

REVIEW PAPER

How do leaf veins influence the worldwide leaf economic spectrum? Review and synthesis

Lawren Sack^{1,*}, Christine Scoffoni¹, Grace P. John¹, Hendrik Poorter², Chase M. Mason³,
Rodrigo Mendez-Alonzo¹ and Lisa A. Donovan³

¹ Department of Ecology and Evolutionary Biology, University of California Los Angeles, 621 Charles E. Young Drive South, Los Angeles, CA 90095, USA

² IBG-2 Plant Sciences, Forschungszentrum Jülich, D-52425 Jülich, Germany

³ Department of Plant Biology, University of Georgia, 2502 Miller Plant Sciences, Athens, GA 30602, USA

* To whom correspondence should be addressed. E-mail: lawrensack@ucla.edu

Received 16 April 2013; Revised 22 August 2013; Accepted 27 August 2013

Abstract

Leaf vein traits are implicated in the determination of gas exchange rates and plant performance. These traits are increasingly considered as causal factors affecting the ‘leaf economic spectrum’ (LES), which includes the light-saturated rate of photosynthesis, dark respiration, foliar nitrogen concentration, leaf dry mass per area (*LMA*) and leaf longevity. This article reviews the support for two contrasting hypotheses regarding a key vein trait, vein length per unit leaf area (*VLA*). Recently, [Blonder *et al.* \(2011, 2013\)](#) proposed that vein traits, including *VLA*, can be described as the ‘origin’ of the LES by structurally determining *LMA* and leaf thickness, and thereby vein traits would predict LES traits according to specific equations. Careful re-examination of leaf anatomy, published datasets, and a newly compiled global database for diverse species did not support the ‘vein origin’ hypothesis, and moreover showed that the apparent power of those equations to predict LES traits arose from circularity. This review provides a ‘flux trait network’ hypothesis for the effects of vein traits on the LES and on plant performance, based on a synthesis of the previous literature. According to this hypothesis, *VLA*, while virtually independent of *LMA*, strongly influences hydraulic conductance, and thus stomatal conductance and photosynthetic rate. We also review (i) the specific physiological roles of *VLA*; (ii) the role of leaf major veins in influencing LES traits; and (iii) the role of *VLA* in determining photosynthetic rate per leaf dry mass and plant relative growth rate. A clear understanding of leaf vein traits provides a new perspective on plant function independently of the LES and can enhance the ability to explain and predict whole plant performance under dynamic conditions, with applications towards breeding improved crop varieties.

Key words: Drought tolerance, functional traits, leaf hydraulics, leaf mass per area, leaf nutrient concentrations, photosynthetic rate, vasculature, vein patterning.

Introduction

Individual plant traits or trait combinations can influence species differences in performance, productivity, and ecology ([McGill *et al.*, 2006](#); [Kattge *et al.*, 2011](#); [Violle *et al.*, 2007](#)). Traits that have received widespread attention include plant height, seed size, and wood density as well as traits related to the ‘leaf economic spectrum’ (LES) ([Westoby *et al.*, 2002](#); [Cornelissen *et al.*, 2003](#); [Diaz *et al.*, 2004](#); [Kattge *et al.*, 2011](#)).

The LES represents a unified axis of leaf trait variation: light-saturated photosynthetic rates are correlated with dark respiration rates and nitrogen concentration, whether expressed on a leaf area or, especially, a mass basis ([Small, 1972](#); [Field and Mooney, 1986](#); [Reich *et al.*, 1997](#); [Wright *et al.*, 2004](#); [Donovan *et al.*, 2011](#); [Lloyd *et al.*, 2013](#); [Osnas *et al.*, 2013](#); [Westoby *et al.*, 2013](#)). The leaf dry mass per area (*LMA*; see

Table 1 for a list of definitions) is a central variable in this trait network, strongly correlated with others across species globally. A well-known generalization is that fast-growing, resource-acquisitive species tend to have lower *LMA*, higher light-saturated rates of photosynthesis per mass (A_{mass}), and higher nitrogen concentration per mass (N_{mass}) and respiration rate per mass (R_{mass}), but shorter leaf lifespan (*LL*), relative to slow-growing, resource-conservative species. These trait correlations tend to be strong across diverse species not only when grown under common controlled conditions (Lambers and Poorter, 1992), but also across communities and biomes (Small, 1972; Reich *et al.*, 1997; Wright *et al.*, 2004, 2005b; Heberling and Fridley, 2012). The strength and ubiquity of these relationships imply mechanistic linkages (Reich *et al.*, 2007). However, there is also a great deal of unexplained variation around the relationships, such that other factors are necessary to explain A_{mass} differences across species (Wright *et al.*, 2005a). A wider framework of trait scaling would therefore be useful.

In recent years, the influence of leaf hydraulic traits on leaf and plant-level function has gained increasing attention, with an important role of vein traits including vein length per unit leaf area (*VLA*, also known as vein density) (Sack and Frole, 2006; Brodribb *et al.*, 2007; Perez-Harguindeguy *et al.*, 2013; Sack and Scoffoni, 2013). The *VLA* is a key feature of a leaf vein system that is typically hierarchical and reticulate in angiosperms, composed of four to eight vein branching orders, including one or more first-order veins (including the central midvein), the second-order veins that branch off from the midrib at intervals, the third-order veins that connect them, and one to several orders of smaller veins that form a mesh among all the lower-order veins. The first three orders of veins are known as ‘major veins’, and the higher order veins are ‘minor veins’. The major veins are large in diameter but low in length per leaf area, whereas the minor veins are very narrow and high in length per leaf area. Although *VLA* is the sum of the major and minor vein lengths per area, *VLA* is determined largely (>80%) by the minor *VLA* (Sack *et al.*, 2012; Sack and Scoffoni, 2013). The aim of this paper is to explore the possible mechanistic linkages of leaf venation with the LES and the impacts on plant performance.

Recently, Blonder *et al.* (2011, 2013) attempted to link leaf venation traits to the LES. Specifically, they proposed a central role for *VLA* in influencing the leaf thickness, volume, and mass, and thus *LMA*, which is equivalent to leaf thickness × leaf dry mass density (Witkowski and Lamont, 1991; Niinemets, 1999b). Blonder *et al.* (2011) therefore proposed that *VLA* was the ‘origin’ of *LMA* and other LES traits across diverse species (Fig. 1), and more recently (Blonder *et al.*, 2013), within given species (Appendix 3). Blonder *et al.* (2013) named their hypothesis, ‘the venation theory’, but as they only focused on a very narrow set of the substantial effects of leaf venation on plant function (see, for example, Table 1 in Sack and Scoffoni, 2013), this review will refer to it as the ‘vein origin’ hypothesis.

This review critically examines the ‘vein origin’ hypothesis by evaluating its assumptions, derivations, and predictions. Because this evaluation did not support the ‘vein origin’

hypothesis, an alternative was synthesized from previous work, the ‘flux trait network’ hypothesis, to explain the function of the leaf venation and its role in the LES. According to the ‘flux trait network’ hypothesis, leaf traits and plant performance are inter-related by many traits, with a key role for structural and physiological variables that influence fluxes. In the ‘flux trait network’, *VLA* has important influences on hydraulic conductance, stomatal conductance, and photosynthetic rate independently of *LMA* (Fig. 2; Sack *et al.*, 2003; Niinemets and Sack, 2006; Sack and Holbrook, 2006; Hao *et al.*, 2010). Thus, according to the best available evidence, this review resolved (i) how *VLA* influences the LES traits and relationships. We additionally considered several details and ramifications of the flux traits hypothesis, including (ii) the specific role of *VLA* in determining leaf hydraulic conductance (K_{leaf}) and A_{area} , (iii) the role of leaf major veins as distinct from the minor veins in influencing LES traits, and (iv) the role of *VLA* in determining A_{mass} and whole-plant relative growth rate. We were careful to be clear about caveats and uncertainties to truly reflect what is known about the combined impacts of traits on higher-level plant function and we propose avenues for further research to clarify the field.

Materials and methods

To test the various assertions and assumptions, a database of *VLA* and LES trait data was compiled from previous studies, for over 350 angiosperm woody and herbaceous species from 88 families diverse in their global origin (Supplementary Tables S1 and S2, available at *JXB* online; sources of vein data: Wylie, 1939; Wylie, 1951, 1954; Philpott, 1956; Sack and Frole, 2006; Dunbar-Co *et al.*, 2009; Blonder *et al.*, 2011; Scoffoni *et al.*, 2011; Walls, 2011; Nardini *et al.*, 2012; Sack *et al.*, 2012; Mason *et al.*, 2013; and gas exchange and climate data: Wright *et al.*, 2004, 2005a). For all analyses, mean values were used when there were replicate values for given species.

Mechanistic trait linkages versus concerted convergence

In examining trait relationships across species, it is important to distinguish which are mechanistic, and the type of mechanism, and to clarify cases when trait correlations arise for other reasons than mechanistic linkages (Sack *et al.*, 2003; Givnish *et al.*, 2005; Niinemets and Sack, 2006; Sack and Scoffoni, 2013). Mechanistic trait correlations require a logical basis such that, all other traits being equal, a substantial shift in one trait would necessitate a shift in another. These mechanistic trait correlations can arise at a range of scales (e.g. within species or across species, within lineages, and/or within communities, and/or across communities and environments). Notably, because traits in combination often affect a number of other traits, and many traits vary within any typical species set, even when traits are mechanistically linked in principle, the strength of the correlation between given traits can differ according to the species set considered (Table 2).

Mechanistic trait correlations include linkages among traits that are intrinsic (i.e. necessary based on physics or physiological integration). Thus, traits are mechanistically correlated if one trait is structurally, functionally, and/or

Table 1. Definitions of traits, variables, and features

Trait, variable, or feature	Symbol	Typical unit
Leaf venation		
Total vein length per leaf area	VLA (or σ)	mm mm^{-2}
Major vein length per leaf area	Major VLA	mm mm^{-2}
Minor vein length per leaf area	Minor VLA	mm mm^{-2}
Interveinal distance	IVD (or d)	μm
Vein-to-epidermal distance	VED	μm
Number of vein areoles per leaf area	VAA (or ξ)	$n \text{ mm}^{-2}$
Maximum distance from vein to stomata	D_m	μm
Total vein length per mass	VLM	m g^{-1}
Vein radius	r_v	μm
Free-ending veins	FEVs	
Bundle sheath	BS	
Bundle sheath extensions	BSEs	
Mesophyll pathways for water flow	MPs	
Leaf structure		
Leaf thickness	LT	μm
Leaf mass density	LD	g cm^{-3}
Leaf economics spectrum and leaf composition		
Leaf mass per area	LMA	g m^{-2}
Mass density of vein tissue	ρ_v	g cm^{-3}
Mass density of non-vein leaf tissue	ρ_L	g cm^{-3}
Light-saturated photosynthetic rate per leaf area	A_{area}	$\mu\text{mol m}^{-2} \text{ s}^{-1}$
Light-saturated photosynthetic rate per leaf mass	A_{mass}	$\text{nmol g}^{-1} \text{ s}^{-1}$
Nitrogen concentration per leaf area	N_{area}	g m^{-2}
Nitrogen concentration per leaf mass	N_{mass}	g g^{-1}
Leaf lifespan	LL	Months
Respiration rate per leaf area	R_{area}	$\mu\text{mol m}^{-2} \text{ s}^{-1}$
Respiration rate per leaf mass	R_{mass}	$\text{nmol g}^{-1} \text{ s}^{-1}$
Mesophyll conductance to CO_2 diffusion	g_m	$\text{mmol m}^{-2} \text{ s}^{-1}$
Hydraulic physiology		
Leaf hydraulic conductance (leaf area basis)	K_{leaf}	$\text{mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$
Leaf hydraulic conductance (leaf mass basis)	$K_{\text{leaf, mass}}$	$\text{mmol kg}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$
Leaf xylem and outside-xylem hydraulic conductance	K_x and K_{ox}	$\text{mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$
Plant hydraulic conductance		
Plant hydraulic conductance	K_{plant}	$\text{mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$
Leaf water potential	Ψ_{leaf}	MPa
Leaf-to-soil water potential gradient	$\Delta\Psi_{\text{leaf-to-soil}}$	MPa
Gas exchange physiology		
Leaf conductance to water vapour	g	$\text{mmol m}^{-2} \text{ s}^{-1}$
Stomatal conductance	g_s	$\text{mmol m}^{-2} \text{ s}^{-1}$
Boundary layer conductance	g_b	$\text{mmol m}^{-2} \text{ s}^{-1}$
Water use efficiency	WUE	$\mu\text{mol mmol}^{-1}$
Maximum stomatal conductance	g_{max}	$\text{mmol m}^{-2} \text{ s}^{-1}$
Electron transport rate	J_{max}	$\mu\text{mol m}^{-2} \text{ s}^{-1}$
Maximum CO_2 assimilation rate	V_{cmax}	$\mu\text{mol m}^{-2} \text{ s}^{-1}$
Transpiration rate	E	$\text{mmol m}^{-2} \text{ s}^{-1}$
Ambient and chloroplastic CO_2 partial pressures	C_a and C_c	Pa
Stomatal anatomy		
Stomatal density	n_s , SD	$n \text{ mm}^{-2}$
Area of stomatal pore	a_s , SS	μm^2
Stomatal pore thickness	t_s	μm
Stomatal pore area index	SPI	%
Stomatal index	SI	cells cell^{-1}
Whole plant growth and biomass allocation		
Leaf mass fraction	LMF	g g^{-1}
Leaf area ratio	LAR	$\text{m}^2 \text{ g}^{-1}$
Unit leaf rate	ULR	$\text{g m}^{-2} \text{ day}^{-1}$

(Continued)

Table 1. Continued

Trait or feature	Symbol	Typical unit
Plant maximum relative growth rate	RGR_{max}	$g\ g^{-1}\ day^{-1}$
Environment		
Mean annual precipitation	MAP	mm
Soil water potential	Ψ_{soil}	MPa
Vapour pressure deficit	VPD	$mol\ mol^{-1}$
Diffusion coefficient of water vapour in air	D	$m^2\ s^{-1}$
Saturation vapour concentration of water in air	c_o	$mol\ H_2O\ m^{-3}$
Relative humidity	h	%
Empirical parameters of equations of Blonder <i>et al.</i> (2011)	k_0, k_1, k_2, k_3	Various

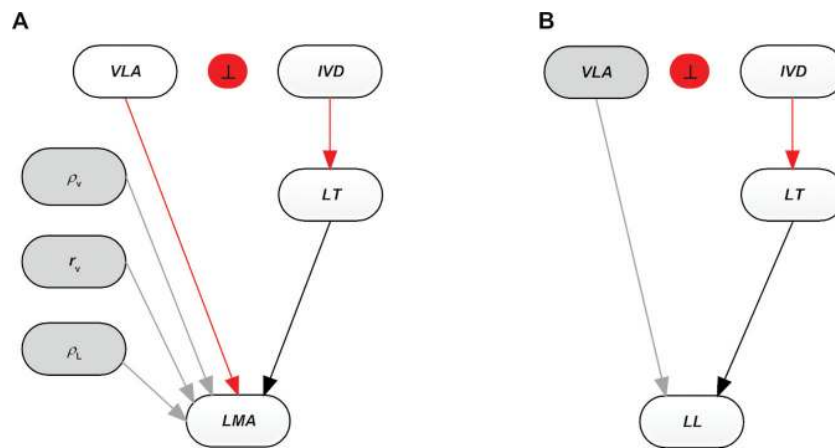


Fig. 1. Schema for the ‘vein origin’ hypothesis for the role of vein traits in determining the leaf economic spectrum, according to [Blonder *et al.* \(2011\)](#) but not supported in these analyses. Arrows represent positive determination. (A) Schema for the determination of leaf mass per area (LMA) by vein length per area (VLA) and interveinal distance (IVD). According to this hypothesis, VLA and IVD are substantially independent (\perp), and a higher VLA directly determines a higher leaf mass per area (LMA), as would a higher mass density of veins (ρ_v), larger vein radii (r_v), and larger mass density of non-vein mesophyll tissue (ρ_L). Traits and linkages in grey indicate that these were treated by [Blonder *et al.* \(2011\)](#) as constants, despite their varying strongly across species in reality, and thus their variation would not scale up to influencing LMA in this formulation. Simultaneously, a higher IVD would correspond to greater leaf thickness (LT), due to mechanistic scaling, and higher LT would drive higher LMA . (B) Schema for the determination of leaf lifespan (LL) by VLA and IVD . According to [Blonder *et al.* \(2011\)](#), a higher VLA directly determines a higher LL by providing damage protection, and a higher IVD would correspond to greater LT , due to mechanistic scaling, and higher LT would correspond to a tougher leaf with higher LL . The linkage of VLA with LL is denoted in grey because [Blonder *et al.*](#) did not pursue it, but only that of IVD with LL in their equation derivations. The traits and arrows indicated in red are predictions of the ‘vein origin’ hypothesis that contrast with those of the ‘flux trait network’ hypothesis ([Fig. 2](#)), in which VLA and IVD are closely negatively related, and VLA and IVD are not generally mechanistically related to LT and LMA across species.

developmentally dependent on another. A first type of mechanistic correlation is a direct mechanistic linkage. An example of a direct mechanistic linkage is the contribution of VLA to K_{leaf} , a ratio expressing the capacity for leaf water transport per leaf area per unit water potential driving force, because the veins are a major component of the leaf water transport pathway ([Sack and Holbrook, 2006](#)). A second type of mechanistic trait linkage is one which arises due to development. For example, across species, major VLA is mechanistically inversely correlated with leaf size, because major veins form early in leaf development, and are spaced apart during leaf expansion, and consequently, larger leaves must have their major veins spaced further apart ([Sack *et al.*, 2012](#)). A third type of mechanistic trait correlation occurs when structurally independent traits co-determine a given function. For

example, leaf veins and stomata occur in distinct tissues, but VLA and stomatal pore area per leaf area tend to be positively correlated, as expected if water supply has to match transpirational demand ([Sack *et al.*, 2003](#); [Dunbar-Co *et al.*, 2009](#); [Brodribb and Jordan, 2011](#)).

