed in Section II, including their functions

Vein features	Measurable traits	Potential function for xylem and phloem transport, gas exchange, leaf support and biomechanical strength
1. Dimensions of vascular cells and of whole veins	Diameters and conductivities of first-, second-, third-, and higher-order (minor) veins Diameters and conductivities of xylem and phloem conduits Xylem and phloem conduit cell wall thicknesses	**Higher xylem conduit numbers or sizes can contribute to higher K_{leaf}, especially in lower-order veins** Larger conduits may be more vulnerable to drought and freeze-thaw embolism Greater wall thickness relative to lumen provides greater mechanical support and resistance to implosion Longer conduits contribute to the spread of bacterial infection **Greater phloem conduit numbers or sizes can contribute to faster phloem transport rates** Sectoriality in the arrangement of xylem and phloem conduits within veins can provide additional drought tolerance and improve homogeneity of pressure distribution across the leaf **Thicker veins may have greater water and sugar transport capacity and greater mechanical resistance but have a greater cost in carbon and nutrients**
2. Vein hierarchy	Decline of vein diameters or conductivity, and increasing vein length per unit area with increasing vein order	Provides cost-efficiency to the venation network Increases contact between vascular and photosynthetic tissues Homogenizes pressures across the leaf Provides tolerance of damage or blockage in higher-order veins because of their greater redundancy in length per unit area, and smaller xylem conduits Provides cost-efficiency for biomechanical support and protection against herbivory
3. Vein tapering	Decline of vein diameters or conductivity, and of xylem and phloem conduit diameters or numbers along the first-order and/or second-order veins	Provides cost-efficiency to the venation network Provides biomechanical advantages, such as more economical leaf support, by shifting the load to the midvein or petiole. In grasses, midrib tapering allows bending so that the center of the leaf is faced towards sunlight
4. Vein length per unit area (VLA)	VLA for minor veins or the total vein system Related traits based on VLA and vein diameters include inter-veinal distance, vein cross-sectional and projected surface area per unit leaf area and vein volume per unit leaf area	**High VLA can enable higher K_{leaf}, greater stomatal density and stomatal conductance and higher rates of gas exchange per unit leaf area** **High VLA can improve phloem loading** High VLA can provide tolerance of fine-scale damage to the leaf High VLA may confer benefits for biomechanical support and protection High VLA may provide a possible advantage against insect herbivory Low VLA can reduce construction costs and lower vein projected area, and potentially enhance mesophyll light capture in shade
5. Major vein density	VLA for major veins Related traits based on major vein density and vein diameters include vein cross-sectional and projected surface area per unit leaf area and vein volume per unit leaf area	**High major VLA contributes to higher K_{leaf} and gas exchange rates across closely related species** **High major VLA acts as water transport superhighways and provides tolerance of disruption to the hydraulic system caused by damage, drought, or freeze–thaw embolism** High major VLA equalizes water potential across the leaf, by reducing the lamina area in between major veins High major VLA provides biomechanical support High major VLA through palmate venation (multiple first-order veins) enables leaves to be larger and thinner, but with the advantages of being protected against disruption of the vein system, and with lower cost of petiole relative to leaf size
6. Topology: areoles, major vein topology, looping	Second- and third-order and minor vein types Number of areoles per leaf area Second-order vein branching angles	High number of areoles per unit leaf area can correlate with, and may provide the advantages of, high VLA **Looping provides optimal transport given fluctuating flow or damage** Brochidodromous vein topology adds second-order VLA compared with craspedodromous, and thus may provide the same benefits as higher major VLA

Table 1 (Continued)

Vein features	Measurable traits	Potential function for xylem and phloem transport, gas exchange, leaf support and biomechanical strength
7. Free-ending veins (FEVs)	Number of FEVs	**Numerous FEVs per unit area can relate to high VLA, and thus higher K_{leaf}** In species where sieve tubes extend to vein endings, a high number of FEVs could facilitate sugar loading
8. Bundle sheath extensions (BSEs)	Length per unit area of veins with bundle sheath extensions Length and width of bundle sheath extensions	**BSEs may play a role in guiding light inside the leaf; species with BSEs tend to have higher photosynthetic rates per unit area** **BSEs may increase K_{leaf} and influence response to irradiance and leaf water status** BSEs may play a role in enabling 'patchy' stomatal responses **BSEs may allow stomata to respond more quickly to changes in epidermal or xylem water potential** BSEs can increase the mechanical strength of the leaf, especially when fibers or sclereids are present, and can also offer an advantage against herbivores
9. Accessory transport and support tissues: sclereids, transfusion tracheids and idioblasts	Number of accessory transport elements per unit leaf area Length and width of accessory transport elements	Accessory transport elements may guide light inside the leaf **Accessory transport elements contribute to K_{leaf}** **Accessory transport elements might act as water storage to buffer cell water potentials from transiently high transpiration rates** Accessory transport elements can increase the mechanical strength of the leaf, and its resistance to shrinkage during dehydration

Functions relating directly to maximum leaf hydraulic conductance (K_{leaf}) and/or rates of transpiration and photosynthesis are in bold.

and projected area are contributed by both major and minor veins, and the bulk of vein volume is in the major veins (Sack *et al.*, 2012). This hierarchy is simplified in earlier-evolved plant lineages (Section VII. 1). Most monocots, including grasses, have a distinct hierarchy of gridded 'parallel' or 'striate' major veins (Fig. 2e,f), with midribs, and large and intermediate longitudinal veins, analogous to major vein orders, and small longitudinal veins and transverse veins analogous to minor veins (Ueno *et al.*, 2006). Several eudicot lineages have also evolved striate vein systems (e.g. *Plantago* and phyllodes of *Acacia*) and certain monocot families have evolved eudicot-like vein systems (e.g. the Araceae and banana families). In palms, the fronds have gridded systems, and the transverse veins can be as large as the large longitudinal veins, and can even be disconnected from some of the longitudinal veins that they cross, suggesting semi-independence within the network (Horn *et al.*, 2009).

As a result of the hierarchy, the diameters of vein segments within a given leaf tend to show skewed frequency distributions when quantified for the whole system, that is, with the majority of segments being of a small size class (Bohn *et al.*, 2002). The hierarchy shows a general scaling across angiosperm species; there are correlations among the diameters of the petiole, midrib and second-order veins (Coomes *et al.*, 2008; Scoffoni *et al.*, 2011; Sack *et al.*, 2012). The hierarchy of external vein dimensions reflects that of the tissues and cells within. From lower to higher vein orders, the xylem and phloem cross-sectional areas decrease with that of the whole vein, as do the numbers, diameters and lengths of xylem conduits, parenchyma and fiber cells, and phloem sieve elements (SEs) and companion cells (CCs), except the diameters of the CCs increase in the minor veins for phloem loading (Plymale & Wylie, 1944; Fisher & Evert, 1982; Russin & Evert, 1985a; Dannenhoffer *et al.*, 1990; Coomes *et al.*, 2008).

The hierarchy of vein dimensions and lengths, and associated shifts in cross-sectional anatomy across vein orders provide multiple benefits (Notes S3). Advantages conferred for water and sugar transport include cost-efficiency; increased contact of higher vein orders with photosynthetic tissues; pressure equilibration across the leaf; and tolerance of damage or blockage in higher-order veins (Zwieniecki *et al.*, 2002; Sack *et al.*, 2004; McKown *et al.*, 2010). Additionally, the vein hierarchy also provides cost-efficiency for biomechanical support and protection against herbivory (Méndez-Alonzo *et al.*, 2013).

3. Vein tapering

In angiosperm leaves, both the first- and second-order veins narrow in diameter along their length (Fig. 1a–h). While in conifers and in some fern pinnae midvein tapering occurs, tapering along the second-order veins is notably absent. Indeed, in many ferns, the second-order (and sometimes first-order) veins widen to supply marginal sori (Fig. 2h,i). Along the tapering veins, the cross-sectional area and number of cells of each tissue type decrease (Fisher & Evert, 1982; Russin & Evert, 1985a; Dannenhoffer *et al.*, 1990).