By contrast with mechanistic trait correlations, a distinct, rather coincidental type of trait correlation is ‘concerted convergence’ ([Givnish *et al.*, 2005](#)), when traits that are independent in function and development appear correlated given their selection across a given set of conditions. For example, in monocots, species with ‘net-venation’ (i.e. branching leaf major veins rather than the striate venation typical of monocots) also tend to possess fleshy fruits, with both traits being advantageous in shade. Unlike mechanistic trait correlations, concerted convergence results in trait correlations typical

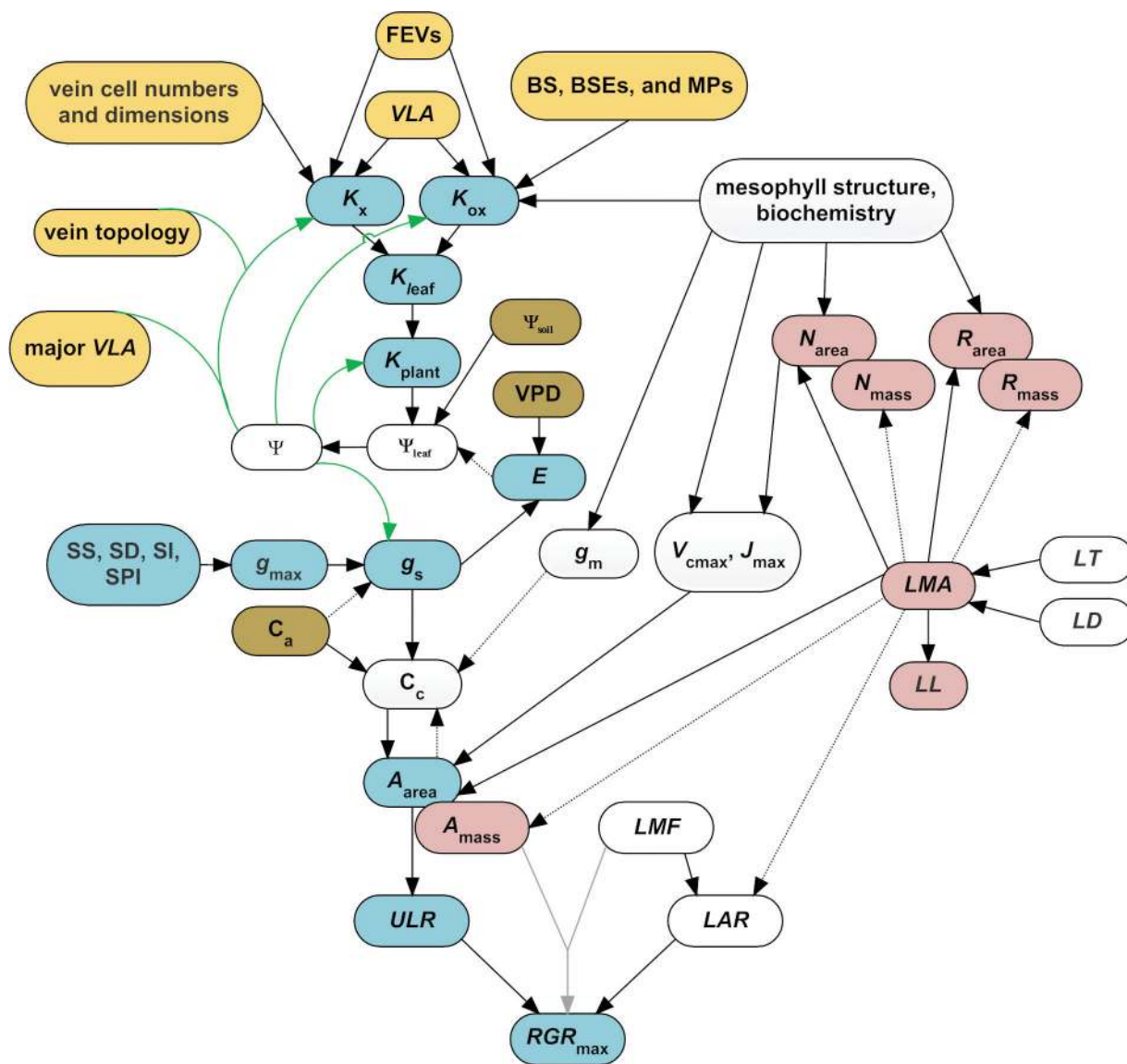


Fig. 2. The influence of vein traits on hydraulic, stomatal, and photosynthetic traits and whole-plant relative growth rate, based on a synthesis of the previous literature and modified from its first presentation by [Sack and Scoffoni \(2013\)](#); see text and [Table 1](#) for definitions). Yellow-shaded variables are vein traits; brown variables are related to soil, climate, and atmosphere; blue variables are flux-related traits that have been found or hypothesized to be correlated across species and to be linked with vein traits; pink variables are other leaf economics variables; and white variables are additional important plant traits. For several traits, both leaf area-based expressions (such as A_{area} , N_{area} , and R_{area}) and leaf mass-based expressions (A_{mass} , N_{mass} , and R_{mass}) are shown. Note that ‘ Ψ ’ represents the water potential of different tissues in the plant, correlated during steady-state transpiration with leaf water potential (Ψ_{leaf}), representing plant water status, which drives impacts on hydraulic conductances and stomatal conductance. Black solid arrows signify positive mechanistic linkages; black dotted arrows indicate negative mechanistic linkages; green arrows indicate dynamics of hydraulic and stomatal conductances with water status; grey arrows indicate an alternative way to consider the impact of physiological traits on RGR_{max} (i.e. showing the more direct influence of A_{mass} on RGR_{max} , than that of A_{area}). Other possible impacts of VLA on A_{area} are not shown, including positive effects via improving sugar and nutrient transport. This scheme is simplified, emphasizing the influence of venation on higher-level traits, and many other influences of traits on other traits are not shown (e.g. the negative influence of R_{area} and R_{mass} on ULR and RGR_{max}).

only in specialized situations, and especially when plants are sampled across particular resource gradients. Such correlations cannot be considered ‘causal’ in the sense that a shift in one trait is necessarily associated with a shift in other traits and cannot thus be used for general prediction.

Recent work has demonstrated that some mechanistic trait linkages, including those among LES variables, arise in part

automatically, or by statistical necessity, given their basis of expression per unit leaf area or per unit leaf dry mass ([Lloyd et al., 2013](#); [Osnas et al., 2013](#)). Traditionally, gas exchange variables have been normalized by dividing by leaf area but have additionally been expressed on a per leaf mass basis by dividing the area-normalized rates by LMA . It is intuitive to normalize flux rates by leaf area, since gas exchange and hydraulic

Table 2. Comparison of sampling strategies to test correlations between traits, such as between VLA and other flux-related traits

Definitions as in Table 1. Note the overall strong support for the linkage of VLA with flux-related traits, although particular results vary depending on the sampling strategy as expected (see text). *Correlation holds when excluding aquatic species outliers.

Sampling strategy (from broadest to narrowest scale)	Power and significance of strategy	Weakness and ambiguity of strategy	Correlation between VLA and other flux traits
Across diverse species (across multiple communities, across biomes, or a compiled global dataset)	High variation in all traits; generality and broad importance; potential ability to compare adaptation in different habitats	Correlations between any given traits may be affected by other trait variation and influenced by the wide range of phylogeny and habitats sampled	Weak for A_{area} (Blonder <i>et al.</i> , 2011; Walls, 2011) No for K_{leaf} (Scoffoni <i>et al.</i> , 2011)
Across diverse species within a given community	High variation in all traits; potential ability to compare adaptation in different habitats within the community	Correlations between any given traits may be affected by other trait variation and influenced by the wide range of phylogeny and habitats sampled; relationships may be relevant only within the given community	Yes for K_{leaf} * (Sack and Frole, 2006)
Across a range of lineages, including a few species within each lineage	High variation in all traits; insight into contrasting biology of different lineages	Potentially conflating other factors that influence trait correlations across lineages, including sun/shade adaptation or other trait variation that would influence the correlation	Yes for K_{leaf} , A_{area} , and g_s (Brodrribb <i>et al.</i> , 2007; Boyce <i>et al.</i> , 2009; Brodrribb <i>et al.</i> , 2010)
Across diverse species within a given lineage (or varieties or populations within a species)	High variation in certain traits; variation among given traits may be easily linked with variation in growth form or adaptive radiation across environments	Certain traits may show constrained variation, resulting in low signal to determine trait correlations; when multiple traits determine a function, one trait with especially high variability may influence the function, reducing the ability to discern the correlation of the other trait with the function	Yes for stomatal pore area per leaf area (Dunbar-Co <i>et al.</i> , 2009) Yes for A_{area} * and g_s * (Feild <i>et al.</i> , 2011b) No for K_{leaf} (Sommerville <i>et al.</i> , 2012)
Across genotypes within a species	Potentially can focus on variation in given traits against a background of trait uniformity; potentially more strongly resolves genetic linkages (if across different clones, varieties, or mutants growing within a given environment)	Certain traits may show constrained variation, resulting in low signal to determine trait correlations; when multiple traits determine a function, one trait with especially high variability may influence the function, reducing the ability to discern the correlation of the other trait with the function	Yes for n_s (Manuel Perez-Perez <i>et al.</i> , 2011)
Across plants of a given species grown in different treatments or of different populations across environments, or across leaf types within a species	Gain insight into the way that individual and clusters of traits lead to plant adaptation and optimization to habitat or climate	Difficult to show that trait relationships are general, as they may have evolved or developed as species-specific responses to contrasting environments	Yes for A_{area} , K_{leaf} , g_s , n_s (Brodrribb and Jordan, 2011; Murphy <i>et al.</i> , 2012)

conductance are both modular with area; all else being equal, additional lamina area adds stomata, vein length, and mesophyll cell tissue of a given depth. However, the advantage of expressing gas exchange variables on a leaf mass basis is that it reflects economic benefit relative to structural cost (i.e. the flux rate normalized by the carbon investment). These principles are fundamental in explaining the LES relationships. Indeed, the most pervasive current understanding of the LES correlations has been that variation in *LMA* and its components (leaf density and leaf thickness) tend to be determined by the layers of cells and their cell walls and protoplasm carbohydrates (Van Arendonk and Poorter, 1994; Roderick *et al.*, 1999; Shipley *et al.*, 2006). All else being equal, if a leaf lamina is thicker and/or contains less airspace and thus higher leaf density, the higher *LMA* would correspond to a higher A_{area} , N_{area} , and

R_{area} , leading to a correlation of these area-based traits with *LMA* and also driving positive relationships among those traits, strengthened by their biochemical interdependence (Niinemets, 1999a). Additionally, investment in cell walls and cell carbon compounds or increased cell packing will increase leaf density and thus *LMA*, but will reduce the metabolic machinery relative to dry mass and thus the A_{mass} , N_{mass} , and R_{mass} will tend to be lower, driving positive biochemical relationships and stoichiometric linkages among these traits (Field and Mooney, 1986; Shipley *et al.*, 2006). The higher allocation to leaf density and thickness, and thus higher *LMA*, would contribute to greater leaf toughness and thus longer leaf lifespan (Wright and Westoby, 2002; Onoda *et al.*, 2011). However, beyond this logic, LES relationships can also arise ‘automatically’ or ‘statistically’ (Lloyd *et al.*, 2013; Osnas *et al.*, 2013).

For example, when traits measured on a leaf area basis that are uncorrelated with LMA are converted to expression on a mass basis by dividing by LMA (e.g. $A_{\text{mass}} = A_{\text{area}}/LMA$), one may generate strong negative correlations with LMA . Similarly, when traits measured on a mass basis that are uncorrelated with LMA are converted to expression on an area basis by multiplying by LMA (e.g. $N_{\text{area}} = N_{\text{mass}} \times LMA$), one may generate strong positive relationships with LMA (Lloyd *et al.*, 2013; Osnas *et al.*, 2013). Some have labelled such correlations negatively, as ‘artefactual’ or ‘spurious’, because they can arise in actual data and, indeed, in randomized data, due to ratio effects such as shared covariance among traits subjected to the same normalization by a variable (LMA) (Jasienski and Bazzaz, 1999; Lloyd *et al.*, 2013; Osnas *et al.*, 2013). Here, such correlations are referred to as ‘innate correlations’. While these linkages certainly arise in part from mathematical necessity, they may still reflect physically based mechanistic processes relevant to trait integration and plant function. For example, according to the logic described above, all else being equal, thicker cell walls will increase LMA , and decrease A_{mass} ; this will occur even if A_{area} were completely random. Such a negative relationship of A_{mass} with LMA is not trivial in meaning and will imply, for example, that high- LMA leaves will tend to have lower maximum photosynthetic return on the mass investment per time (Westoby *et al.*, 2013). Further, it follows that when LES relationships are found on an area basis, then they are less likely to be found on a mass basis, and vice versa (Niinemets and Sack, 2006; Lloyd *et al.*, 2013; Osnas *et al.*, 2013). The importance of one case or the other can provide insights into mechanisms of adaptation and species’ relative performances within and across communities (Westoby *et al.*, 2013). Thus, even if they arise in part innately, the LES relationships are considered by most to represent mechanistic trait linkages with ecological significance.

Examination of the ‘vein origin’ hypothesis for the LES

The ‘vein origin’ hypothesis of Blonder *et al.* (2011) was proposed as ‘a comprehensive framework for the origin of the leaf economics spectrum based on venation-mediated economic strategies’ (Blonder *et al.*, 2011). According to this hypothesis, LMA , A_{mass} , N_{mass} , and LL are directly and mechanistically determined by VLA and two other traits, the intervein distance (IVD) and ‘loopiness’ (vein areoles per leaf area, VAA). (For ease of interpretation, we have used simple three-letter acronyms for vein traits, where Blonder *et al.* used σ , d , and ξ , respectively). Further, according to this hypothesis, the relationships among the vein traits are the main cause of the relationships among LES traits. On the one hand, Blonder *et al.* emphasized the causal nature of their hypothesis: ‘venation traits are mechanistically linked to the leaf functional traits’ and ‘mechanistically predict key leaf functional traits’, and ‘constraints on the geometry of the venation network generate tradeoffs among these functional traits’. On the other hand, at the end of their paper they referred to their hypothesis as only a ‘useful “zeroth-order” approximation’. Here, their statements are evaluated.

The ‘vein origin’ conceptual hypothesis of Blonder *et al.* (2011) can be described as four key assertions, untested in that paper, of mechanistic correlations among vein traits and LES traits. Based on these assertions, Blonder *et al.* presented four equations that predicted LES variables based on VLA and IVD . However, these equations also required direct or implicit input of other LES variables, raising the possibility that the predictions were circular and the conclusions erroneous. These assertions are examined in detail in the following sections.

Assertion 1: VLA and IVD are independent enough to support contradictory linkages with other traits

The first assertion of Blonder *et al.* (2011) was that three vein traits, VLA , IVD , and VAA , are linked with LMA and LL , but in ways that seem at first sight to be contradictory (Fig. 1). In fact, these three vein traits are well known to be geometrically auto-correlated; in a regular vein network a higher VLA will correspond to a lower IVD . Indeed, previous studies showed negative correlations of VLA and IVD within species (Uhl and Mosbrugger, 1999) and across species (Philpott, 1953). Although Blonder *et al.* (2011) recognized that VLA , IVD , and VAA ‘are not strictly independent of each other’ and thus that ‘there should be characteristic relationships between them within every species’, they advised treating these three traits as distinct metrics sufficient to describe the venation system when comparing different species. Blonder *et al.* (2011) further asserted that VLA and IVD might be both positively correlated with a third trait, rather than being positively and negatively, respectively, correlated with the third trait. However, in our analysis of the Blonder *et al.* (2011) data and previous datasets, the VLA , IVD , and VAA were very closely inter-correlated. In particular, VLA and IVD were so tightly negatively related, with r -values ranging from -0.5 to -0.9 , that they could not both be strongly positively correlated with a third trait. Indeed, all other analyses found VLA and IVD to be correlated with other traits in opposite ways (Table 3, row 1).

Assertion 2: VLA and/or IVD directly determine LMA

The second assertion of Blonder *et al.* (2011) was that LMA is directly positively influenced by VLA and/or negatively by IVD . Blonder *et al.* proposed that ‘maximizing carbon assimilation requires a large carbon investment in vein construction and thus a high LMA ’, and that ‘high [minor vein] density [i.e. high VLA] implies high carbon investment in venation’. This assertion assumes that (i) VLA is a major determinant of the vein mass per leaf area (VMA), and (ii) that the VMA makes a substantial contribution to leaf thickness (LT) and/or density (LD), the components of LMA . Although Blonder *et al.* recognized that major veins may contribute to LMA , they focused only on minor veins.

The idea that minor veins contribute substantially to LT or LD , or to LMA , was not supported in our database analyses. Just as previous studies found no significant positive correlation of VLA with LMA across species (Sack and Frole, 2006; Dunbar-Co *et al.*, 2009; Nardini *et al.*, 2012), there was no support for this assertion across diverse species

Table 3. Tests of correlations that were asserted in the conceptual hypothesis of *Blonder et al. (2011)*

Definitions as in [Table 1](#). Correlations were tested for phylogenetically diverse sets of angiosperms, except when specified. Note: For tests using the data of [Blonder et al. \(2011\)](#), three erroneous data values were excluded: a very large leaf thickness value for *Ficus carica* and two very low leaf dry mass values for *Magnolia grandiflora* and *Quercus agrifolia*. All data excluded for *Pinus canariensis*, as it was the only non-angiosperm, and given its single vein, the calculation of vein traits such as IVD and VLA were non-standard.

Row	Assertion	Traits	Dataset, <i>r</i> -value, <i>P</i> -value (number of species)	Supported?
1	Assertion 1: VLA and IVD are independent enough to support contradictory linkages with other traits	VLA, IVD, VAA	Global compiled dataset: VLA and IVD: $r=-0.57$; $P<0.001$ (63) Blonder et al. (2011) : VLA and IVD: $r=-0.76$, $P<0.001$ (24) Philpott (1953) (<i>Ficus</i> species): VLA and IVD: $r=-0.89$, $P<0.001$ (47)	No: VLA and IVD are too strongly correlated to realistically support contradictory correlations with other traits
2	Assertion 2: LMA is directly positively influenced by VLA or IVD	VLA, LMA	Global compiled dataset: IVD and LMA: $r=0.12$, $P=0.47$ (38) Global dataset (individual lineages): <i>Acer</i> : VLA and LMA: $r=0.40$, $P=0.023$ (32) <i>Helianthus</i> , <i>Quercus</i> , Hawaiian <i>Plantago</i> and <i>Viola</i> : VLA and LMA: $r=0.22-0.65$, $P=0.058-0.64$ (7-23) Blonder et al. (2011) : VLA and LMA: $r=-0.41$, $P=0.046$ (24) IVD and LMA: $r=0.09$, $P=0.68$ (24)	No: LMA is generally independent of VLA, except weakly within some lineages, due to apparent concerted convergence across a light gradient
3	Assertion 3: IVD is linearly related to LT due to a general, universal mechanistic scaling of IVD and VED and of VED with LT	IVD, VLA, LT, VED	Global compiled dataset: IVD and LT: $r=0.36$, $P<0.001$ (158) Blonder et al. (2011) : IVD and LT: $r=0.40$, $P=0.053$ (24) Wylie (1946) : IVD and LT: $r=0.33$, $P=0.001$ (90) Philpott (1953) (<i>Ficus</i> species): IVD and LT: $r=0.48$, $P<0.001$ (47) Wylie (1946) : IVD and LT: $r=0.12$, $P=0.46$ (38) Philpott (1956) : IVD and LT: $r=-0.25$, $P=0.22$ (27) Wylie (1951) : VLA and IVD: $r=0.37$, $P=0.29$ (10) Wylie (1956) : VLA and IVD: $r=0.081$, $P=0.52$ (66) Brodribb et al. (2007) (angiosperms, mosses, gymnosperms and ferns): IVD and VED: $r=0.15$, $P=0.35$ (42) Brodribb et al. (2007) (angiosperms only): IVD and VED: $r=-0.39$, $P=0.27$ (10) Noblin et al. (2008) : IVD and VED: $r=0.99$, $P<0.001$ (30) Nardini et al. (2012) : VLA and VED: $r=0.76$, $P=0.08$ (6)	No: A positive correlation of IVD and LT or VED is found some datasets, but not in others, and weakly across the global compiled dataset, implying no single, universal scaling; when the pattern arises, it may be due to one of several mechanisms or to concerted convergence rather than mechanistic correlation
4	Assertion 4: Mechanistic correlations of VLA and IVD to LL via correlations of the vein traits with LT, LD, or LMA	VLA, IVD, LL	Global compiled dataset: LL and VLA: $r=-0.34$, $P<0.001$ (93) LL and LMA: $r=0.43$, $P<0.001$ (91) LL and VLA, partialling out LMA: $r_{\text{partial}}=-0.37$, $P<0.001$ (91) Blonder et al. (2011) LL and VLA: $r=-0.66$, $P=0.011$ (14) LL and IVD: $r=0.62$, $P=0.019$ (14) LL and LMA: $r=0.27$, $P=0.35$ (14) LL and LT: $r=0.38$, $P=0.18$ (14) LL and LD: $r=-0.13$, $P=0.66$ (14)	No: While LL tends to be weakly correlated negatively with VLA and positively with IVD across species, this trend has no clear mechanistic basis, since it occurs independently of LMA (i.e. the trend exists even when LMA is partialled out); the trend likely arises from concerted convergence; this is apparent when the trend disappears for <i>Helianthus</i> when mean annual precipitation is partialled out

(Continued)

Table 3. Continued

Row	Assertion	Traits	Dataset, <i>r</i> -value, <i>P</i> -value (number of species)	Supported?
			<i>LL</i> and <i>VLA</i> , partialling out <i>LMA</i> : $r_{\text{partial}} = -0.62$, $P < 0.05$ (14) <i>LL</i> and <i>IVD</i> , partialling out <i>LMA</i> : $r_{\text{partial}} = 0.61$, $P < 0.05$ (14) <i>Helianthus</i> : <i>LL</i> and <i>VLA</i> : $r = -0.90$, $P = 0.002$ (8) <i>LL</i> and <i>LMA</i> : $r = -0.79$, $P = 0.020$ (8) <i>LL</i> and <i>VLA</i> , partialling out <i>LMA</i> : $r_{\text{partial}} = -0.85$, $P = 0.02$ (8) <i>LL</i> and <i>VLA</i> , partialling out <i>MAP</i> : $r_{\text{partial}} = -0.24$, $P > 0.31$ (8)	

using the data of [Blonder et al. \(2011\)](#); here, in fact, a negative trend was found (Table 3, row 2). In the global database, *VLA* and *IVD* were independent of *LMA* (Table 3, row 2; Fig. 3A). Consequently, as expected from an area-based variable independent of *LMA* (as discussed in the section, ‘Mechanistic trait linkages versus concerted convergence’), when expressed on a mass basis by dividing by *LMA*, vein length per mass ($VLM = VLA/LMA$) was negatively related to *LMA* (Fig. 3B). There were also no significant correlations between *VLA* and *LMA* within four of the five individual lineages in the global database represented by seven or more species or populations (Table 3, row 2.) For *Acer*, the one genus to show a weak relationship of *VLA* and *LMA*, this appeared to reflect concerted convergence of mechanistically independent traits, consistent with more shade-tolerant species having adapted both lower *LMA* and lower *VLA* than light-demanding species ([Sack and Scoffoni, 2013](#)).

This independence of *LMA* from *VLA* is consistent with leaf anatomy and the allocation of mass to tissues within leaves. Cross-sections of leaves reveal a minimal contribution of minor veins to the lamina volume (Fig. 4). Indeed, the *VLA* is not even a strong determinant of leaf vein volume per leaf area, which is equal to the sum across vein orders of the product of vein length per area and vein cross-sectional area, and which is driven by the major veins rather than the minor veins. In a global meta-analysis ($n = 58$ angiosperm species), the minor veins, although they accounted for the bulk of *VLA*, accounted for a mean \pm SE of only $17 \pm 0.2\%$ of the total vein volume per leaf area and $2 \pm 0.02\%$ of the whole leaf volume per area (*LT*) ([Sack et al., 2012](#)). Even if the mass density of vein tissue should be 5-fold higher than that of mesophyll cells ([Poorter et al., 2009](#)), and if we assume for calculation that the leaf includes up to 15% airspace by volume ([Sack et al., 2003](#)), then the minor veins would contribute only $< 11\%$ of *LMA* on average. Some species develop mechanical tissue around the veins, and these tissues too make only a small contribution to *LMA*. Thus, one previous study of 14 grass species found *LMA* to be statistically related to the volume of non-vein sclerenchyma per leaf area, which accounted for $< 10\%$ of leaf volume ([Van Arendonk and Poorter, 1994](#)), and another study of 14 grass species found *LMA* to be statistically related to the

thickness of sclerenchyma plus vascular tissues, but that trend was lost after removing two *Brachypodium* species ([Garnier and Laurent, 1994](#)). Similarly, a third study of 52 species of woody seedlings found that while *LMA* increased with the combined thickness of sclerenchyma and vascular tissues, this was not a causal correlation ([Castro-Diez et al., 2000](#)); those tissues accounted for $< 10\%$ of leaf volume. In contrast to the minor veins, the major veins, which account for a small proportion of *VLA*, contribute the bulk of total leaf vein volume and can contribute a substantial minority portion of *LMA* in many leaves, especially large leaves (as will be discussed in the section, ‘Detail of synthetic conceptual model: the impact of major vein length per area on leaf mass per area’).

Assertion 3: IVD is a linear driver of LT due to general mechanistic scaling

The third assertion of [Blonder et al. \(2011\)](#) was that *IVD* (a negative correlate of *VLA*) should be greater in thicker leaves. This assertion was contradictory to assertion 2 that a higher *VLA* would result in greater *LT*, *LD*, and *LMA*, since *VLA* and *IVD* are strongly negatively correlated. A positive relationship of *IVD* to *LT* has been shown for several species sets ([Wylie, 1946](#); [Philpott, 1953](#); [Noblin et al., 2008](#)). One explanation of this trend, proposed by [Noblin et al., 2008](#), is that, for optimal hydraulic design, leaves with higher *VLA* (and lower *IVD*) should also have a shorter vertical distance from the vein to the epidermis (*VED*) containing the stomata. According to that argument, reducing *IVD* (or increasing *VLA*) would increase K_{leaf} by shortening the pathways for water flow outside the veins to the sites of evaporation, but that would only be effective if the leaves were also thinner, or else vertical flow from vein to epidermis (where water was assumed to evaporate) would become more limiting of K_{leaf} . Given the positive correlation of *VED* with *LT* across species ([Nardini et al., 2012](#); G.P. John, C. Scoffoni and L. Sack, unpublished data; C. Scoffoni, D. Chatelet, M.J. Donoghue, E. Edwards, L. Sack, unpublished data), *IVD* would correlate positively with *LT*. That idea for a mechanistic correlation of *IVD* and *VED* may not, in fact, be general, because it would be valid only when water evaporates near the stomata

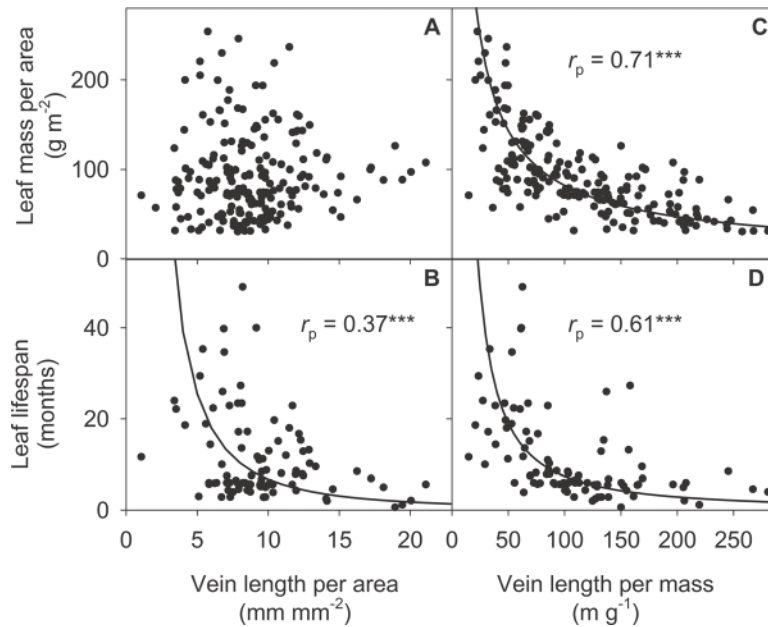


Fig. 3. Plots of leaf economic spectrum traits: (A) leaf mass per area (LMA) and (B) leaf lifespan (LL) against vein length per area (VLA) in a global database for angiosperm species (Supplementary Table S1). Strong, negative relationships (C and D) were observed of these traits with vein length per mass ($VLM=VLA/LMA$). Lines are power laws fitted using standard major axes: (B) $LL=558VLA^{-1.92}$; (C) $LMA=3289VLM^{-0.80}$; (D) $LL=3908VLM^{-1.36}$. For panels A–D, n -values were 196, 93, 196, and 91, respectively.

and that may not be true for all species in all conditions (as will be discussed in the section ‘Detail of synthetic conceptual model: how does vein length per area influence K_{leaf} ?’). However, there may be additional explanations for a positive relationship of IVD to LT . A developmental explanation is that, all else being equal, species that develop cells larger in all dimensions will simultaneously push minor veins further apart and develop thicker mesophyll tissues. Indeed, across species, thicker leaves tend to have larger cells (Pyankov *et al.*, 1999; John *et al.*, 2013), and among closely related species, those with larger cells may tend to have greater IVD (Brodrigg *et al.*, 2013). However, the development of different numbers of cells between minor veins would destabilize any general positive trend between cell size and IVD across diverse species. A third explanation for the positive relationship of IVD to LT is concerted convergence in specific sets of species. As one scenario, more light-demanding species may develop high VLA (and low IVD) for greater hydraulic supply to enable higher gas exchange rates, and also thinner leaves would be selected for improved competitive ability (Sack and Scoffoni, 2013). As another scenario, succulent-leaved species may develop low VLA (and high IVD), relative to non-succulents, reflecting a reduced need for hydraulic water supply, and also thicker leaves, corresponding to their water storage tissues (Sack and Scoffoni, 2013).