Tapering provides benefits previously described for venation hierarchy, that is, increased hydraulic capacity relative to construction costs (Zwieniecki *et al.*, 2006; McKown *et al.*, 2010). Vein tapering also provides economical mechanical support, because the load is borne at the base of the petiole and midvein, which operate as a cantilevered beam (Niklas, 1992). Similarly, the loads on second-order veins are mostly borne at the thickened base. Tapering can also allow specialized biomechanical function. In long grass leaves, midvein tapering contributes to the bending of the blade such that the center of the leaf faces sunlight (Moulia & Fournier, 1997).
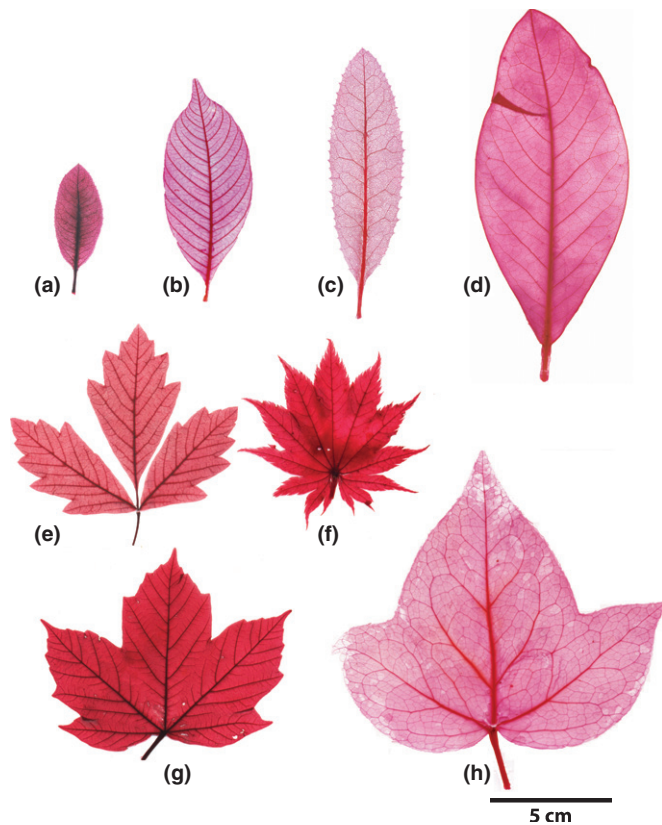
Fig. 1 Diversity in angiosperm leaf vein systems visible in chemically cleared leaves, emphasizing leaf vein tapering, major vein length per unit area, vein topology and leaf size. (a) *Comarostaphylis diversifolia*: simple pinnate leaf with brochidodromous secondary (second-order) veins. (b) *Trichilia elegans*: simple pinnate leaf with eucamptodromous second-order veins. (c) *Heteromeles arbutifolia*: simple pinnate leaf with brochidodromous second-order veins. (d) *Magnolia grandiflora*: simple pinnate leaf with brochidodromous second-order veins. (e) *Acer griseum*: compound pinnate leaf with craspedodromous second-order veins. (f) *Acer japonicum*: simple palmate leaf with craspedodromous second-order veins. (g) *Acer diabolicum*: simple palmate leaf with craspedodromous second-order veins. (h) *Hedera canariensis*: simple palmate leaf with brochidodromous second-order veins. In (a–d), note that larger-leaved species have major veins spaced further apart, and declining major vein length per unit area. Photo: M. Rawls (b).
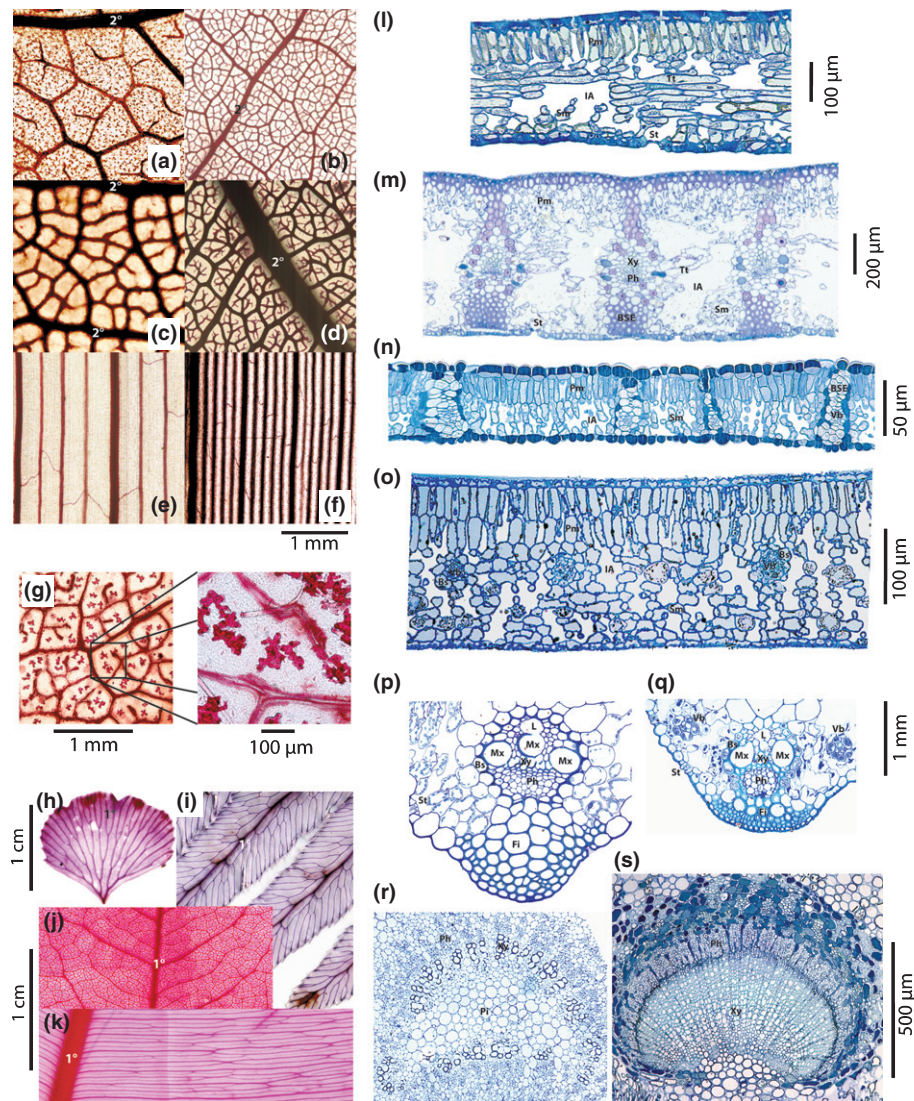
4. Vein length per unit area ('vein density')

The leaf vein length per unit area (VLA; also known as 'vein density') varies strongly across species (Fig. 2a–f). In the typical angiosperm, except for those with striate vein systems, VLA is mainly determined by the minor vein length per unit area (minor VLA), which accounts for >80% of the total vein length (Sack *et al.*, 2012). Across species or genotypes of a given species, high VLA can correspond to a lower number of epidermal and mesophyll cells between veins, and/or to smaller cells (McKown & Dengler, 2007; Manuel Perez-Perez *et al.*, 2011; Smillie *et al.*, 2012).

To quantify VLA, X-ray imaging can be applied for some leaves (Blonder *et al.*, 2012), but for many others, it is necessary to chemically clear the leaf (Sack & Frole, 2006; Scoffoni *et al.*, 2011)

or prepare paradermal sections (Brodribb *et al.*, 2007) and microscopically image the minor vein network. Using software, images can be measured manually or automatically, although it is essential to validate automatic methods, and measurement of low-resolution images of venation can underestimate the VLA (see Rolland-Lagan *et al.*, 2009; Price *et al.*, 2011; Dhondt *et al.*, 2012; Sack *et al.*, 2012). The interveinal distance (IVD) has been often measured as a proxy for VLA, as, across species, the two are reciprocally related, although the relationship can vary depending on the topology of the minor veins, and IVD can be much more variable within leaves than VLA (Uhl & Mosbrugger, 1999; Blonder *et al.*, 2011).

A high VLA (Fig. 2b,d,f) has multiple benefits (Notes S4). All else being equal, a high VLA can enable higher K_{leaf}, greater stomatal density and stomatal conductance and higher rates of gas exchange per leaf area (Sack & Frole, 2006; Brodribb *et al.*, 2007, 2010; Boyce *et al.*, 2009; McKown *et al.*, 2010; Feild *et al.*, 2011; Walls, 2011). By hypothesis, a high VLA can enable greater phloem transport efficiency (Russin & Evert, 1984), but this depends on the distribution of phloem in the vein network, and on species' loading strategies among other factors. A high VLA may also provide tolerance of fine-scale damage or vein blockage (Raimondo *et al.*, 2003; Sack & Holbrook, 2006; Nardini *et al.*, 2010). Additionally, a high VLA may confer benefits for biomechanics and protection, although, given the low volume of minor veins, this effect would be subtle relative to the influence of major veins and the composition (Choong *et al.*, 1992). In grasses and palm leaves, the small transverse veins reinforce against bending forces (Niklas, 1999). A high VLA may also provide an advantage against herbivory, forcing insects to spend energy to cut through more veins (Vincent 1990; Read & Stokes, 2006). Finally, by contrast, a low VLA (Fig. 2a,c,e) has possible benefits for certain species, for example, species under shade, or high water supply, corresponding to a reduced construction cost and displacement of mesophyll (Sporck & Sack, 2010).