Another type of concerted convergence of IVD with LT may arise indirectly due to a negative relationship between IVD and the palisade:spongy mesophyll thickness ratio. This trend may indicate a need for greater hydraulic and sugar transport capacity in leaves with greater photosynthetic capacity in high irradiance, which tends to relate to high palisade:spongy mesophyll thickness ratio (Wylie, 1946, 1951; Philpott, 1953; Sack and Frole, 2006). The relationship of IVD to palisade:spongy

mesophyll thickness ratio is much stronger than the relationship of IVD to LT in the studies that have measured both (Wylie, 1946, 1951; Philpott, 1953; Sack and Frole, 2006), and in some species sets, LT too may correlate with palisade:spongy mesophyll thickness ratio (Philpott, 1953). On balance, previous work points to several possible bases for such a relationship of IVD to leaf thickness when it does occur, and to the potential for this relationship not to have a general universal basis and for the relationship to be absent in some species sets. Certainly this relationship does not apply to sun and shade leaves of given species, as sun leaves generally tend to have higher VLA , and lower IVD , yet greater thickness in all mesophyll tissues than shade leaves (Wylie, 1951).

Indeed, database analyses showed that the relationship of VLA or IVD with LT tended to appear in only some species sets and not others (Table 3, row 3). In four datasets, IVD was correlated weakly or strongly with VED or LT (24–90 species; Wylie, 1946; Philpott, 1953; Noblin *et al.*, 2008; Blonder *et al.*, 2011). However, the IVD was not related to LT across diverse species in six datasets with 10–66 species (Wylie, 1939; Wylie, 1951, 1954; Philpott, 1956; Brodrigg *et al.*, 2007; Nardini *et al.*, 2012). Finally, IVD and LT were significantly but weakly related in the compiled global dataset for 158 angiosperm species (Table 3, row 3). This strong variation in the relationship is consistent with no single general universal scaling across species.

Assertion 4: Mechanistic correlations of VLA and IVD to LL

The fourth assertion of Blonder *et al.* (2011) was that LL scales positively with IVD . This assertion was based on assertion 3 (i.e. the positive scaling of IVD with LT) and additionally the idea that LL should scale positively with LT and

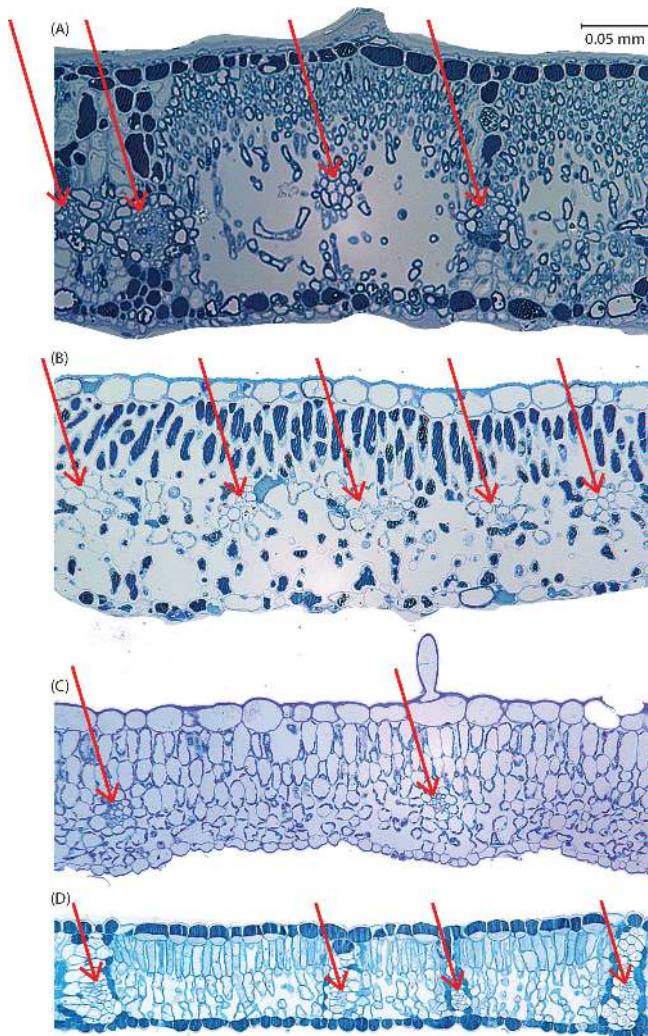


Fig. 4. Leaf cross-sections, showing the small volume of mesophyll space taken up by minor veins (indicated by arrows): (A) *Cercocarpus betuloides* (Rosaceae); (B) *Platanus racemosa* (Platanaceae); (C) *Salvia canariensis* (Lamiaceae); and (D) *Bauhinia galpinii* (Fabaceae). Bar, 0.05 mm.

LMA. Thus, [Blonder *et al.* \(2011\)](#) stated, ‘very dense or closely spaced veins [i.e. low *IVD*, or higher *VLA*] implied shorter life spans’ ([Fig. 1B](#)). However, they also argued that ‘denser venation may also resist damage... and long life span can also result from... high reticulation in leaf venation’. That idea would lead to the opposite prediction that *LL* would be negatively related to *IVD* and positively related to *VLA* ([Fig. 1B](#)). In developing their equations, [Blonder *et al.*](#) utilized the first, but not the second, predicted relationship (i.e. that *LL* correlated positively with *IVD* and negatively with *VLA*; see the next section).

Our database analyses did find a negative correlation of *LL* with *VLA* across species, but this relationship appeared to arise from concerted convergence rather than to the mechanistic correlation via leaf thickness or *LMA* that was predicted by [Blonder *et al.* \(2011\)](#). Indeed, a negative concerted convergence of *VLA* and *LL* would be expected for species sets in which shorter *LL* is associated with drought tolerance.

Across diverse species of mixed leaf habit, species with higher *VLA* tend to be associated with low moisture supply and higher irradiance, as expected given selection for rapid gas exchange during the times when high moisture pulses are available ([Sack and Scoffoni, 2013](#)), and such conditions also select for shorter *LL* ([Walters and Reich, 1999](#); [Wright *et al.*, 2005b](#)).

The assertion of a correlation of *IVD* or *VLA* with *LL* due to their mechanistic correlation with *LMA* was tested using three datasets—the data of [Blonder *et al.*](#), that of the global database, and that for eight populations of sunflowers of three species sampled across a gradient of mean annual precipitation (MAP) from 580 to 1580 mm ([Mason *et al.*, 2013](#)). In all three datasets, *LL* was correlated negatively with *VLA* and/or positively with *IVD* ([Table 3](#), row 4). In the sunflower dataset and the global dataset, *LL* was positively related to *LMA*; thus in the global dataset, *LL* was negatively related to *VLM*, which, as already described, was negatively correlated to *LMA* ([Table 3](#), row 4; [Fig. 3B, D](#)). However, in the [Blonder *et al.*](#) dataset, *LL* was not significantly related to *LMA*, *LT*, or *LD* ([Table 3](#), row 4). Further, in all three datasets, partial regression analysis showed that the relationship of *LL* with *VLA* was independent of *LMA*, remaining significant when *LMA* was partialled out ([Table 3](#), row 4). These findings are consistent with a concerted convergence of *VLA* and *LL* rather than a mechanistic linkage via *LMA*. Notably, the data for sunflower populations pointed to concerted convergence according to selection for drought tolerance. Across the sunflower populations, *LL* was negatively correlated with MAP, and *VLA* was positively correlated with MAP ($r=0.94$ and -0.93 respectively, $P=0.001$, $n=8$), and partial correlation analyses showed that at a given MAP, *LL* was not related to *VLA* ($r_{\text{partial}}=-0.24$, $P>0.5$). Thus, the negative correlation of *LL* and *VLA* within certain species sets is consistent with a basis in concerted convergence due to adaptation across a resource gradient, independently of *LMA*, rather than arising from any intrinsic mechanistic relationship.

*Sensitivity analysis of the ‘vein origin’ equations of Blonder *et al.* shows the failure to predict LES traits from VLA and IVD*

Based on the above four assertions, additional assumptions, and arguments from leaf geometry, [Blonder *et al.* \(2011\)](#) derived equations to predict the four LES traits *LMA*, *LL*, A_{mass} , and N_{mass} from *VLA* and *IVD*, with the input of other traits, including the LES traits themselves (their equations 4–7; summarized in [Appendix 1](#)). In deriving the equations, they emphasized roles of *VLA* and *IVD* to account for variation in *LT*, *LD*, and *LMA*. Additionally, in estimating A_{mass} , they asserted a role for *IVD* in the diffusion of gases through the leaf airspaces; the veins were considered to have a negligible hydraulic importance in their formulation ([Appendix 1](#); see also [Appendix 3](#) for treatment of an additional model for estimating A_{mass} with an alternative equation based on a different simplification; [Blonder *et al.*, 2013](#)). [Blonder *et al.* \(2011\)](#) applied these equations to dummy trait data and also to their dataset for 25 species of vein traits and LES traits. They found weak relationships of predicted with observed

values and inferred these relationships to provide support for their assumptions and equations, and more broadly, for the ‘vein origin’ hypothesis: i.e. that *VLA* drives the LES.

However, overall in the [Blonder *et al.* \(2011\)](#) dataset, the *VLA* and *IVD* were not correlated with *LMA*, N_{mass} , and A_{mass} in the ways they asserted ([Figs. 3 and 5](#) of this paper), thus raising the question of how such a model could result in correct predictions. In fact, the predictive power of equations 4–7 arose due to the circularity of the equations because they included LES variables as well as vein traits, and the vein traits made a negligible contribution to the prediction. Thus, careful analysis of these equations in fact proves the independence of LES traits from the structural influence of *VLA* and *IVD*. We applied a partial derivative sensitivity analysis typically used to assure validity and quality in modelling studies ([Saltelli *et al.*, 2009](#)). These sensitivity analyses showed that three of the four equations of [Blonder *et al.*](#) were insensitive to *VLA* and orders of magnitude more sensitive to the LES variables and *LT* and *LD* ([Appendix 2](#)). Consistent with that conclusion, randomizing the species’ *VLA* values in the [Blonder *et al.*](#) database did not change the values for *LMA*, A_{mass} , and N_{mass} predicted for those species by their equations 4, 6, and 7 respectively ([Fig. 6; Supplementary Table S3](#)). In the case of the prediction of *LL* by the fourth equation (their equation 5), this was simply an asserted linear dependency of *LL* on *IVD*, with a slope fitted to their data. As described above, in consideration of assertion 4, the weak correlation of *LL* and *IVD* apparently arises from concerted convergence of mechanistically independent traits, and the linear equation with a fitted parameter reflects this coincidental trend. Such trends cannot be used for general prediction because they occur for different reasons across datasets and

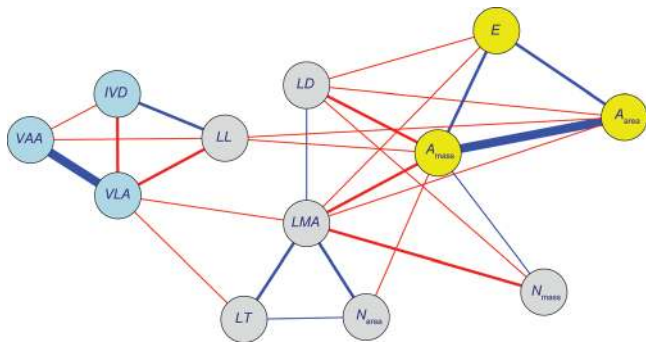


Fig. 5. Network diagram of the inter-correlations among vein traits and leaf economic and structural traits for 24 species based on the data of [Blonder *et al.* \(2011\)](#), emphasizing the separation of the correlations of vein traits and leaf economic traits (analysis conducted using igraph; <http://cran.r-project.org/web/packages/igraph/citation.html>; [RDC Team, 2007](#)). Definitions as in [Table 1](#). Before analysis, data were corrected, as explained in the note to [Table 3](#). Blue nodes represent vein traits; grey nodes represent leaf structure and composition; and yellow nodes represent gas exchange rates. Blue and red lines indicate positive and negative correlations, respectively. Line thickness represents the strength of the correlations between traits: thin lines, $|r|=0.4\text{--}0.6$; intermediate lines, $|r|=0.6\text{--}0.8$; thick lines, $|r|>0.8$.

thus would have no clear predictive capacity for any particular set of species. The same circularity and negligible predictive value was found for the additional equations proposed to extend the ‘vein origin’ hypothesis of the LES by [Blonder *et al.* \(2013\)](#) ([Appendix 3](#)).

[Blonder *et al.* \(2011\)](#) concluded that *VLA* and *IVD* predicted not only LES traits but also their inter-correlations. They drew this conclusion because when they determined the LES variables for their species based on their equations 4–7 and plotted these on top of global LES correlations, the directions of the LES trends were similar, and within the range of those of the global trends. However, just as their predictions of LES variables were circular, so were the inter-relationships among the predicted variables. Their equations 4–7 were based on common LES variables, and equations were derived incorporating the other equations, creating a co-dependence or circularity among these equations, not due to any significant role of *VLA* or *IVD*. Some of the correlations among variables would also have arisen innately by common normalization of area-based measurements by mass. These findings highlight the absolute imperative that studies which propose models should perform sensitivity analyses to ensure that those variables in the model presumed to be important are in fact important, lest the authors and readers be misled and reach invalid conclusions.

Conclusions regarding the Blonder et al. model and the ‘vein origin’ hypothesis

In conclusion, this review found no support for the ‘vein origin’ hypothesis of direct, mechanistic determination of the LES by vein traits. Rather, consideration of leaf anatomy and empirical data, deeper inspection of the assertions, and sensitivity analyses of the model showed this to be erroneous and indicated that LES traits are not directly determined by *VLA* and *IVD*. This review rejects the ‘vein origin’ hypothesis for the LES of [Blonder *et al.* \(2011\)](#) and also rejects the extended version of [Blonder *et al.* \(2013\)](#), which contains additional invalid assertions and derivations ([Appendix 3](#)). However, there are still good reasons that *VLA* and *IVD* have strong, although indirect, influences on the LES and on leaf and plant performance.