Famously, VLA is often higher for C_4 relative to C_3 species. C_4 species typically have high VLA so that mesophyll cells are at most two cells away from veins to allow diffusion of sugars via plasmodesmata to their enlarged bundle sheath (BS) for decarboxylation and photosynthesis (Ogle, 2003; Sage, 2004; Griffiths *et al.*, 2012). The importance of VLA was clear in the evolution of C_4 within eudicot genera *Cleome* and *Flaveria*, as VLA increased from C_3 to C_3/C_4 intermediate and C_4 species (Marshall *et al.*, 2007; McKown & Dengler, 2007). However, the linkage is not entirely necessary; some species perform C_4 within a single cell, and no difference in VLA was found on average between C_3 and C_4 eudicots sampled from sister lineages (Muhaidat *et al.*, 2007). The diffusion of C_4 sugars would be more limited by the number of cells between veins than by distance *per se*, as transport through plasmodesmatata would be the major resistance to the flux of solute (Haritatos *et al.*, 2000b), and species with larger cells may maintain efficient C_4 despite lower VLA. The consistently higher VLA of C_4 grasses (Ueno *et al.*, 2006) suggests a particular mechanistic basis in that lineage. By contrast, CAM plants, with their typically succulent habit, have low VLA (Section VI. 2).

Fig. 2 Diversity in leaf vein systems, emphasizing leaf vein diameters, numbers of vein orders, vein length per unit area (VLA), free-ending veins (FEVs), xylem conduit sizes, accessory transport tissues and bundle sheath extensions. (a–f) Micrographs of chemically cleared leaves of eudicots (a–d) and monocots (e, f). (a) *Hedera canariensis*; (b) *Machaerium acutifolium*; (c) *Comarostaphylis diversifolia*; (d) *Talisia esculenta*; (e) *Triticum aestivum*: C_3 grass; (f) *Panicum virgatum*: C_4 grass. (g) *Scaevola gaudichaudii* leaf imaged at lower and higher resolution; note the sclereids in the lamina. (h–j) Variation in the complexity of vein hierarchy. (h, i) Chemically cleared frond pinnae of ferns with dichotomously branching veins, *Adiantum jordanii* (h) and *Woodwardia fimbriata* (i). (j) Chemically cleared leaf of *Machaerium scleroxylon*, which has a hierarchical vein system with reticulation. (k) Chemically cleared leaf of *Micropholis melinoniana*, which has dense, thin parallel secondary veins branching off the midrib. (l) Cross-section of the lamina of cycad *Cycas szechuanensis*, with transfusion tracheids and sunken stomata. (m) Cross-section of the lamina of cycad *Zamia furfuracea*, with multiple parallel first-order veins and transfusion tracheids, sclerified bundle sheath extensions and sunken stomata. (n) Cross-section of the lamina of *Bauhinia galpinii*, heterobaric. (o) Cross-section through the lamina of *Camellia sasanqua*, homobaric. (p) Cross-section through the midrib of the C_3 grass *Triticum aestivum*, with large xylem conduits, and leaf hydraulic conductance (K_{leaf}) of 17.5 mmol m^{-2} s^{-1} MPa^{-1}. (q) Cross-section through the midrib of the C_4 grass *Echinochloa crus-galli*, with smaller xylem conduits, and K_{leaf} of 0.409 mmol m^{-2} s^{-1} MPa^{-1}. (r) Cross-section through the midrib of shade-grown *Clermontia parviflora*, with few midrib xylem conduits, and K_{leaf} of 5.09 mmol m^{-2} s^{-1} MPa^{-1}. (s) Cross-section through the midrib of *Raphiolepis indica*, with numerous xylem conduits arranged in rays and K_{leaf} of 11.0 mmol m^{-2} s^{-1} MPa^{-1}. (K_{leaf} data from Scoffoni *et al.* (2011); Guyot *et al.* (2012) and C. Scoffoni & L. Sack, unpublished.) Symbols: 1°, midrib; 2°, second-order vein; BS, bundle sheath; BSE, bundle sheath extension; Fi, fibers; IA, intercellular airspace; L, lacuna; Mx, metaxylem; Ph, phloem; Pi, pith; Pm, palisade mesophyll; Sm, spongy mesophyll; St, stomata; Tt, transfusion tracheids; Xy, xylem. Photographs: C. Creese (h, f); G. John (n, o, s); A. McKown (g); M. Ng (k–m); M. Rawls (b, d, j); C. Vuong (e, f, p, q).

5. Major vein length per unit area ('major vein density')

The major vein length per area (major VLA; Fig. 1a–h) has multiple key functions (Notes S5). All else being equal, a high major VLA can contribute to: higher K_{leaf} and gas exchange (Sommerville *et al.*,

2012); tolerance of the leaf vein system to disruption by damage, drought, and, potentially, freeze–thaw embolism (Sack & Holbrook, 2006; Scoffoni *et al.*, 2011); equalizing of water potential across the leaf (Zwieniecki *et al.*, 2004b; Ocheltree *et al.*, 2012); and biomechanical support (Lucas *et al.*, 1991; Choong *et al.*,
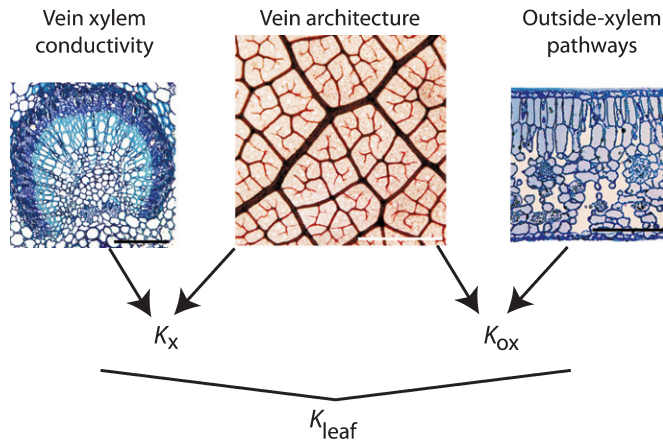
Fig. 3 Determinants of leaf hydraulic conductance (K_{leaf}): K_{leaf} can be partitioned into two components: the hydraulic conductance of the vein xylem (K_x) which is determined by the vein xylem conductivities and vein lengths per unit area (VLA) of the different vein orders, and the hydraulic conductance outside the xylem (K_{ox}), which is determined by the VLA and cellular and biochemical properties of the living tissues outside the xylem. Higher VLA and wider and more numerous xylem conduits will increase K_x and thus K_{leaf}, while higher VLA will also increase K_{ox} by reducing the distance for water to move from the xylem to the site of transpiration, and K_{ox} will also be increased by the permeability of cells and cell walls in the pathway of water movement through the mesophyll. From left to right: midrib cross-section of *Viburnum molle* (bar, 0.1 mm); micrograph of a chemically cleared leaf of *Ampelocera ruizii* (bar, 1 mm; photograph: M. Rawls); lamina cross-section of *Camellia sasanqua* (bar, 0.1 mm; photograph: G. John).

1992; Niklas, 1999). However, the major veins also involve a substantial construction cost in carbon and nutrient investment (see Section III. 1).

These functions of high major VLA would benefit palmate-veined relative to pinnately veined species, that is, those that have multiple first-order veins branching from the base of the leaf (Fig. 1e–h) (Sack *et al.*, 2008). The hydraulic and mechanical protection provided by major vein redundancy would be very important in thinner and larger leaves that are not already protected. Indeed, palmate-veined species occur more commonly in temperate than in tropical floras and are more frequent in large leaves with thin laminae (and low leaf mass per unit area) (Sack *et al.*, 2008; Walls, 2011).

Smaller leaves tend to have higher major VLA intrinsically as a result of their development (Section IV. 1). The drought tolerance and protection conferred by higher major VLA in smaller leaves provides an explanation for the greater abundance of smaller leaves in drier and more exposed habitats (Scoffoni *et al.*, 2011; Fig. 1a–d).