Synthesis of the linkage of *VLA* and the LES as part of the flux trait network

Having concluded that the ‘vein origin’ hypothesis of [Blonder *et al.*](#) contained misconceptions, we synthesized an alternative conceptual model, the ‘flux trait network’ hypothesis for the influence of vein traits on gas exchange traits, including certain LES traits, based on previous studies of a wide range of species sets (see also [Supplementary Information to Sack and Scoffoni, 2013](#)). This schema elaborates on causal trait networks proposed in the earlier literature ([Ehleringer and Clark, 1988](#)). A number of these studies have shown strong correlations across species (typically for light-exposed fully expanded leaves of mature plants) of vein traits with hydraulic, photosynthetic, anatomical, and compositional traits which generally relate to fluxes of water and carbon in and out of the leaf

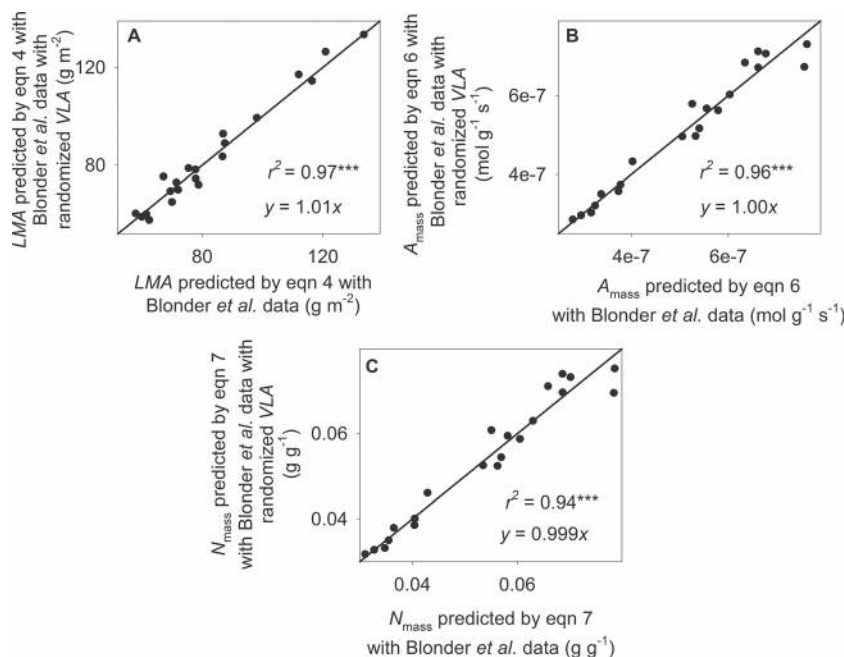


Fig. 6. Debunking the model of [Blonder *et al.* \(2011\)](#) for predicting leaf economic spectrum traits from vein length per area (VLA). Their equations 4, 6, and 7 of [Blonder *et al.* \(2011\)](#) were apparently able to weakly predict LES traits leaf mass per area (LMA), light-saturated photosynthetic rate per mass (A_{mass}), and nitrogen concentration per mass (N_{mass}) from VLA . However those equations also included LES traits, and therefore were circular (see text and [Appendix 1](#) and [2](#)). Partial derivative sensitivity analyses showed these equations to be insensitive to VLA . That fact is also demonstrated by plots of the predicted values of LES traits based on applying those equations 4, 6, and 7 to the empirical data of [Blonder *et al.* \(2011\)](#) against predicted values of LES traits based on applying the equations to the same empirical data, but after randomizing the VLA data (panels A, B, and C; also see [Supplementary Table S3](#)). The identity of the predicted values even after randomizing the VLA data indicates insensitivity of these equations to VLA and that the predictions of LES traits are circular and based on the LES traits themselves. In these analyses, the k_0 values were double those provided by [Blonder *et al.* \(2011\)](#), after we corrected an error in their calculation (see [Supplementary Table S3](#)). Note that randomizing the interveinal distance data (IVD) did affect the predicted LES variables when using their equations 4, 6, and 7. However, that effect was due to the k_0 term in these equations, which is equal to $IVD/0.5LT$; thus, altering the IVD data in these equations also affected the influence of LT . Using equivalent, simplified equations 4a, 6a, and 7a ([Appendix 2](#)), which eliminate both k_0 and IVD , also showed that randomizing the VLA data leads to identical predictions. Before analysis, data were corrected, as explained in the note to [Table 3](#). Plotted lines are forced through the origin so that the slopes test for bias (i.e. deviation from 1.0). The presented r^2 are for lines not forced through the origin. $n=24$.

([Wright *et al.*, 2004](#); [Sack and Holbrook, 2006](#); [Brodrigg *et al.*, 2007](#); [Dunbar-Co *et al.*, 2009](#)). Additionally, numerous studies have clarified correlations among photosynthetic traits and leaf structure and composition. In brief, vein traits are important in determining hydraulic conductance, which in turn is correlated with stomatal conductance (g_s) and photosynthetic rate per leaf area (A_{area}), leading to an influence on photosynthetic rate per leaf mass (A_{mass}) and plant relative growth rate ([Fig. 2](#)). Here is provided a description of the statistical and mechanistic evidence in the literature for each step.

Leaf vein traits affect the vein xylem hydraulic conductance (K_x) and the outside-xylem hydraulic conductance (K_{ox}), which together determine K_{leaf} ([Cochard *et al.*, 2004](#); [Sack *et al.*, 2004, 2005](#); [McKown *et al.*, 2010](#); [Sack and Scoffoni, 2013](#)):

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

The xylem cell numbers and dimensions in each vein order determine the vein cross-sectional conductivities of each vein order, which influence K_x . The VLA and the number and size

of free-ending veins positively influence K_x and K_{ox} ; and the sizes, numbers, and permeability of the bundle sheath and bundle sheath extensions and the transport properties of the mesophyll pathways for water flow influence K_{ox} . Two additional vein features can reduce the sensitivity of K_x to xylem embolism: a higher major vein length per area (major VLA) and a topology of the vein system with greater redundancy, both of which enable greater conductivity around blocked xylem ([Scoffoni *et al.*, 2011](#); [Sack and Scoffoni, 2013](#)). A higher minor VLA and thus higher total VLA contribute to a higher K_{leaf} by positively influencing both K_x and K_{ox} . A higher VLA increases K_x by contributing more water flow pathways in parallel and increases K_{ox} by providing more exit pathways from the xylem through the bundle sheath and/or shortening the pathways for water to flow outside of the xylem, both by reducing the distance for hydraulic flow of liquid water, and, if water evaporates from the bundle sheath or bundle sheath extensions, by increasing evaporative surface within the leaf ([McKown *et al.*, 2010](#); as discussed further in the section, ‘Detail of synthetic conceptual model: how does

vein length per area influence K_{leaf} ?). Consistent with this influence of VLA on leaf hydraulic capacity, across tropical rainforest angiosperm tree species, K_{ox} , and K_{leaf} correlated with VLA (Sack and Frole, 2006), and K_{leaf} correlated negatively with IVD across a wide range of bryophyte, pteridophyte, gymnosperm, and angiosperm species from different lineages in high- and low-irradiance habitats (Brodribb *et al.*, 2007). Importantly, VLA is not the only trait that influences K_{leaf} (Fig. 2), and in several species sets, no relationship was observed between K_{leaf} and VLA , corresponding to a stronger role of other vein traits (e.g. vein cross-sectional conductivities, or extra-xylem anatomy and/or physiology) in determining K_{leaf} differences (Scoffoni *et al.*, 2011; Nardini *et al.*, 2012; Sommerville *et al.*, 2012).

The leaf is an important bottleneck in the whole-plant hydraulic system, and thus, the impact of vein traits on K_{leaf} scales up to the whole plant hydraulic conductance (K_{plant}). According to the Ohm's law analogy, K_{plant} determines leaf water potential (Ψ_{leaf}) at a given transpiration rate (E):

$$\Delta\Psi_{\text{leaf-to-soil}} = E / K_{\text{plant}} \quad \text{Equation 2}$$

$$\Psi_{\text{leaf}} = -E / K_{\text{plant}} + \Psi_{\text{soil}} \quad \text{Equation 2a}$$

where $\Delta\Psi_{\text{leaf-to-soil}}$ is the water potential gradient between leaf and soil and Ψ_{soil} is the soil water potential. E , in turn, depends on the diffusive conductance to water vapour of the leaf (g) and the evaporative driving force, the vapour pressure deficit (VPD)

$$E = g \times \text{VPD} \quad \text{Equation 3}$$

Where g is determined by the stomatal conductance (g_s) under moderate wind speeds, when the boundary layer conductance (g_b) is sufficiently high:

$$\Psi_{\text{leaf}} = -(g_s \times \text{VPD}) / K_{\text{plant}} + \Psi_{\text{soil}} \quad \text{Equation 4}$$

Thus, for Ψ_{leaf} to be maintained within narrow limits at a given range of VPD and Ψ_{soil} , within and across species g_s must be coordinated with K_{plant} (i.e. for a correlation of hydraulic demand with supply; Tyree and Zimmermann, 2002; Sack *et al.*, 2005; Sack and Holbrook, 2006).

The g_s is a function of the maximum stomatal conductance (g_{max}) and the degree that stomata close as Ψ_{leaf} declines. The g_{max} is determined by a function of stomatal dimensions and numbers (which can be quantified as stomatal size, stomatal density, stomatal index, and stomatal pore area per leaf area index; Sack *et al.*, 2003; Franks and Farquhar, 2007). The decline of g_s with leaf dehydration may arise from several possible mechanisms. First, leaf dehydration may lead to stomatal closure in part related to the accumulation of abscisic acid and/or ethylene, or increased tissue sensitivity to hormones, in response to the leaf dehydration experienced. The hormone response may be triggered by osmosensing cells. Another possible mechanism is the hydraulic-mechanical hypothesis for stomatal control, proposed based on mathematical

models, and on experiments involving direct measurement of the turgor of guard cells and epidermal pavement cells, and of stomatal VPD responses (e.g. Franks, 2004; Buckley *et al.*, 2011). According to this hypothesis, g_s does not depend directly on bulk Ψ_{leaf} but rather on the water potential at or near the guard cells or epidermis (Ψ_{gce}), as stomatal opening is driven by the guard cell turgor against the pressure of surrounding epidermal cells (Buckley *et al.*, 2003; Franks, 2004). A third possible mechanism for stomatal closure during leaf dehydration is a direct sensitivity of g_s to humidity near the stomatal pore (Peak and Mott, 2011).

Ultimately, the g_s , influenced by the vein and hydraulic traits as described above, is a strong determinant of photosynthetic rate per leaf area (A_{area}). The A_{area} depends on the chloroplastic CO_2 concentration (C_c) and biochemical parameters including the electron transport rate (J_{max}) and the maximum carboxylation capacity of Rubisco (V_{cmax}), and, at a given ambient CO_2 concentration (C_a), C_c is dynamically determined by g_s and mesophyll conductance (g_m ; Farquhar *et al.*, 2001; Flexas *et al.*, 2012). The photosynthetic parameters depend on the concentration of photosynthetic pigments and of enzymes, including Rubisco, and of metabolites as well as mesophyll anatomy, which in turn are related to leaf nitrogen concentration per area (N_{area}), as does respiration rate (R_{area}) (Evans *et al.*, 2000). Shifts in these parameters can thus alter the coordination among A_{area} and hydraulic and venation traits.

Declining water potentials throughout the plant system lead to reductions of K_x and K_{ox} and hydraulic conductance elsewhere in the plant due to embolism, tissue collapse, and/or changes in membrane properties. Thus the 'vulnerability' of hydraulic conductance, itself determined in part by vein traits, including major VLA , will impact on gas exchange and its dynamics (Sack and Scoffoni, 2013). As already described, the declining water potential in or around the guard cells may drive a decline of g_s (Hubbard *et al.*, 2001; Guyot *et al.*, 2012; Scoffoni *et al.*, 2012).

In contrast to the 'vein origin' hypothesis for the LES, the 'flux-trait network' concept indicates that the LES arises from other key traits, independently of vein traits (Fig. 2). The LMA , determined by leaf thickness and density (LT and LD respectively), dilutes with dry mass the protoplasmic biochemical constituents and thus negatively influences mass-based nitrogen concentration and rates of respiration and photosynthesis (N_{mass} , R_{mass} , and A_{mass} , respectively). Additionally, mechanically, the LMA tends to increase leaf lifespan (LL ; Wright *et al.*, 2004). The linkages among area-based and mass-based traits that are interconvertible by LMA may partially arise innately from statistical effects, as explained previously, in the section 'Mechanistic trait linkages versus concerted convergence'.

Vein traits can thus scale up to an important influence on plant relative growth rate. A_{area} contributes to the time-integrated dry mass accumulation per leaf area (unit leaf rate, ULR), which, with leaf area ratio (LAR , leaf area/plant mass), determines plant maximum relative growth rate ($RGR_{\text{max}} = ULR \times LAR$). The LAR is equivalent to the leaf mass fraction (LMF , leaf mass/plant mass) divided by LMA (Evans, 1972; Poorter *et al.*, 2009, 2012).

Supporting this synthesis of the importance of vein traits in determining K_{leaf} and its importance in determining g_s and A_{area} , numerous correlations of gas exchange with vein traits have been reported across species (Table 2). A number of studies reported correlations of VLA and/or K_{leaf} with stomatal pore area per leaf area across diverse species or across closely related species within a genus (Sack *et al.*, 2003, 2005), or of g_s with K_{leaf} across diverse species (Feild *et al.*, 2011b), or of g_s with VLA (Boyce *et al.*, 2009; Feild *et al.*, 2011b) or stomatal density or stomatal pore area with VLA , across species, or between shade and sun leaves within a given species (Edwards, 2006; Sack and Frole, 2006; Dunbar-Co *et al.*, 2009; Brodribb and Jordan, 2011; Murphy *et al.*, 2012; Zhang *et al.*, 2012; Table 2). Notably, across sun and shade leaves of some, but not all, species, and among species within a genus, the expected functional coordination of vein and stomatal traits (i.e. the matching of hydraulic supply with demand) also has a further developmental basis, as the coordination arises due to genetic and developmental linkages of vein and stomata during leaf expansion (Murphy *et al.*, 2012; Sack *et al.*, 2012; Sack and Scoffoni, 2013).

This conceptual hypothesis represents the possible relationships among vein, hydraulic, stomatal, and gas exchange traits and will not be entirely supported in all species sets in the same way. Given that the coordination of traits depends on narrow variation across species in operating Ψ_{leaf} , Ψ_{soil} , and VPD, this framework predicts that the trait coordination will shift or weaken if species are considered from across different environments. Indeed, species of moister forests are expected to achieve higher g_s relative to K_{leaf} and VLA than species of drier forests, as should aquatic plants as compared with terrestrial plant species (Sack *et al.*, 2005; Feild *et al.*, 2011b). Further, the importance of given traits within the cluster of traits that drive other traits will depend on the species set. Thus, because A_{area} depends not only on g_s , but also on J_{max} and V_{cmax} , which are related to nitrogen allocation, the modulation of these parameters can shift the coordination of A_{area} and hydraulic and venation traits. Such shifts are thus consistent with the framework of flux-related traits.

This framework did not explicitly include additional mechanisms for influence of vein traits on A_{area} that have been proposed in the recent literature, although these can readily be added. Classic and recent work have suggested mesophyll structure and traits associated with photosynthetic processes (e.g. g_m) are coordinated with hydraulic traits. For example, aquaporin activity would increase both K_{ox} and g_m (Ferrio *et al.*, 2012; Flexas *et al.*, 2013). Additionally, recent work has suggested a possible role of phloem loading and translocation rate in determining A_{area} (Fu *et al.*, 2011; Nikinmaa *et al.*, 2013) and that, in some species, VLA may provide additional phloem delivery capacity and increase A_{area} , especially in species with symplastic loading (Sack and Scoffoni, 2013). Recent work also suggests that nutrient delivery rates, which would be influenced by vein traits, may also determine A_{area} , given the need not only to supply developing leaves but also to maintain mature leaves (Girardin *et al.*, 1985; Niinemets *et al.*, 2004). Nutrient delivery rates may depend on E and thus on vein traits (Shabala *et al.*, 2002; Kerton *et al.*, 2009; Gilliam *et al.*,

2011), and E in turn may be modulated to achieve given nutrient delivery rates (Cramer *et al.*, 2008; Cramer *et al.*, 2009).

Overall, this conceptual hypothesis indicates the roles for individual vein traits in scaling up to impact on hydraulic, stomatal and photosynthetic function in tandem with additional traits. The following sections examine three details and the ramifications of this synthetic conceptual model.

Detail of synthetic conceptual model: how does vein length per area influence K_{leaf} ?

According to our conceptual model, the VLA affects higher-level traits via its influence on K_{leaf} . This influence can arise in several possible ways (Sack and Holbrook, 2006; Coomes and Sack, 2009; Sack and Scoffoni, 2013). A higher VLA can increase K_x because it corresponds to a larger number of xylem flow pathways in parallel. A higher VLA can also increase K_{ox} by corresponding to (i) a greater bundle sheath surface area and thus higher total permeability for water flow out of the veins, (ii) a geometric effect reducing the hydraulic flow distances for water to the site of evaporation, and/or (iii) a greater surface area from which water evaporates into the vapour phase in the mesophyll before diffusing from the stomata.