6. Topology: vein 'types', loopiness, areolation, and branching angles

Variation in vein topology, that is, the arrangement of network elements, accounts for much of the visually striking diversity of leaf vein systems. Vein systems have been classified with 'types' for each order (Hickey, 1973; Ellis *et al.*, 2009). For first-order veins, types include palmate vs pinnate. For second-order veins, types include craspedodromous systems, with the veins running to the margin (Fig. 1e–g), brochidodromous systems with looping second-order

veins (Fig. 1a,c,d,h), and eucamptodromous, an intermediate type (Fig. 1b). For third-order veins, types include percurrent, ramified and reticulate.

Some differences in vein types correspond to differences in 'loopiness', that is, how often veins of given orders interconnect. Early researchers hypothesized that reticulation (higher-order loopiness) protects against herbivory (Haberlandt, 1914; Wagner, 1979) and, indeed, in leaves lacking reticulation, severing veins kills the distal lamina (Shull, 1934), and models showed that looping hierarchical systems become optimal for transport and distribution only when there are fluctuations in flow, or damage (Bohn & Magnasco, 2007; Corson, 2010; Katifori *et al.*, 2010). An index of loopiness is areolation, with an areole defined as the smallest area of leaf enclosed by veins (Ellis *et al.*, 2009). Across species, number of areoles per unit leaf area correlates with total VLA (Blonder *et al.*, 2011). However, areoles vary in their shape and size within given leaves, some leaves have no areoles (i.e. those with freely ramifying high-order veins), and many species have several tiers of areolation, loops within loops. Recent work has focused on developing other descriptors for loopiness (Katifori & Magnasco, 2012; Mileyko *et al.*, 2012).

Some differences in vein types correspond to differences in VLA for given vein orders. For example, palmate systems have higher first-order VLA than do pinnate systems. Other topologies signify greater connectivity and may provide greater hydraulic conductance and support for a given VLA (Roth-Nebelsick *et al.*, 2001); thus, brochidodromous leaves have greater second-order vein connectivity than do craspedodromous leaves of the same second-order VLA, and have greater leaf mass per unit area and leaf lifespan on average globally (Walls, 2011), probably reflecting their greater abundance among tropical evergreen species (Roth-Nebelsick *et al.*, 2001). Biomechanical support may be provided by the numerous parallel second-order veins in some eudicots (Fig. 2k), as in species of *Calophyllum* and *Micropholis*, and the striate system of grasses (Lucas & Pereira, 1990; Onoda *et al.*, 2011).

Vein branching angles are also diverse across species. Across the whole vein system, the angle within each three-way vein junction is proportional to the radii of the connecting veins: smaller veins meet at more acute angles (Bohn & Magnasco, 2007). Mathematical analyses showed this property to be consistent with minimizing flow resistance relative to vein surface area, but also that it could arise during leaf expansion if tensions develop according to the relative strength of the veins and contribute to the final angles (Durand, 2006). In some species, the veins guide the folding of the developing leaf in the bud, and branching angles reflect the manner of folding (Couturier *et al.*, 2012), with implications for bud size and the economics of leaf growth. Leaves with higher second-order vein branching angles can be folded more compactly but require more energy for unfolding (Kobayashi *et al.*, 1998).

7. Free-ending veins

Leaves vary strongly in the presence and number of free-ending veins (FEVs; Fig. 2a–d). The morphology of the cells in the FEVs is diverse, often including one to three tracheids with large lumens, and cell walls thickened in discrete hoops (Strain, 1933). FEVs may

account for a significant portion of VLA, and the number of FEVs correlates with the total VLA across *Arabidopsis thaliana* genotypes and across diverse species (Scoffoni *et al.*, 2011; Dhondt *et al.*, 2012). The FEVs may in fact contribute disproportionately to K_{leaf}, as pathways for bulk water flow, and represent a substantial evaporative surface area. In most species, FEVs have little or no phloem inside (Van Bel, 1993); in the few species that do, VLA tends to be lower and the FEVs might facilitate sugar export (Adams *et al.*, 2007).

8. Bundle sheath and bundle sheath extensions (BSEs)

The BS surrounding the veins is made up of cells distinct from surrounding mesophyll cells in their elongate morphology, gene expression and chloroplast development (Kinsman & Pyke, 1998). The BS plays numerous roles, including water and nutrient transport via channels and pumps, phloem loading and water storage (Leegood, 2008; Griffiths *et al.*, 2012). In maize, BS development involved gene expression in common with the root endodermis (Slewinski *et al.*, 2012). The BS may be a locus for the sensitivity of K_{leaf} to decline with dehydration (Shatil-Cohen *et al.*, 2011; Pantin *et al.*, 2013). C4 species typically have 'Kranz anatomy', that is, enlarged BS cells dense with chloroplasts for CO_2 assimilation (Sage, 2004).

Bundle sheath extensions are achlorophyllous cells that in some species extend from the BS to the epidermis, and separate the leaf into chambers. Leaves with relatively dense BSEs are known as 'heterobaric' (Fig. 2m,n), and those without are known as 'homobaric' (Fig. 2l,o). BSEs have important roles (Notes S6), influencing light absorption (Nikolopoulos *et al.*, 2002; Liakoura *et al.*, 2009); K_{leaf} and its dynamics with irradiance and leaf water status (Zwieniecki *et al.*, 2007; Scoffoni *et al.*, 2008; Sommerville *et al.*, 2012); stomatal control (Terashima, 1992; Buckley *et al.*, 2011); and biomechanical support (Haberlandt, 1914; Read & Stokes, 2006). A function of BSEs in sugar transport has not been supported by their anatomy or chemical composition; BSEs may have few plasmodesmatal connections with other cells, and their low osmotic concentrations point to a low sugar content (Russin & Evert, 1985b).

9. Accessory transport and support tissues: sclereids, transfusion tracheids and idioblasts

Numerous conifers, cycads and angiosperms have specialized lamina tissue beyond the vein network, including sclereids (Fig. 2g), transfusion tracheids (Fig. 2l,m), fibers or idioblasts; these may be isolated, or form a network throughout the lamina. These tissues may also guide light to deeper mesophyll layers (Karabourniotis, 1998); contribute to higher K_{leaf} by conducting water, or by providing additional evaporative surface; shorten the hydraulic pathways; and/or contribute to water storage (Brodribb *et al.*, 2010). The transfusion tracheids that radiate from the midvein in conifer needles, or extend from the midrib parallel to the leaf surface in podocarps and in cycads, provide a higher K_{leaf} than expected solely from VLA (Brodribb *et al.*, 2007). This tissue may also contribute biomechanical support, especially during dehydration, by reducing leaf shrinkage (Cutler, 2005).

10. Leaf size

Leaf size is developmentally related to the venation architecture (see Section IV). Larger leaves tend to have larger petioles and major veins, which contain more numerous and larger xylem and phloem conduits, allowing transport capacity per unit leaf area to be independent of leaf size (Fig. 1a–d; McKown *et al.*, 2010). However, the minor vein diameter and cell sizes tend to be independent of leaf size (Sack *et al.*, 2012). These scaling trends allow K_{leaf} and gas exchange to be independent of leaf size, and to be separately optimized to the environment (Sack *et al.*, 2012).

Larger leaves may be intrinsically vulnerable to drought or freeze–thaw embolism, given their lower major VLA and their larger xylem conduit diameters. The venation architecture might limit maximum leaf size in certain cases, if low major VLA renders the leaf especially vulnerable to drought or overheating. In single-veined conifers, lamina area is linked with environmental moisture as expected from a direct hydraulic limitation of size (Zwieniecki *et al.*, 2004a; Brodribb & Feild, 2008). This can be remedied with multiple veins, or by the accessory transport and support tissues (Brodribb *et al.*, 2010).

The greater sizes of major veins in larger leaves contributes to their higher LMA, that is, the 'diminishing returns' in leaf area per mass invested in larger leaves (Grubb, 1998; Niinemets *et al.*, 2007a; Niklas *et al.*, 2007; Poorter *et al.*, 2009; Sack *et al.*, 2012).

IV. Development of the leaf vein system

1. Overall framework of vein development during leaf expansion

The algorithm of angiosperm vein development is very general, from *A. thaliana* to a wide range of diverse eudicotyledons (Sack *et al.*, 2012). This generality indicates genetic obstacles to evolution and redundant developmental pathways (Kang & Dengler, 2004; Kang *et al.*, 2007), consistent with the finding of low number of vein mutants in natural *A. thaliana* ecotypes and mutagenized populations (Candela *et al.*, 1999).