Which of these factors determines the influence of VLA on K_{ox} depends on the unresolved question of where water evaporates in the leaf and whether the location differs across species and environmental conditions (Meidner, 1983; Sack and Tyree, 2005; Sack and Holbrook, 2006). For example, if water evaporates throughout the leaf, then a higher VLA would translate into smaller hydraulic flow distances from the xylem to evaporation sites. However, in this case, other leaf structural anatomical characteristics would also influence the flow pathway. For example, all else being equal, a thicker leaf would have a higher K_{ox} , as there would be more parallel pathways for water flow outside the xylem.

By contrast, if water tends to evaporate near the stomata, whether within the substomatal cavity or from the guard cells or inner surface of the surrounding epidermal cells, then, all else being equal, a thicker leaf may have a lower K_{ox} , as water would travel a longer distance. In one formulation of the anatomical basis for water flow pathways, assuming water evaporation near the stomata, K_{ox} should relate negatively to the mean distance that water moves across the mesophyll from the vein to the epidermis (D_m), estimated as a diagonal line in the leaf cross-section, the hypotenuse of half the IVD and the vertical vein-to-epidermis distance (VED ; Brodribb *et al.*, 2007). The consideration of the D_m as a proxy for K_{ox} or K_{leaf} and as a primary mechanism for the influence of VLA on A_{area} is a valuable working hypothesis, proposed in one study which found strong negative correlation of both K_{leaf} and A_{area} with D_m across a set of 43 species of diverse major plant lineages: mosses, ferns, cycads, gymnosperms, and angiosperms from shade and sun-exposed habitats (Brodribb *et al.*, 2007). Further, experiments with an artificial 'leaf' made of a microchannel 'vein system' subtending a gel 'mesophyll system' from which water evaporated found that its hydraulic conductance was driven by the structural analogies to IVD and VED (Noblin *et al.*, 2008). However, those

findings do not necessarily support a general importance of D_m beyond its being a strong correlate of IVD and VLA . Indeed, across most species sets, the D_m is primarily determined by IVD , rather than by VED . Thus, in the dataset of Brodrribb *et al.* (2007) the reported correlations of high K_{leaf} and A_{area} with low D_m (r for log-transformed data = -0.68 and -0.59 respectively, $P < 0.001$) were statistically driven by the correlations of K_{leaf} and A_{area} with low IVD ($r = -0.62$ and -0.55 , $P < 0.001$) rather than with low VED ($r = -0.07$ and 0.001 , $P = 0.66-0.995$). Further, the relationships among IVD , K_{leaf} , and A_{area} in that dataset may not have been directly mechanistic, because the species spanned major plant lineages and habitats, and additional factors may also have contributed to differences in K_{leaf} and A_{area} . For example, later-evolved lineages (e.g. angiosperms) would have had not only higher VLA and lower IVD , but also may have had larger xylem conduits and higher values for n_s , N_{mass} , J_{max} , and V_{cmax} than earlier-evolved lineages (e.g. mosses, ferns, and cycads). This is especially true because the plants of early-branching lineages were sampled from relatively shaded habitats. Additionally, several other datasets indicated that a high VED did not significantly increase K_{ox} or K_{leaf} . For example, K_{leaf} is greater in thicker leaves of deciduous angiosperms and in sun relative to shade leaves within canopies (Aasamaa *et al.*, 2001; Sack *et al.*, 2003), even disproportionately greater than expected from their VLA , despite their greater thickness (Brodrribb and Jordan, 2011).

Indeed, the exact influence of VLA on K_{ox} may differ across species and environmental conditions. Leaves vary enormously in cross-sectional anatomy and possibly in where the water principally evaporates. In leaves with extensive spongy mesophyll airspaces (e.g. Fig. 4B), all water flowing vertically across this layer to the lower epidermis for evaporation does not seem likely. In other species, bundle sheath extensions or extra-xylem networks of sclereids or tracheids may create additional

pathways for water flow and/or an additional evaporative surface (Wylie, 1952; Brodrribb *et al.*, 2007, 2010; Sommerville *et al.*, 2012; Sack and Scoffoni, 2013). Equally importantly, the flow pathways might vary with the external environment. For leaves in the dark, water might tend to evaporate near the stomata, driven by the vapour pressure difference across the epidermis between stomatal cavity and outside air. However, for leaves illuminated from the top and experiencing a thermal gradient through the leaf corresponding to pigment light absorption, water would evaporate preferentially from warmer inner tissues (Sheriff, 1977). Also, as leaves dehydrate during transpiration, tissues may shrink and flow pathways outside the xylem may be further reduced. New research on many species is needed to clarify the important impacts of VLA on K_{leaf} , and thus on A_{area} across species and environmental conditions.

Detail of synthetic conceptual model: the impact of major vein length per area on leaf mass per area

The evidence supports VLA being mechanistically independent of LMA and LL . However, it is important to distinguish the major from the minor veins. The major and minor vein systems, while integrated in the mature leaf, are highly distinct in their evolution, genetics, and development and they contrast in many features (Sack and Scoffoni, 2013). The major VLA accounts for a minority (<20%) of VLA , which is thus determined predominantly by minor VLA in virtually all angiosperms (Sack *et al.*, 2012). Thus, major VLA did not correlate with total VLA in the global dataset ($r = -0.10$, $P = 0.40$, $n = 71$).

Despite this, the major veins *can* have a direct, although often small, influence on LMA across species (Fig. 7). Given their

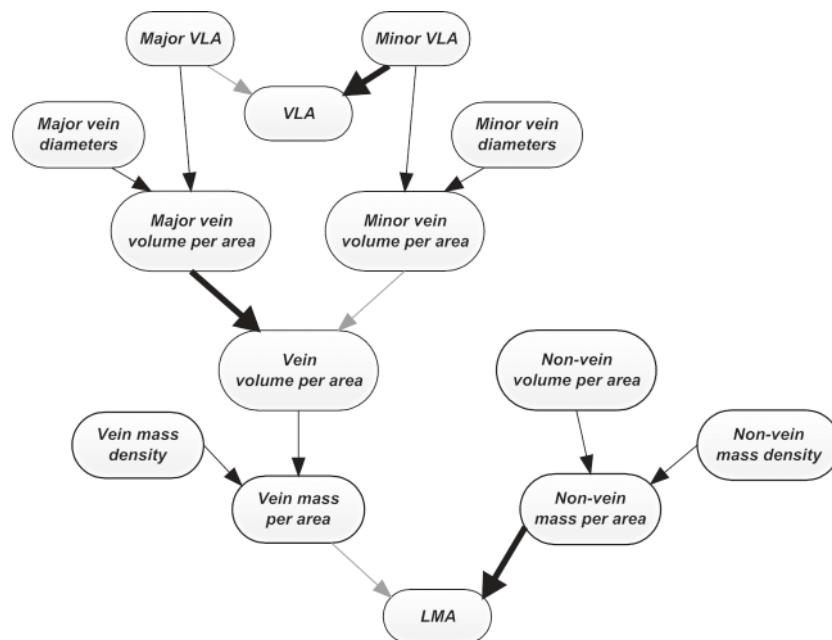


Fig. 7. Schema for the impact of major but not minor vein length per area (VLA) on leaf mass per area (LMA) and thereby on the leaf economic spectrum, and diminishing returns in larger leaves. Thick black arrows indicate great influence; thin black arrows indicate small influence; and thin grey arrows indicate very small influence, based on analysis of a global database (Supplementary Table S2).

very large diameters relative to those of minor veins, major veins contribute the bulk of total vein volume per leaf area (mean \pm SE, $82 \pm 1.3\%$ of the total vein volume in the global database; $n=58$) and thus are the primary determinant of the total vein volume per leaf area ($r=0.996$, $P<0.001$, $n=58$). Thus, major veins contribute the bulk of the mass of the leaf vein system and would be expected to directly influence *LMA*. For 21 species of a wide range of textures from California vegetation, just the midrib mass accounted for 9–33% of leaf mass (mean \pm SE, $18 \pm 1.5\%$), disproportionately to their fraction of leaf area (Méndez-Alonzo *et al.*, 2013). In our analysis of the global dataset, the *LMA* measured on whole leaves was positively correlated with major vein volume per leaf area ($r=0.38$, $P=0.003$, $n=58$). Further, a higher major *VLA* might contribute to *LL* due to both biomechanical and hydraulic effects; a higher major *VLA* can additionally improve *LL* by providing redundant pathways around sites of damage or embolism during drought (Sack *et al.*, 2008; Scoffoni *et al.*, 2011). Still, the mesophyll accounts for the majority of the leaf volume and mass, and its properties apparently contribute the most to species differences in *LMA* (Poorter *et al.*, 2009), and likely to *LL*. In fact, across diverse species, leaf biomechanical strength and *LL* were negatively related to the allocation of mass to the midrib relative to lamina and higher order veins (Niinemets *et al.*, 2007b; Méndez-Alonzo *et al.*, 2013).

The impact of major veins on *LMA* and *LL* has functional consequences for leaf size. Leaf size is variable across species by many orders of magnitude and labile in evolution, with benefits that depend on the availability of moisture, irradiance, and nutrients, as well as on herbivory (Peppe *et al.*, 2011; Scoffoni *et al.*, 2011). Thus, shifts in leaf size are favoured by multiple selective agents. One of the costs of larger leaves is biomechanical support. According to the ‘diminishing returns’ hypothesis, larger leaves may have higher *LMA*; this trend is observed within some species (Milla and Reich, 2007; Arcand *et al.*, 2008), across species within some but not all lineages (Grubb, 1998), across species within communities (Price and Enquist, 2007), and globally (Niklas *et al.*, 2007). While the minor veins would not contribute substantially to the greater *LMA* of larger leaves, major veins may contribute significantly: a study of global scaling of vein traits with leaf size showed that major vein diameters were greater in larger leaves, whereas major *VLA* decreased with leaf size due to a conserved developmental algorithm. On balance, major vein volume per area increased significantly with leaf size, resulting in an increase of total vein volume per leaf area with increasing leaf size (Sack *et al.*, 2012). Consistent with this finding, larger leaves invest more mass in petiole and midrib (Niinemets *et al.*, 2007a). As discussed previously, the *LMA* is more strongly affected by the properties of the mesophyll (i.e. tissue and cell-wall thicknesses and airspace) than by those of the vasculature, and higher *LMA* in larger leaves would also be due to investment in thicker mesophyll cell walls and extra-vein sclerenchyma (Arcand *et al.*, 2008). Nonetheless, the increase of vein volume per area would contribute quantitatively to higher *LMA* (i.e. the general decline of photosynthetic leaf area per investment of carbon in larger leaves; Niinemets *et al.*, 2007b; Niklas *et al.*, 2007). Despite their

greater carbon cost per photosynthetic surface and their greater drought sensitivity, large simple leaves are known to be highly competitive in resource-rich environments (Givnish, 1987; Beerling and Franks, 2010; Feild *et al.*, 2011a), likely for the maximization of leaf area for capture of diffuse light produced per investment in primordia and in support biomass.

In conclusion, major veins have a much stronger influence on bulk leaf structure and composition than the minor veins, an influence that tends to increase with leaf size across species. However, the properties of the lamina outside the veins have an even stronger influence on bulk leaf properties.

Implications of the synthetic conceptual model for the linkages of vein traits with gas exchange and plant performance

The importance of vein traits such as *VLA* in determining hydraulic supply gives them a crucial role in supporting leaf gas exchange and whole-plant growth. Indeed, the key importance of *VLA* in enabling higher g_s and A_{area} (Fig 8A) indicates a possible importance in influencing A_{mass} and *RGR*. A_{mass} is the key trait to scale leaf gas exchange to whole plant carbon

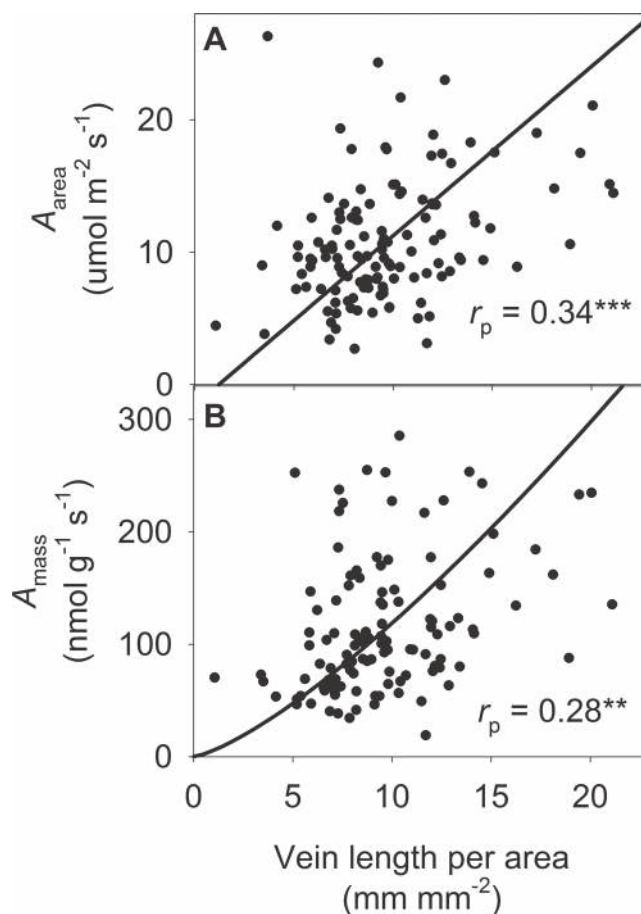


Fig. 8. Importance of leaf vein length per area in determining (A) light-saturated photosynthetic rate per leaf area and (B) per leaf mass in a global database for angiosperm species (Supplementary Table S1). Lines were fitted using standard major axes: (A) $A_{\text{area}}=1.28VLA-1.54$; (B) $A_{\text{mass}}=5.60VLA^{1.33}$.

balance, as it determines maximum plant relative growth rate for a given allocation to leaf mass (Fig. 2; Quero *et al.*, 2006). A_{mass} is driven by ‘metabolics’ (A_{area}) and economics (LMA):

$$A_{\text{mass}} = A_{\text{area}} / LMA \quad \text{Equation 5}$$

Analysis of our global database showed for the first time a correlation of A_{mass} with VLA (Fig. 8B). Notably, A_{mass} was driven by its negative correlation with LMA (Fig. 9A) and its positive correlation with A_{area} (R^2 values were 0.34 for both in the database, $P < 0.001$, $n = 119$, data log-transformed before testing); both relationships are due to potential mechanistic bases and at least in part arise innately from statistical effects given A_{mass} is calculated from those variables (Equation 5, also previously discussed in the section ‘Mechanistic trait linkages versus concerted convergence’). A model predicting A_{mass} from LMA and VLA thus could explain more of the variance than one based on VLA alone (Fig. 10). While the relationships of A_{area} and A_{mass} to VLA are weak, there is a substantial signal (Fig. 8A and B). The scatter in such a dataset representing

diverse species and growth forms implies a lack of universal scaling of gas exchange with VLA , as expected because multiple vein traits influence K_{leaf} , and species vary in the coordination of K_{leaf} with A_{area} , depending on their lineage and the environment to which they are adapted (Sack and Scoffoni, 2013; see previous section, ‘Synthesis of the linkage of VLA and the LES as part of the flux trait network’). A future research direction is to determine phylogenetic and ecological groupings in these relationships. According to the mechanisms described above, not only VLA but other vein traits (e.g. vein conductivities or bundle sheath extensions) and extra-xylem traits can allow increased g_s for a given Ψ_{leaf} and thus provide the hydraulic conductance necessary for high A_{area} and thus high A_{mass} . The non-linearity of the relationships (i.e. saturation of the effect on A_{area} and A_{mass} of increasing VLA) is consistent with increasing limitations of other factors influencing photosynthetic rates as VLA increases, again supporting the expectation that multiple traits within the flux trait network contribute to determining photosynthetic gas exchange and RGR .

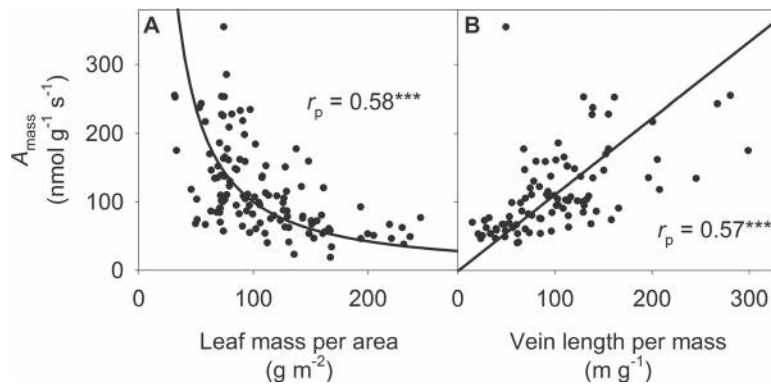


Fig. 9. (A) The negative relationship of light-saturated photosynthetic rate per mass (A_{mass}) and leaf mass per area (LMA) expected from the leaf economic spectrum, observed in a global database for angiosperm species (Supplementary Table S1). (B) Given the negative relationship of vein length per mass (VLM) with LMA (Fig. 3C), A_{mass} correlated positively with VLM . Lines were fitted using standard major axes: (A) $A_{\text{mass}} = 29.648LMA^{-1.24}$; (B) $A_{\text{mass}} = -0.92VLM + 1.11$. For A and B, n -values were 126 and 119 respectively.

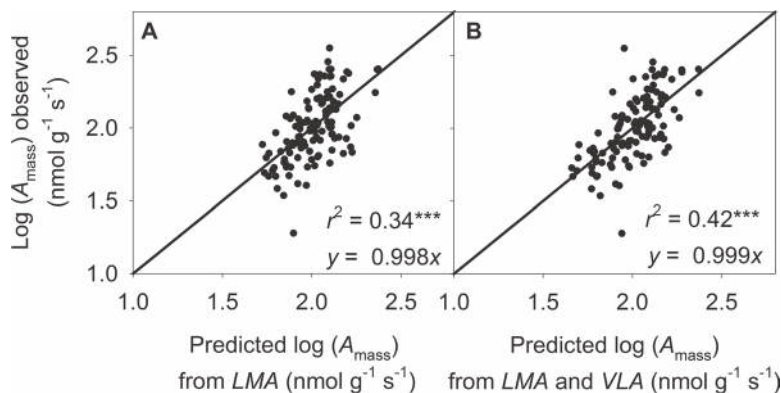


Fig. 10. The power of vein length per area (VLA) to predict light-saturated photosynthetic rate per mass (A_{mass}) in addition to leaf mass per area (LMA). (A) $\log(A_{\text{mass}})$ data from a global database for angiosperm species (Supplementary Table S1) plotted against values predicted from a model based on $\log(LMA)$: $\log(A_{\text{mass}}) = 3.52 - 0.722\log(LMA)$ ($R^2 = 0.34$, $P < 0.001$; $n = 119$). (b) $\log(A_{\text{mass}})$ data from a global database for angiosperm species plotted against values predicted from a model based on $\log(LMA)$ and $\log(VLA)$: $\log(A_{\text{mass}}) = 3.10 - 0.727\log(LMA) + 0.378\log(VLA)$ ($R^2 = 0.42$, $P < 0.001$; $n = 119$). Note the tighter fit and improved R^2 for (b) over (a). Lines are forced through the origin so that the slope tests for bias (i.e. deviation from 1.0). The presented R^2 are for lines not forced through the origin. $n = 119$.

To completely describe how flux-related traits affect the LES and scale up to *RGR*, we may describe additional flux-related traits that have been traditionally considered on a leaf area basis, also on a leaf mass basis. Hydraulic supply to leaves has been traditionally only expressed on a leaf area basis; however, recent studies have begun also to normalize K_{leaf} on a leaf mass basis (Niinemets and Sack, 2006; Nardini *et al.*, 2012; Simonin *et al.*, 2012).

Many flux-related traits, such as *VLA*, K_{leaf} , n_s , *SPI*, and g_s , are typically expressed on an area basis for at least two reasons. First, characters such as the total absolute (non-normalized) vein length, leaf hydraulic conductance, leaf stomatal pore area, and leaf photosynthetic rate tend to increase tightly with leaf size within species and across species, and thus, normalizing by leaf area typically strongly reduces or removes their dependence on leaf size (Ackerly and Reich, 1999; Sack *et al.*, 2004; McKown *et al.*, 2010; Simonin *et al.*, 2012). Secondly, and more profoundly, these flux-related traits depend on networks and structures that are horizontally modular with leaf area (e.g. vein length and stomatal pores being evenly distributed across the leaf surface), and/or that depend on layers of tissues per area (A_{area}); in either case, these traits are intrinsically related to leaf area (as previously explained in ‘Mechanistic trait linkages versus concerted convergence’). However, recent studies have extended the LES by dividing other flux-related traits by *LMA*, to calculate mass-based versions of typically area-based traits. Thus, recent studies have focused on $K_{\text{leaf, mass}}$ ($= K_{\text{leaf}}/LMA$; Nardini *et al.*, 2012; Simonin *et al.*, 2012). Given that K_{leaf} and *LMA* are independent, it follows, at least in part innately (i.e. by statistical necessity), that $K_{\text{leaf, mass}}$ typically shows a negative relationship with *LMA* and *LL* (Nardini *et al.*, 2012; Simonin *et al.*, 2012). However, the relationship does emphasize an important insight. Leaves of higher *LMA* achieve less K_{leaf} for their mass investment, because the additional investment of leaf mass per area which contributes to *LL* is principally in the thickness of leaf tissues and cell walls outside the vein system. Similarly the same argument can be made for vein length (i.e. based on $VLM = VLA/LMA$). There are negative relationships of *LMA* and *LL* with *VLM* (Fig. 3C and D), which demonstrate that, in leaves of higher *LMA*, less vein length is constructed per leaf mass investment.

Another advantage of calculating traits on a leaf mass basis is that by including the effect of *LMA*, they scale up more directly to influencing *RGR*. Thus, we found a tight correlation of A_{mass} with *VLM* (Fig. 9B). This relationship arises from the relationship of *VLA* with A_{area} and is strengthened innately or statistically by the independence of *VLA* from *LMA* (see previous section, ‘Mechanistic trait linkages versus concerted convergence’). Notably, these new correlations of mass-based flux-related traits with other LES traits are consistent with the conceptual synthesis based on flux traits expressed in Fig. 2, which could be redrawn with mass-based traits calculated using *LMA* (as shown for A_{mass} in Fig. 2). Given that *LMA* is unrelated to area-based flux-related traits, structurally, physiologically, developmentally, or genetically,

the relationship of the mass-based traits to *LMA* is innate, simply another expression of this hypothesis.

Further work and broader implications

This review rejected the ‘vein origin’ conceptual hypothesis and provided support for the ‘flux trait network’ conceptual hypothesis in which *VLA* contributes to the LES via K_{leaf} , and thereby to g_s and A_{area} , and further, to A_{mass} and thus to *RGR*. This conceptual hypothesis is based on a synthesis of numerous previously published studies conducted on many species sets, computer simulation modelling, and theory based on physical principles. Truly validating this second conceptual hypothesis will ideally be done by collecting data for the traits in Fig. 2 for a given set of species, or genotypes of a given species, in common growth conditions, or using manipulative experiments to test the causality at each step.

The importance of *VLA* and other vein traits that influence gas exchange and *RGR* may be even stronger when one considers net leaf carbon balance, rather than light-saturated A_{mass} . A_{mass} is driven by *LMA* and A_{area} (equation 5), and A_{area} gains in importance relative to *LMA* during natural fluctuations in resource availability when these occur at a more rapid frequency than can be accommodated by plasticity in leaf form and physiology (Villar *et al.*, 1998, 2005). In addition, other vein traits are important in determining plant responses to dynamic conditions. Vein traits such as *VLA* do not only influence maximum K_{leaf} , but also its environmental responses. Thus, K_{leaf} can be affected by the light response of K_{ox} , the effect of xylem embolism during leaf dehydration, and the collapse of cells and deactivation of aquaporins in the bundle sheath and mesophyll. These dynamics in turn can influence stomatal opening and photosynthetic rates (Sack and Scoffoni, 2013). Such dynamics of K_{leaf} will thus be influenced by *VLA* and other vein traits, such as vein conductivities, and air-seeding pressures in the veins.

The relationships of vein traits to gas exchange and plant performance are important for understanding past as well as present vegetation. Vein traits have been used as gas exchange proxies in paleobiology, and the strong linkage of A_{area} and g_s with *VLA* has been suggested to allow estimation in the fossil record and to investigate the trends of plant features with changing climate and atmospheric CO_2 (Boyce *et al.*, 2009; Brodribb and Feild, 2010; Feild *et al.*, 2011a; Boyce and Zwieniecki, 2012). In some applications, A_{area} has been reconstructed from single vein traits (i.e. *IVD* or *VLA*; Brodribb and Feild, 2010; Boyce and Zwieniecki, 2012). We note that the precise coordination of traits is subject to shifting, as described above, and models based on larger numbers of traits will have greater predictive value as many other vein traits can impact on leaf function, independently of *VLA* (Fig. 2). The potential for such estimation based on leaf trait proxies is enormous, especially for generating refined hypotheses of vegetation function in the deep past (Jordan, 2011). For example, *VLA* increased strongly on average from early branching lineages of seed plants to early and to modern angiosperms (Boyce *et al.*, 2009; Brodribb and Feild, 2010; de Boer *et al.*, 2012; Sack

and Scoffoni, 2013), coinciding with the decline of CO₂ in the atmosphere. Simultaneously there was an increase in stomatal pore area. These trends indicate that the causal framework of coordinated flux-based traits (Fig. 2) also applied across macroevolutionary trajectories. Notably, *LMA* and *LL* have also diversified strongly. Previous studies have provided contrasting evidence that early angiosperms had high *LMA* and high *LL* (Feild *et al.*, 2004, 2009) or low *LMA* and low *LL* (Royer *et al.*, 2010). As these traits diversified in angiosperms, simultaneously with the evolution of high *VLA*, the mechanistic independence of vein traits from *LMA* would allow angiosperms to achieve many combinations of these traits and thus occupy a wide range of niches relative to earlier branching lineages. Similarly, a previous study showed that the mechanistic independence of *VLA* from leaf size (Sack *et al.*, 2012) indicated a strong capacity for angiosperms to simultaneously diversify in both *VLA* and leaf size and thereby gain ecological advantages.

Given increasing recognition of the influence of vein traits such as *VLA*, in addition to LES traits, in determining whole plant performance in crops and wild plants alike, new work is needed to develop a full framework of traits to test the adequacy of the synthesis in Fig. 2. Ultimately, there is the need to quantitatively model the impact of differences in vein traits and other key traits on leaf level gas exchange and plant growth and to test the hypothesis that they are strongly influential during dynamic environmental conditions. More work is needed to determine the genetic and developmental bases for vein traits to test the hypothesis that they are strongly shared across species. Further work is also essential to determine the evolution of given traits and trait clusters in a wide range of plant lineages. Those processes generated the enormous diversity across species in vein traits, which scales up to functional differences and responses to environment. These approaches will not only improve predictions of plant function and tolerances of environmental challenges, including climate change, but can provide a trait-based approach for breeding improved crop varieties.

Supplementary material

Supplementary data are available at *JXB* online.

Supplementary Table S1. Database compiled from published studies of vein traits, leaf economic spectrum traits, leaf structural traits, and climate.

Supplementary Table S2. Database for leaf vein allocation.

Supplementary Table S3. Data and equations of *Blonder et al.* (2011), and test of the impact of randomizing vein trait data.

Acknowledgements

The authors thank Colin Osborne for organizing the symposium 'Evolution of Physiological Traits' at the Society of Experimental Biology meeting in July 2012, during which this paper was conceived and designed, and two anonymous reviewers for their comments which improved the paper. This work was supported by the National Science Foundation (grants IOS-0753233 and IOS-1147292).

Appendix 1. Equations derived by *Blonder et al.* (2011)

The equations are presented after slight simplification, and using definitions as in Table 1, with equation numbers as presented in that paper:

$$LMA = \pi r_v^2 VLA (\rho_v - \rho_L) + \frac{2\rho_L IVD}{k_o} \quad \text{Equation 4}$$

$$LL = k_1 \times IVD \quad \text{Equation 5}$$

$$A_{\text{mass}} = \frac{c_o(1-h)WUE}{\pi r_v^2 VLA (\rho_v - \rho_L) + \frac{2\rho_L IVD}{k_o}} \times \frac{1}{\frac{1}{\frac{\pi D \times VLA}{2 \log \frac{IVD}{k_o r_v}} + \frac{1}{D \frac{a_s n_s}{t_s + \sqrt{a_s/\pi}}}}} \quad \text{Equation 6}$$

and

$$N_{\text{mass}} = k_2 A_{\text{mass}} + \frac{k_3}{k_o} \frac{2 \times IVD - k_o \pi r_v^2 \times VLA}{LMA} \quad \text{Equation 7}$$

where r_v , ρ_v , and ρ_L are the vein bundle radius, the mass density of veins, and the mass density of lamina, respectively; k_o is $IVD/0.5T$, where T is leaf thickness; and c_o , h , WUE , D , a_s , n_s , and t_s are the saturation vapour concentration of water in air, relative humidity, water use efficiency, the diffusion constant of water in air, stomatal pore area, stomatal density, and stomatal pore thickness. In applying these equations, for r_v , ρ_v , ρ_L , a_s , n_s , and t_s , *Blonder et al.* used constants based on a typical or an average value in the literature, although values in fact vary enormously among species. For the other variables, *Blonder et al.* made measurements of gas exchange and vein traits for 25 species or used published values. The k_1 , k_2 , and k_3 were set to constant values to allow predictions of realistic *LL* and N_{mass} values. With the exception of equation 5, this review found no error in the derivation of these equations, although k_o values were miscalculated by a factor of two (see Appendix 2 for discussion of the erroneous theory used for deriving equation 5).

Appendix 2. Sensitivity analysis of the model of *Blonder et al.* demonstrate the negligible direct role of *VLA* in driving the LES according to this model

Equations are presented using definitions as in Table 1, with equation numbers as used by *Blonder et al.* (2011).

Prediction of *LMA*

According to *Blonder et al.* (2011; see main text), *LMA* can be predicted from the equation,

$$LMA = \pi r_v^2 VLA (\rho_v - \rho_L) + \frac{2\rho_L IVD}{k_o} \quad \text{Equation 4}$$

where r_v , ρ_v , and ρ_L are respectively the vein bundle radius, the mass density of vein, the mass density of non-vein lamina, and $k_o = IVD/0.5LT$, where LT is leaf thickness. In applying this equation, for r_v , ρ_v , and ρ_L , constants were used, based on the literature, of 2×10^{-5} m, 1×10^6 g m⁻³, and 3×10^5 g m⁻³ respectively. By the definition of k_o , the second term simplifies to $LT \times \rho_L$, and thus

$$LMA = \pi r_v^2 VLA (\rho_v - \rho_L) + (LT \times \rho_L) \quad \text{Equation 4a}$$

The first term gives the added mass per area of the veins (i.e. the volume per area of the veins multiplied by the difference in density between veins and non-vein lamina); the second term gives the non-vein lamina density multiplied by its thickness. Notably, when applying equation 4 for prediction, *Blonder et al.* used a constant for k_o . Doing this may have suggested a sensitivity of LMA to IVD and allowed incorporation into this equation of the sometimes-observed correlation of IVD with LT . However, a correlation of IVD with LT is not necessarily mechanistic or reliable across datasets (see discussion of Assertion 3 and Table 3, row 3). Further, even in a dataset in which IVD correlates with LT over a wide range of values, the use of a constant k_o for linear prediction of one from the other can be fallacious and lead to inaccuracy and imprecision (*Nee et al., 2005*). In fact, treating k_o as a constant is not appropriate given that it varies widely (over 5-fold in the dataset of *Blonder et al.* alone). Equally, predicting LT from IVD and k_o is impractical, as LT can be determined much more simply by direct measurement. In the simulations in this study, equation 4 was used with actual measured k_o values, rather than a constant. For sensitivity analyses, the simplified version, equation 4a, was used, which removed those issues and additionally allowed the isolation of VLA as an independent vein trait for sensitivity analysis.