Vein development begins as the primordium takes shape. Indeed, vascular parenchyma might act as driver cells, providing the impetus for leaf expansion (Van Volkenburgh, 1999). Within the ground meristem, vascular identity gene expression and signal transduction begin the process of vein formation, followed by the differentiation of procambial strands (long cells with few organelles and dense cytoplasm) from central ground meristem cells. The developing vein pattern can be assessed once procambial cells are identifiable (Nelson & Dengler, 1997; Scarpella *et al.*, 2006) or earlier by visualizing the 'preprocambial' markers of gene expression (Kang & Dengler, 2004; Scarpella *et al.*, 2004; Sawchuk *et al.*, 2007; Notes S7).

Veins of given orders form in specific 'windows' of development time. Leaf development can be separated into two phases, a 'slow', limited expansion phase principally as a result of cell proliferation, and a 'rapid' and dramatic increase phase resulting mainly from cell expansion, although cell divisions continue. The first- and second-order veins are formed during the slow leaf expansion phase, the

third-order veins next, and the minor veins principally during the rapid phase (Sack *et al.*, 2012). In the typical sequence in *A. thaliana*, the leaf and midvein are initiated by preprocambial signals within the surface of the shoot apical meristem (SAM) by polar auxin transport (Notes S7). Marginal cells express the auxin efflux carrier protein PIN1 asymmetrically, that is, predominantly on one side, polarized toward a 'convergence point' on the SAM epidermis, causing it to accumulate auxin (Scarpella *et al.*, 2010). From this convergence point, the midvein preprocambium appears: a basipetally developing strand of cells with PIN1 expressed asymmetrically along their basal or inwardly directed cell surfaces, and this developing strand extends linearly into the SAM, where it signals the placement of the primordium and thus contributes to phyllotaxis (Sawchuk *et al.*, 2007; Bayer *et al.*, 2009; Scarpella *et al.*, 2010). Next, the midvein procambium develops progressively along that strand of cells, extending acropetally from the SAM into the leaf priomordium as it develops (Nelson & Dengler, 1997). The second-order veins form as the marginal meristem grows outward. Preprocambial genes are expressed in the dermal tissue at the leaf margin, and again PIN1 carriers are oriented toward convergence points that accumulate auxin; the preprocambial signal for the second-order veins begins from these convergence points, and spreads in lines of cells from the margin toward the midvein, incrementally recruiting ground meristem cells at their terminus (Scarpella *et al.*, 2006; Sawchuk *et al.*, 2007). The procambium then differentiates from the midrib to the margin progressively along those paths (Kang & Dengler, 2004; Scarpella *et al.*, 2004).

Higher-order veins form in between lower-order veins as the leaf expands. The initiation of procambial strands for each vein order is limited by the need to maintain a critical cell number or distance between new strands and by the duration that the ground meristem can perceive vascular-forming signals (Sack *et al.*, 2012). The preprocambium for each minor vein order appears as cell files throughout the lamina, each extending in two directions from a cell with a bipolar distribution of PIN1 toward an earlier formed vein; the procambium for each vein order differentiates from those cell files (Scarpella *et al.*, 2006, 2010). Leaf expansion proceeds simultaneously, such that the VLA of each order peaks as procambium forms, then declines as leaf expansion pushes veins apart, but in *A. thaliana* the minor VLA stabilizes as minor veins continue to be initiated in between pre-existing vein strands during ongoing expansion (Kang & Dengler, 2004; Kang *et al.*, 2007). In some species, after all vein strands are laid down, the leaf continues to expand, causing a decline in total VLA (Sack *et al.*, 2012). The FEVs initiate separately in the mesophyll, and grow to attach in the minor vein network. This general pattern of vein formation is superimposed on a basipetal wave of lamina expansion, so that higher orders of venation are formed earlier in the apical portion of the leaf and later in the basal portion (Nelson & Dengler, 1997; Scarpella *et al.*, 2006). Termination of the initiation of procambium from ground meristem is linked to mesophyll cell differentiation (Scarpella *et al.*, 2004; Kang *et al.*, 2007).

Vein diameter expansion depends on vein order. The first- and second-order veins have prolonged diameter growth, whereas third-order veins and minor veins rapidly attain maximum diameter (Nelson & Dengler, 1997; Sack *et al.*, 2012; Taneda & Terashima, 2012). In herbs, the thickening of minor veins is the result of cell proliferation, but in woody species, the first- and second-order veins can thicken by cambial growth (Plymale & Wylie, 1944). In some species, second-order veins may develop branches that form loops later in development; in other species, third-order veins can be thickened and thus transformed into second-order veins, but this accounts for a small portion of the network.

Finally, the phloem and then the xylem mature within the veins (Esau, 1977; Scarpella *et al.*, 2004; Sawchuk *et al.*, 2007). The phloem of the major veins functions in unloading sugars for leaf development, until the minor veins are fully formed, and then phloem unloading ceases, as the minor veins become functional for phloem loading (Turgeon, 2006).

In monocots with striate venation (and in eudicots such as pitcher plants; Franck, 1976), a similar process unfolds. The large longitudinal veins develop within the primordium, and as the leaf expands, these veins are spaced apart and the small longitudinal veins form in between, followed by the transverse veins (Denne, 1960). In grasses, the small longitudinal veins are connected apically and basally with adjacent veins within the leaf blade, and do not extend into the sheath region. Dicotyledons with exceptional venation also exhibit departures from the typical developmental sequence. Thus, in *Calophyllum* spp., the dense, parallel second-order veins branching from the midrib develop during the rapid expansion phase, as is the case for minor veins in the typical dicot leaf (Ramji, 1967).

The major and minor veins differ not only in the timing of development, but also in their gene expression during development, procambial anatomy, and xylem and phloem formation, just as they differ in many aspects of their function and evolution (see Table S5 in Sack *et al.*, 2012).

The BS tissue differentiates early with the developing vascular strand and cells divide and elongate in concert with vascular cells (Kinsman & Pyke, 1998). In species with multilayered BSs (e.g. grasses, which have an inner 'mesome sheath'), the inner layer can arise from the procambium, whereas the outer layers may arise from the ground meristem, which produces the mesophyll tissue (Bosabalidis *et al.*, 1994; Trivett & Evert, 1998). Accessory xylem tissues tend to form after the vein system is fully differentiated and the mesophyll has matured. Sclereids and mesophyll fibers can form independently from initials that appear late in mesophyll tissue differentiation (Arzee, 1953; Tomlinson & Fisher, 2005).

2. Coordination of vein traits with other cell and leaf traits in development and evolution

The developmental algorithm for vein formation during leaf expansion provides a basis for trait correlations within and across species. First, there are trait correlations within given leaves. Thus, in many species, the second-order veins are spaced further apart in the center of leaves, which expands most; their spacing can approximate the appearance of wavefronts, with their separation corresponding to development time (Pietak, 2009; Rolland-Lagan

et al., 2009). Further, because cell expansion after veins are patterned reduces minor VLA, areas of the leaf with smaller cells have higher VLA, for example, the tips and margins (Zwieniecki *et al.*, 2004b; Nardini *et al.*, 2008).

Across species, vein traits scale characteristically with mature leaf size as predicted by the developmental algorithm. Across a global dataset, major VLA declined with final leaf size, and major vein diameter increased with leaf size; this is because major vein formation occurs in the slow phase of leaf expansion, and these veins are subsequently pushed apart, although they can be thickened during most of their development. By contrast, minor VLA and diameter were independent of leaf size, as these can be initiated progressively during rapid leaf expansion, and rapidly achieve final diameter (Sack *et al.*, 2012; Notes S8). The scaling trends would be reinforced by additional adaptation to the environment. For example, when a larger leaf is selected for a given ecological advantage, this would reinforce the global scaling trends, because that leaf would automatically have a lower major VLA, according to the developmental mechanism. Further, this larger leaf would require a larger midrib for biomechanical and hydraulic support, and that would arise from the linked development of midrib diameter and leaf size. Selection for higher or lower minor VLA, independently of the final leaf size, would reinforce the independence of VLA from leaf size (Sack *et al.*, 2012). Vein chemistry also differs across the venation network, as expected from the developmental algorithm. The first- and second-order veins have less negative carbon isotope ratios than lamina, indicating preferential allocation of stored 'sink' phloem sugars rather than 'source' sugars in the growing leaf, as the major veins develop while the leaf is a sink (Badeck *et al.*, 2009).

The functional coordination of vein traits and stomatal traits (Section III. 4) also has a genetic and developmental basis. Studies of the fossil record and of modern adaptive radiations have shown correlations of vein traits and stomatal traits in evolution (Upchurch, 1984; Dunbar-Co *et al.*, 2009; Zhang *et al.*, 2012). Mutant studies also suggested that vein and stomatal patterning overlap in genetic basis (Jover-Gil *et al.*, 2012). Stomatal density is determined during the same development time as the venation (Pantin *et al.*, 2012) and in the mature leaf depends on epidermal cell numbers and sizes. *Arabidopsis thaliana* genotypes with larger cells at leaf maturity tend to have both lower VLA and lower stomatal density (Manuel Perez-Perez *et al.*, 2011). Similarly, in some species the sun-shade plasticity of vein traits is linked with shifts in cell and leaf size. Shade leaves of many species have greater cell and leaf expansion after the vein strands are fully formed, and thus develop a lower VLA and stomatal density than sun leaves (Brodribb & Jordan, 2011; Murphy *et al.*, 2012). However, this developmental coordination of vein and epidermal traits can be decoupled by shifting the amount and/or timing of vein development and stomatal initiation relative to cell and leaf expansion. Thus, in other species, shade leaves may develop lower, or similar, VLA, with larger, similar-sized, or even smaller leaves (Sack *et al.*, 2012).

Vein development can also set the pattern for other specialized tissues. In *Lithops*, the vein areoles guide the formation of unique epidermal 'windows' for light entry (Korn, 2011).

V. Function of the coordinated vein system and scaling up

Multiple vein traits in coordination with other leaf traits can influence K_{leaf} and plant performance (Table 1). Thus, the leaf venation is a model for how one or more higher-level properties can be determined by clusters of multiple traits, that is, 'many-to-one mapping' (Wainwright *et al.*, 2005). The trait that has the strongest impact in determining species differences will be one that varies most and/or that has an intrinsically stronger impact. The ability to predict the function of the whole system from some or all of its parts is assisted by the optimality principle that, under selection, multiple traits tend to shift simultaneously toward improving overall performance. Thus, selection for high gas exchange rate should cause a shift in a number of features that contribute to high K_{leaf} (Table 1), as increasing only one would lead to a greater limitation by other features (McKown *et al.*, 2010). Thus, vein traits are often intercorrelated with other structural and functional traits relating to leaf gas exchange. Vein traits may scale up to influence not only maximum rates of leaf gas exchange, but also stomatal responses to water supply, and plant water use and growth (Sack & Frole, 2006; Brodribb *et al.*, 2007; Boyce *et al.*, 2009; Brodribb *et al.*, 2010; McKown *et al.*, 2010; Feild *et al.*, 2011; Walls, 2011; Notes S9), and thus may have increasing application in predicting plant demographics and responses to climate change (Notes S10).

VI. Plasticity, evolution and assembly of leaf vein traits across growth forms, environments and biomes

1. Plasticity of vein traits within canopies and across environments

Vein traits, like other leaf traits, including leaf size and stomatal density, are determined by signals before and during leaf development (Zwieniecki *et al.*, 2004b; Pantin *et al.*, 2012). Many studies have demonstrated the plasticity of vein traits within canopies and for plants of given species across environments. Theoretically, leaves acclimated to higher irradiance, temperatures and nutrient supplies would have vein traits associated with higher K_{leaf} and rates of gas exchange (Table 1). However, leaves acclimated to *lower* water supply would also often have vein traits associated with higher K_{leaf} and rates of gas exchange, and also with greater drought tolerance. Such leaves can better 'gear up' to higher function when water is available, and to 'gear down' with stomatal closure and/or dormancy during periods of extended water shortage (Maximov, 1931; Grubb, 1998; Scoffoni *et al.*, 2011). This theory was supported by our synthesis of studies of vein trait plasticity (Table S1).

### 2. Variation in vein traits with growth form, habitat and biomes

Species adaptation across habitats drives strong shifts in leaf vein traits. Adaptation followed the same patterns described for plasticity across environments in a synthesis of studies of species variation in vein traits across habitats, for species within and across lineages (Table S1).

As a first test for worldwide trends in vein adaptation to climate, we compiled global data on VLA for 796 species (Table S3). This analysis resulted in critical novel trends (Figs 4, 5), consistent with those reported so far for smaller species sets and individual lineages (Table S1). Within given biomes and growth forms, deciduous species had higher mean VLA than evergreens. Further, within biomes, mean VLA increased from herbs to shrubs to trees, paralleling a trend previously shown for stomatal density (Beaulieu *et al.*, 2008). Within biomes and growth forms, sun-adapted species had higher VLA than shade-adapted species. Across biomes, there was a strong correlation of mean VLA with aridity (i.e. with the Priestly–Taylor α, an index of moisture supply relative to demand; $r_p = -0.93$, $P = 0.003$; inset in Fig. 4). These trends for VLA were also observed when we plotted individual species means against climate variables (Fig. 5); VLA correlated positively with mean annual temperature (MAT) and negatively with mean annual

precipitation (MAP) ($|r_p| = 0.17$, $P = 0.03$). The VLA also decreased with species' shade tolerance index. The global trend of increasing mean VLA with aridity matches the smaller-scale trends reported within smaller species sets and individual lineages (Table S1), and supports the idea that, while species vary strongly in their mechanisms of adaptation to aridity, a very common mechanism is to accomplish more photosynthesis during pulses of high water availability (Maximov, 1931; Grubb, 1998; Scoffoni *et al.*, 2011). This trend does not imply that forest species cannot have high VLA. Indeed, pioneer species of wet tropical forests frequently have high VLA (Sack & Frole, 2006; Feild *et al.*, 2011). However, many tropical wet forest canopies are dominated by species that establish as understory shade-tolerators, and their moderate vein densities persist even as they reach the canopy (Sack & Frole, 2006), and would lower the biome mean. This global analysis, based on the substantial available data, provides a baseline against which forests, growth forms, and lineages should be compared.

We also identified shifts in VLA associated with specific functional types, consistent with expectations (Fig. 5). Aquatic herbs had very low VLA, particularly submerged aquatics. Crop herbs and alpine herbs had similar VLA to temperate herbs. Succulent-leafed species had very low VLA, consistent with their low g_s, and, in many cases, CAM photosynthesis. As previously observed, C_4 grasses tended to have higher VLA than C_3 grasses.

Major VLA also showed adaptation to climate and habitat, in part corresponding to its linkage with leaf size (Fig. 5). Leaf size but not major VLA was positively related to MAT. Leaf size also correlated with MAP, as expected from previous meta-analyses, while major VLA declined ($r_p = -0.35$; $P = 0.02$). Thus, species of drier habitats tended to have smaller leaves, with greater major VLA, conferring redundancy and drought tolerance (Section III. 5). Leaves of shade-tolerant species tended to have larger leaves with lower VLA (Fig. 5).
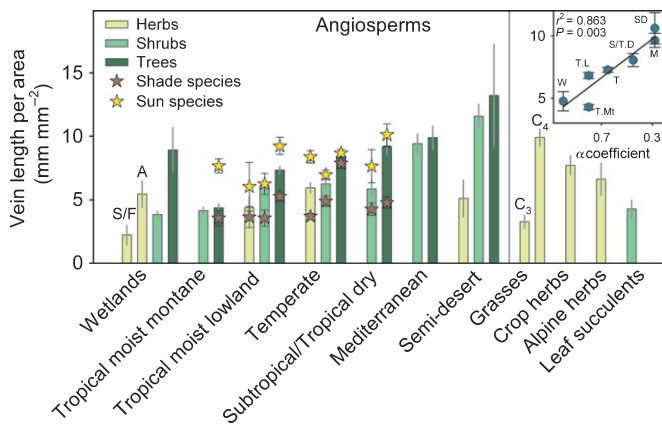

Fig. 4 Meta-analysis of the diversity of vein length per unit area (VLA) across growth forms, habitats and biomes from a global database compiled from published values (Table S3). Mean VLA and standard errors are given for species that establish in sun vs shade for different growth forms across biomes. Biomes are arranged from left to right in order of increasing aridity, with means for additional growth forms on the far right. Mean and standard errors for sun and shade species are represented by stars for each biome and growth form. Across biomes, woody species had, on average, 1.3-fold higher VLA than herbs, and VLA in trees was, on average, 1.3-fold higher than shrubs (one-way ANOVAs, $P < 0.0001$). VLA was significantly higher for sun than for shade species, and higher for trees than for shrubs and herbs across biomes (three-way ANOVA, with sun/shade nested within growth form, nested within biome; all $P < 0.0001$). Deciduous species tended to have higher VLA than evergreen species across biomes and growth forms (three-way ANOVA, with leaf habit nested within growth form, nested within biome, all $P < 0.0001$). From left to right, respectively, $n = 6, 6, 2, 3$ for wetland species (A, aerial leafed; S/F, submerged and floating leafed aquatics); 28 (4, 19 for sun and shade means), 36 (12,18) for tropical moist montane species; 14 (5, 7), 46 (16, 17),118 (78, 22) for tropical moist lowland species; 56 (27, 28), 88 (45, 36), 129 (108, 17) for temperate species; 27 (5, 7), 46 (16, 17) for subtropical/tropical dry species; 14, 13 for mediterranean-climate species; 2, 6, 2 for semi-desert species; 18, 27 for grasses; 12 for crop herbs; 4 for alpine herbs; 3 for leaf succulents. The inset shows mean VLA ± standard error averaged across growth forms for each biome and plotted against the Priestley–Taylor coefficient of annual moisture availability, indicating higher VLA in drier biomes (α; Prentice *et al.*, 1992). SD, semi-desert; M, Mediterranean; S/T.D, subtropical/tropical dry; T, temperate; T.L, tropical moist lowlands; T.Mt, tropical moist montane; W, wetlands.

VII. Evolution of the leaf vascular system across plant lineages, paleohistory and biogeography

1. Evolution of vein traits in major plant lineages and paleohistory

The leaf venation appeared in the earliest true leaves. The earliest land-plant lineages lacked vasculature, although some may have had analogous structures. Certain mosses evolved laminate structures that deliver some water through hydroids in their costae. The multiple evolution of leaves is analogous to that of wood, which evolved five or more times by the mid-Carboniferous (320 million yr ago (Mya)). In the early Devonian, *c.* 400 Mya, leaf-like organs evolved independently in two lineages. In lycophytes, microphylls (containing a single vascular bundle) evolved through enations, small outgrowths of tissue later becoming vascularized, or through the sterilization of sporangia (Crane & Kenrick, 1997; Floyd & Bowman, 2006). In euphyllophytes, megaphylls probably originated multiple times as lateral branches (Floyd & Bowman, 2006; Tomescu, 2009). This phenomenon has been hypothesized to involve infilling ('webbing') between side branches on the same plane, according to Zimmermann's 1930 telome theory (Wilson,
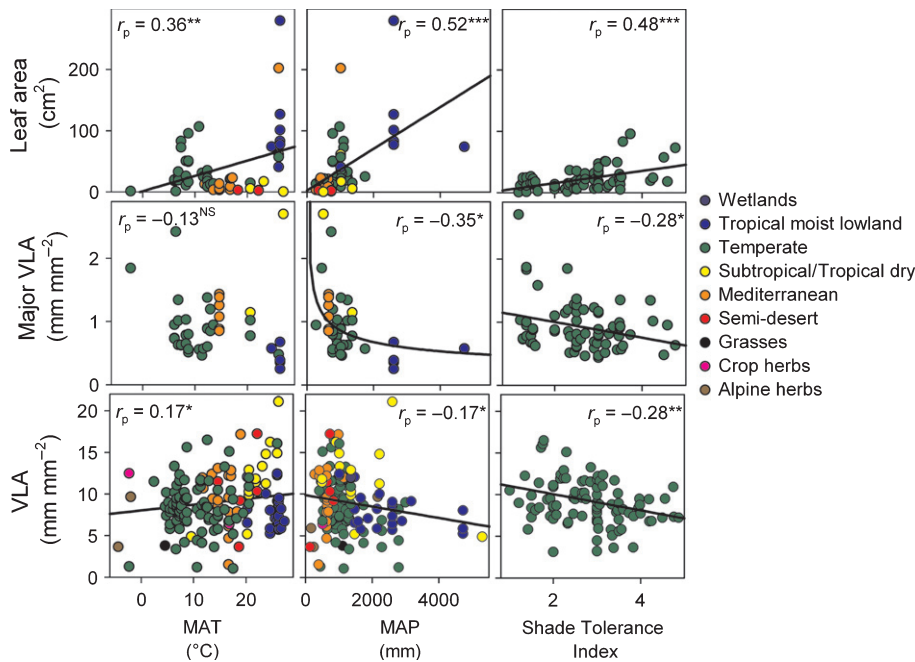
Fig. 5 Relationships from a global database (Table S3) compiled from published values of leaf size, major vein length per unit area (major VLA) and total vein length per unit area (VLA) with climate (mean annual temperature (MAT) and precipitation (MAP)), and with shade tolerance index for temperate species (Niinemets & Valladares, 2006). Climate data are not those of the plants sampled for measurements, but were taken for field populations of the given species from GLOPNET (Wright *et al.*, 2004, 2005); trends would probably be even stronger if climate data were available from the sampled plants of given species. From left to right: leaf area, $n = 55, 55, 69$; major VLA, 44, 44, 68; VLA, 159, 159, 94. ^{NS}, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

1953). Webbing may arise as a continuation of meristematic activity as a marginal meristem, rather than splitting of meristems during dichotomy in the ancestral branched system (Doyle, 1998; Beerling & Fleming, 2007).

Vein reticulation and hierarchy, simpler than that for angiosperms, emerged in earlier lineages via different evolutionary and developmental pathways (Table S2; Alvin & Chaloner, 1970; Trivett & Pigg, 1996). We provide a synthesis of leaf venation in the major plant groups, highlighting vein complexity, reticulation and presence of FEVs and BSEs (Fig. 6; Table S2).

2. Vein traits as key innovations in angiosperms

Angiosperms evolved numerous distinctive vein traits contributing to their greater performance than earlier-evolved lineages. Early angiosperms tended to have low-order veins with less organization, correlating with stomata varying strongly in the numbers and arrangement (Upchurch, 1984). Later evolution of higher VLA (> 5 mm mm^{-2}; Boyce *et al.*, 2009; Brodribb & Feild, 2010; Feild *et al.*, 2011) corresponded with increasing numbers of vein orders (Brodribb & Feild, 2010) and higher stomatal density (Franks & Beerling, 2009). Angiosperms required the evolution of the plate meristem to produce higher vein orders (Doyle & Hickey, 1976; Boyce, 2008; Sack *et al.*, 2012). A high VLA would also require the evolution of a vein hierarchy and vessels, because a high-VLA system built of large veins comprising tracheids would be prohibitively expensive (Beerling & Franks, 2010; McKown *et al.*, 2010). Further, the evolution of simple perforation plates from scalariform plates may have been essential (Feild & Wilson, 2012). Sieve tubes may also have been a necessary advance. In *Amborella trichopoda*, sister to all other angiosperms, the minor veins

showed anatomical and physiological specialization for both apoplastic and symplastic loading via polymer trapping, indicating versatile efficiency (Turgeon & Medville, 2011).

These innovations made possible large leaves with large major veins for mechanical support and a high VLA that enables transpirational cooling and high photosynthetic rates (Niklas, 1999; Osborne *et al.*, 2004; Boyce, 2008; Brodribb *et al.*, 2010; Walls, 2011; Sack *et al.*, 2012). Indeed, the angiosperm system allows for a dramatic range of leaf sizes with a wide spectrum of VLA, vein conductivities, and photosynthetic rates (Wright *et al.*, 2004), and enables dominance in a far greater range of habitats than other plant lineages (Boyce *et al.*, 2009; Brodribb *et al.*, 2010; McKown *et al.*, 2010, 2010; Peppe *et al.*, 2011).

This advanced venation, in addition to a more sophisticated stomatal control system and the floral organ, set the stage for the rise of angiosperms to global dominance.

3. Links of vein evolution with atmosphere and climate and influence on the biogeography of key vegetation types

The evolution of venation, like that of wood and leaves, may have been critically coupled to changes in atmospheric CO_2 (Sperry, 2003; Brodribb & Feild, 2010; de Boer *et al.*, 2012). Indeed, the evolution of megaphylls is linked to the drop in atmospheric CO_2 in the late Devonian (Beerling *et al.*, 2001; Fig. 6). Megaphylls evolved higher stomatal densities, enabling the fixation of sufficient CO_2 to survive, and transpiration rates adequate to cool their planate laminae (Beerling *et al.*, 2001; Shougang *et al.*, 2003; Osborne *et al.*, 2004). Similarly, the evolution of high VLA in angiosperms may have been precipitated by declining CO_2, enabling greater stomatal apertures and thus maintaining or increasing photosynthetic performance (Beerling & Franks, 2010;
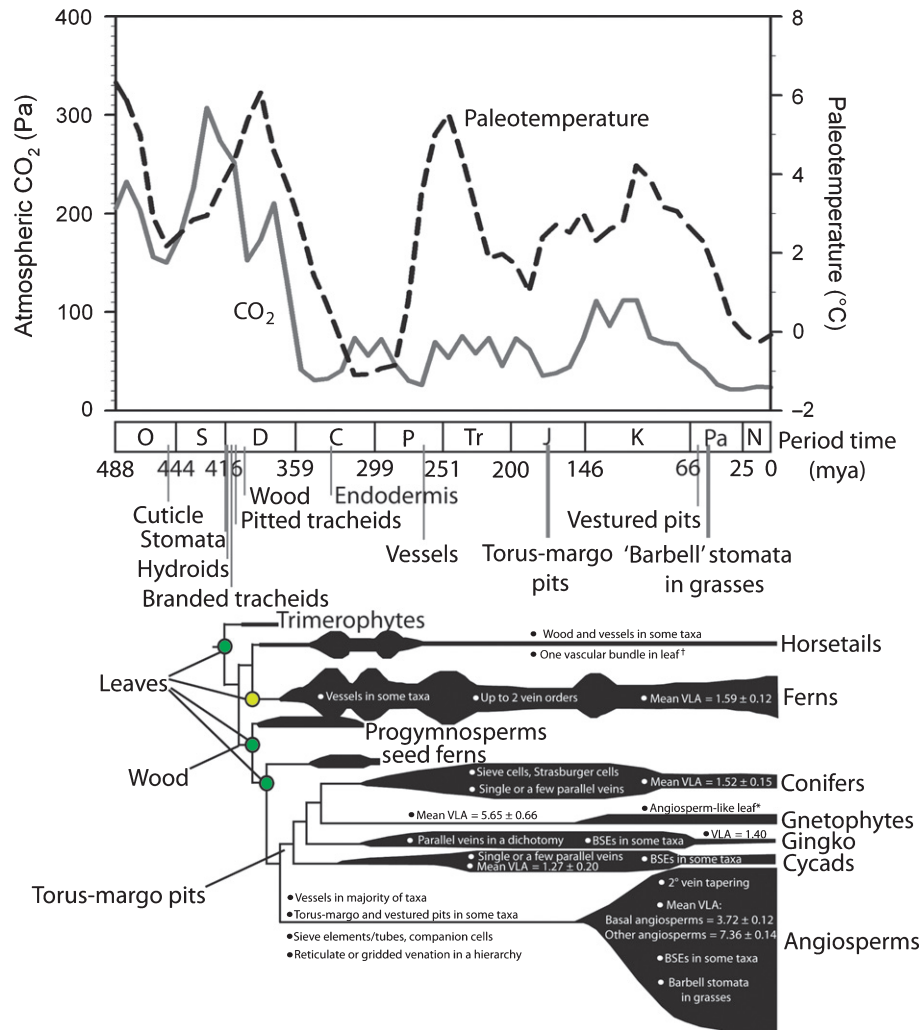
Fig. 6 Evolution of terrestrial plants and their traits, including leaf vein traits against geological periods and time (million years ago, Mya). The top graph shows fluctuations through time of atmospheric CO_2 according to Berner (2008) and temperature deviations relative to today according to Royer (2006). The phylogeny is from Pittermann (2010) where relative tracheophyte abundances in the fossil record are shown by line thickness. The morphological features important to the evolution of plant water transport are from Pittermann (2010) with additions relating to leaf venation architecture (Table S2); traits marked on the *x*-axis indicate first evidence in the fossil record, whereas traits marked on the phylogeny represent those common in given lineages. Leaves have evolved multiple times, represented by green dots. The first known megaphyll species was the Trimerophyte *Eophyllophyton bellum*. The light green dot in ferns refers to the multiple evolution of leaves within this lineage (three to six events, Tomescu, 2009). Mean vein lengths unit per area (VLA) ± standard errors given for each lineage are from a newly compiled global database (Table S3). Ferns ($n = 67$); cycads ($n = 19$); *Gingko* ($n = 1$); conifers ($n = 6$); gnetophytes ($n = 4$); basal angiosperms ($n = 73$); other angiosperms ($n = 627$). †, *Equisetum* leaves are microphylls organized in whorls and are believed to have evolved from sphenophylls, megaphyll-like precursors (Rutishauser, 1999). *, Gnetophytes evolved leaves similar in all ways to angiosperm leaves, with reticulate venation, tapering and bundle sheath extensions (BSEs) in some taxa. Note their higher vein length per unit area (VLA) compared with other gymnosperms.

de Boer *et al.*, 2012), providing a strong advantage over competing plant groups (Brodribb & Feild, 2010; Feild *et al.*, 2011; Fig. 6). There is some debate about the ability of VLA to drive differences among groups in photosynthetic rates as atmospheric CO_2 changed, with predictions depending on the traits and assumptions used to estimate A_{area} from VLA (de Boer *et al.*, 2012; Boyce & Zwienecki, 2012).

The leaf venation may have impacts on the distribution of vegetation systems. The evolution in angiosperms of higher VLA and transpiration rates may have driven the establishment of angiosperm-dominated rainforests, and influenced the global water budget (Boyce *et al.*, 2009, 2010; Boyce & Lee, 2010). Vein traits

also likely played a role in the evolution of grasslands. Higher VLA in grasses probably evolved in response to declining CO_2 in the Miocene and with the colonization of drier, more exposed habitats (Edwards *et al.*, 2010). High VLA probably potentiated the repeated evolution of C_4 and enabled the emergence of this key biome (Sage, 2004; Osborne & Sack, 2012).

VIII. Applications of leaf venation architecture

Leaf venation has increasing usefulness in paleoecology and paleoclimatology, agriculture, urban ecology, and design and technology (Notes S10).

IX. Conclusions

Our synthesis of the broad range of recent research on leaf venation highlighted simplifying principles with wide-ranging implications:

1 The leaf vein features responsible for water, nutrient, and sugar transport, and biomechanical support and protection are optimized according to several similar principles, and tightly coordinated in their genetic and developmental basis, and their evolution.

2 The major and minor vein systems of angiosperms form an integrated transport network, but are disjunct in numerous aspects of their function, and in their development, evolution and paleohistory.

3 Multiple genetic, structural and physiological vein traits combine to determine higher-level traits that influence whole-plant performance and adaptations across environments.

4 In angiosperms, leaf venation develops according to a typical algorithm, and shows strong and predictable plasticity and adaptation across environments, resulting in global trends in vein traits across growth forms, habitats and biomes.

5 Leaf vein traits have shown repeated evolutionary trajectories across major plant groups and have probably influenced climate and shifts of dominance in world vegetation.

6 Leaf vein traits have numerous and increasing applications across a wide range of activities.

Further integration of research on leaf venation across fields will hasten discoveries and generate a unified knowledge base across genetics, development, and structure and function. This integration will contribute to new understanding that extends across whole-plant biology and the ecology of current, past and future ecosystems.

Acknowledgements

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Synthesis of studies of vein features, testing their plasticity (differences across canopies, or for plants of given species grown in different conditions) or adaptation (differences found across species, native to different habitats, likely mainly genetically driven)

Table S2 Synthesis of vein features in land plant lineages, emphasizing variation in hierarchy, reticulation, and vein length per area, and presence or absence of free ending veins and bundle sheath extensions, with key examples and notes

Table S3 Vein traits compiled from previous studies for globally-distributed species, including information of lineage, growth form, sun/shade distribution, leaf area, mean annual temperature and precipitation for plants in native communities, and shade tolerance index data

Notes S1 Establishing the function of a vein trait.

Notes S2 The functional consequences of variation in dimensions of vascular cells within veins.

Notes S3 The functional consequences of the leaf vein hierarchy.

Notes S4 The functional consequences of vein length per area (VLA).

Notes S5 The functional consequences of major vein length per area (major VLA).

Notes S6 The functional consequences of bundle sheath extensions (BSEs).

Notes S7 Genetic pathways and signals for vein patterning during leaf development.

Notes S8 The independence of minor vein length per area from leaf size across species.

Notes S9 Scaling up the coordinated function of the leaf vein system.

Notes S10 Applications of leaf venation architecture.

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