Applying sensitivity analyses to equation 4a shows that the first term which includes VLA is negligible because the volume of veins per leaf area (neglecting the major veins) is very small (see main text), and the second term, $(LT \times \rho_L)$, determines LMA . The second term is very close to LMA (which equals $LT \times$ total leaf density). If a constant is used for ρ_L as done by *Blonder et al.*, then the term is proportional to LT , and thus LMA is simply predicted as a function of LT .

In partial derivative sensitivity analysis, examining the influence of traits that were not considered as constants (i.e. VLA and LT), and using values for constants from *Blonder et al.*:

$$\delta LMA / \delta VLA = \pi r_v^2 (\rho_v - \rho_L) = \pi \times (2 \times 10^{-5} \text{ m})^2 (1 \times 10^6 - 3 \times 10^5 \text{ g m}^{-3}) = 8.8 \times 10^{-4} \quad \text{Equation 4b}$$

$$\delta LMA / \delta LT = \rho_L = 3 \times 10^5 \quad \text{Equation 4c}$$

Thus $\delta LMA / \delta LT \gg \delta LMA / \delta VLA$, and LMA is effectively a function of LT and not of VLA .

Consistent with this result, randomizing the VLA data did not affect the LMA values predicted from equation 4 (the

slope of the regression fitted through the origin of LMA predicted from equation 4 against the LMA predicted from equation 4 after randomizing the VLA data was 1.01, $R^2=0.97$, $P<0.001$; Fig 6A; Supplementary Table S3). These analyses indicated that, despite the apparent ability of equation 4 to predict observed LMA values significantly and VLA being a term in equation 4, there was no direct role for VLA in determining LMA .

Prediction of A_{mass}

According to *Blonder et al. (2011)*; see main text), A_{mass} can be predicted from the equation,

$$A_{\text{mass}} = \frac{c_o(1-h)WUE}{\pi r_v^2 VLA (\rho_v - \rho_L) + \frac{2\rho_L IVD}{k_o}} \times \frac{1}{\frac{1}{\frac{\pi D \times VLA}{2 \log \frac{IVD}{k_o r_v}} + \frac{1}{D \frac{a_s n_s}{t_s + \sqrt{a_s/\pi}}}}} \quad \text{Equation 6}$$

where c_o , h , WUE , D , a_s , n_s , and t_s are saturation vapour concentration of water in air, relative humidity, water use efficiency, the diffusion constant of water in air, stomatal pore area, stomatal density, and stomatal pore thickness, respectively. In applying this equation, for a_s , n_s , and t_s , constants were used based on the literature (although values in fact vary enormously among species), and values for the other variables were based on measurements. In the simulations in this study, equation 6 was used with actual measured k_o values, rather than the constant k_o value used by *Blonder et al.*, and for sensitivity analyses the equation was simplified using the definition $k_o = IVD/0.5LT$, which allowed the isolation of VLA as an independent vein trait for sensitivity analysis with the advantages as explained in the section 'Prediction of LMA' above,

$$A_{\text{mass}} = \frac{WUE}{LMA} \frac{c_o(1-h)}{\frac{1}{\frac{\pi D \times VLA}{2 \log \frac{0.5T}{r_v}} + \frac{1}{D \frac{a_s n_s}{t_s + \sqrt{a_s/\pi}}}}} \quad \text{Equation 6a}$$

Blonder et al. derived equation 6 beginning with the definition of A_{area} equalling transpiration rate per leaf area (E) \times WUE , and then dividing both sides by LMA . Next, E was expressed as a function of VLA (the second term in equation 6a) by modelling diffusion in the leaf. However, this modelling was not theoretically or physiologically valid. The E was given as $C_o(1-h)g$ (leaf conductance to water vapour), with g then decomposed into g_s , g_x (xylem hydraulic conductance), and g_v (the diffusion conductance of vapour from vein to stomata). This derivation is based on a fundamental misunderstanding of water transport in leaves, which occurs in two phases, i.e. liquid phase through the veins and apoplast, via bulk flow

transport, and vapour phase, from the sites of evaporation to the stomata. It is critical to separate the two modes of water transport, as they depend on different driving forces (pressure and concentration gradients, respectively), and although the term ‘conductance’ can be used for both modes of water transport, they are in different units with different meanings. The assumption that g can be decomposed in the way described is at odds with current understanding of water transport. These conductances cannot be combined unless they were re-expressed all in terms of water potential and in that case, the g_s will be the main limitation (Cowan, 1972; Sack and Tyree, 2005; Sack and Holbrook, 2006). Thus, this expansion of g loses physical realism and leads to wrong conclusions.

Next, Blonder *et al.* assumed that g_x is negligible (an invalid assumption, given that g_x was intended to represent the leaf vein xylem hydraulic conductance, K_x , which is low, accounting for, on average, half the bottleneck in the leaf; Sack and Holbrook, 2006), removing any hydraulic basis for the function of leaf venation from their formulation. Blonder *et al.* then assumed that g_v and g_s should be of the same order (although in terms of diffusion conductances, g_s would actually be far lower than the diffusion through leaf airspaces). Blonder *et al.* then used a new geometric argument to express g_v as a function of VLA and IVD and a classical model to express g_s as a function of stomatal density and pore dimensions. This formulation is erroneous physically. According to this formulation, the only importance of vein traits in influencing gas exchange is by modifying the diffusion of water through air from veins to stomata. However, in reality, such diffusion would not be limiting.

In any case the prediction of A_{mass} in this formulation does not in fact depend on vein traits, as can be seen from a partial derivative sensitivity analysis. Examining the influence of leaf traits that were not considered as constants, i.e. VLA , LMA and LT , and using values for constants from Blonder *et al.*:

$$\delta A_{\text{mass}} / \delta VLA = \frac{2\pi \times \ln(10) \times a_s^2 n_s^2 D \times c_o (1-h) \times (\ln(T) - \ln(r_s) - \ln(2)) WUE}{LMA \times (2\sqrt{\pi} (\sqrt{\pi} \times t_s + \sqrt{a_s}) \times (\ln(LT) - \ln(r_s) - \ln(2)) \times VLA + \ln(10) a_s n_s)^2}$$

Equation 6b

$$\delta A_{\text{mass}} / \delta LT = \frac{2\pi \times \ln(10) \times a_s^2 n_s^2 D \times c_o (1-h) \times VLA \times WUE}{LMA \times T \times (2\sqrt{\pi} (\sqrt{\pi} \times t_s + \sqrt{a_s}) \times (\ln(LT) - \ln(r_s) - \ln(2)) \times VLA + \ln(10) a_s n_s)^2}$$

Equation 6c

$$\delta A_{\text{mass}} / \delta LMA = \frac{-c_o (1-h) WUE}{LMA^2 \times \left(\frac{\ln(10)}{2\pi D \times (\ln(LT) - \ln(r_s) - \ln(2)) + VLA} + \left(\frac{t_s + \sqrt{a_s/\pi}}{a_s n_s D} \right) \right)}$$

Equation 6d

We calculated these partial derivatives for the mean values for each species in the Blonder *et al.* (2011) dataset and found that the mean \pm SE for $\delta A_{\text{mass}} / \delta VLA$ was $2.49 \times 10^{-13} \pm 3.16 \times 10^{-14}$, while that for $\delta A_{\text{mass}} / \delta T$ was $7.73 \times 10^{-7} \pm 9.06 \times 10^{-8}$ and that for $\delta A_{\text{mass}} / \delta LMA$ was $-6.72 \times 10^{-9} \pm 6.47 \times 10^{-10}$. Thus $\delta A_{\text{mass}} / \delta T \gg \delta A_{\text{mass}} / \delta LMA \gg \delta A_{\text{mass}} / \delta VLA$, and A_{mass} is effectively a function of T and LMA , but not of VLA .

Consistent with this result, randomizing the VLA data had a negligible effect on the A_{mass} values predicted from equation

6 (the slope of the regression fitted through the origin of A_{mass} predicted from equation 6 against the A_{mass} predicted from equation 6 after randomizing the VLA data was 1.0, $R^2=0.96$; $P<0.001$; Fig 6B; Supplementary Information 3). These analyses indicated that despite the apparent ability of equation 6 to predict observed A_{mass} values significantly, and VLA being a term in equation 6, there was no direct role for VLA in determining A_{mass} according to this equation.

Prediction of N_{mass}

According to Blonder *et al.* (2011; see main text), N_{mass} can be predicted from the equation

$$N_{\text{mass}} = k_2 A_{\text{mass}} + \frac{k_3}{k_0} \frac{2 \times IVD - k_0 \pi r_v^2 \times VLA}{LMA}$$

Equation 7

where k_2 and k_3 are constants, chosen by Blonder *et al.* such that predicted N_{mass} values would fall in the range of observed values. The simulations in this study used equation 7 with actual measured k_0 values, rather than the constant k_0 value used by Blonder *et al.*, and for sensitivity analyses the equation was simplified using the definition $k_0 = IVD/0.5LT$, which allowed the isolation of VLA as an independent vein trait for sensitivity analysis with the advantages as explained in the section ‘Prediction of LMA’ above,

$$N_{\text{mass}} = k_2 A_{\text{mass}} + \frac{k_3 (T - \pi r_v^2 \times VLA)}{LMA}$$

Equation 7a

The causality of this equation—predicting N_{mass} from A_{mass} —seems counterintuitive. However, Blonder *et al.* reasoned that N_{mass} can be determined based on the assumption that A_{mass} across species should be linearly related to N_{mass} in the photosynthetic lamina, and adding the nonphotosynthetic nitrogen for the lamina minus the veins. This formulation adds a supposed dependence on the veins, although in fact the second term is only negligibly affected by the minor veins, which account for the bulk of VLA but contribute minimally to the volume or mass of the leaf lamina. Thus, this equation simply relates N_{mass} to A_{mass} and LMA as previously known from the LES.

In partial derivative sensitivity analysis, examining the influence of leaf traits that were not considered as constants (i.e. A_{mass} , VLA , LMA , and LT) and using values for constants from Blonder *et al.*:

$$\delta N_{\text{mass}} / \delta A_{\text{mass}} = k_2 = 1 \times 10^5$$

Equation 7b

$$\delta N_{\text{mass}} / \delta VLA = \frac{-k_3 \pi r_v^2}{LMA} = -1 \times 10^3 \times \pi \times (2 \times 10^{-5})^2 / LMA$$

Equation 7c

$$\delta N_{\text{mass}} / \delta LMA = -k_3 (LT - \pi r_v^2 \times VLA) / LMA^2$$

Equation 7d

$$\delta N_{\text{mass}} / \delta LT = k_3 / LMA = 1 \times 10^3 / LMA$$

Equation 7e

We calculated these partial derivatives for the mean values for each species in the [Blonder *et al.* \(2011\)](#) dataset and found that the mean \pm SE for $\delta N_{\text{mass}}/\delta VLA$ was $-1.20 \times 10^{-8} \pm 1.09 \times 10^{-9}$, while that for $\delta N_{\text{mass}}/\delta LMA$ was $-2.48 \times 10^{-5} \pm 5.04 \times 10^{-6}$ and that for $\delta N_{\text{mass}}/\delta T$ was 9.57 ± 0.865 . Thus $\delta N_{\text{mass}}/\delta A_{\text{mass}} \gg \delta N_{\text{mass}}/\delta T \gg \delta N_{\text{mass}}/\delta LMA \gg \delta N_{\text{mass}}/\delta VLA$, and in this scheme, N_{mass} is effectively a function of A_{mass} , LT , and LMA , but not of VLA .

Consistent with this result, randomizing the VLA data did not affect the N_{mass} values predicted from equation 7 (the slope of the regression fitted through the origin of N_{mass} predicted from equation 7 against the N_{mass} predicted from equation 7 after randomizing the VLA data was 0.999, $R^2=0.94$, $P<0.001$; [Fig 6C](#); [Supplementary Table S3](#)). These analyses indicated that, despite the apparent ability of equation 7 to predict observed N_{mass} values significantly and VLA being a term in equation 7, there was no direct role for VLA in determining leaf N_{mass} .

Appendix 3. Addressing the ‘extended vein origin’ hypothesis of [Blonder *et al.* \(2013\)](#)

While [Blonder *et al.* \(2011\)](#) developed their vein origin hypothesis to apply to differences found across diverse species, in a more recent paper, [Blonder *et al.* \(2013\)](#) applied the hypothesis to the case of different clones of *Populus tremuloides*. The same core set of assertions from [Blonder *et al.* \(2011\)](#) were maintained as a framework to explain correlations among A_{mass} , LMA , and VLA . In addition, [Blonder *et al.*](#) added the prediction that differences in LES traits that were driven by VLA could be done so because of shifts in VLA with water supply, where plants would have higher VLA if they were adapted to, or developed under, higher water supply.

[Blonder *et al.*](#) found that, across clones of *Populus tremuloides*, A_{mass} and LMA were negatively correlated, consistent with previous work within species and across species. [Blonder *et al.*](#) also found that clones under higher water supply also had higher VLA . However, assigning water supply a causal influence was not easily justified, as in this system, multiple environmental gradients were conflated; higher water supply coincided with lower temperature and higher elevation, and further, the irradiance of the sampled leaves was not reported. Indeed, previous work has shown that minor VLA , and thus, total VLA , are generally higher for plants grown in drier soil, and further, higher for plants grown under higher nutrient supply or temperature, or higher VPD, and higher for sun than shade leaves (reviewed in [Sack and Scoffoni, 2013](#); [Supplementary Table S1](#)).

Most importantly, [Blonder *et al.*](#) found for the *Populus* clones that A_{mass} and LMA were not correlated with VLA in the ways predicted by the vein origin hypothesis. Analyzing their data yielded no general correlation between LMA and VLA (for all data pooled, $r=-0.09$, $P=0.084$ and $r=-0.06$, $P=0.23$ with and without log-transformation respectively; for the seven clones with sufficient replication (>6) considered separately, with log-transformation, r -values ranged from <0.001 to -0.37 , $P=0.09-0.92$ for six clones; and a significant

correlation for one clone; $r=-0.71$, $P=0.03$; $n=6-80$ leaves per clone), or between A_{mass} and VLA (for all data pooled, $r=0.057$, $P=0.36$ and $r=0.04$, $P=0.52$ with and without log-transformation respectively; for the six clones with sufficient replication (>6) considered separately, with log-transformation, r -values ranged -0.20 to -0.032 , $P=0.098-0.87$ for 4/6 clones; and significant correlations in opposite directions for 2/6 clones; $r=0.33$ and $r=-0.61$, $P<0.001$ to 0.011). As explained in the main text, such lack of correlation would be expected based on the rejection of the ‘vein origin’ hypothesis. A lack of correlation of LMA from VLA is expected based on their being independent ([Fig. 2](#)), and a lack of correlation of K_{leaf} (and/or A_{area} and/or A_{mass}) and VLA is expected when considering plants sampled across a strong environmental gradient (main text). Despite the lack of these correlations, [Blonder *et al.*](#) maintained that the ‘covariation of $A_{\text{mass}}-LMA$ is linked to venation network geometry’, based on a structural equation modelling exercise, in which they tested 4096 models for possible correlation networks among A_{mass} , LMA , leaf area, and VLA , and selected a model in which LMA was negatively related to VLA , but A_{mass} was independent of VLA .

Rather than reject their ‘vein origin’ hypothesis, [Blonder *et al.*](#) described an extension of their unsupported hypothesis, with alternative equations for the prediction of A_{mass} and LMA . The alternative prediction of A_{mass} was developed by replacing all the terms other than $1/LMA$ in equation 6 with a new formulation:

$$A_{\text{mass}} = \frac{A_0}{(LT^2 + 4VLA^{-2})^{0.5}} \times \frac{1}{LMA} \quad (\text{Equation 2 in } \a href="#">Blonder *et al.*, 2013$$
)

The new term on the left was based on the assumption that photosynthesis per leaf area is equal to a constant A_0 divided by the distance from leaf veins to stomata, assumed to ‘reflect the hydraulic path length for diffusion of water away from the veins’. Thus, in this formulation, as in that of [Blonder *et al.* \(2011\)](#), the A_{area} would be determined by limitations on the diffusion of water vapour ([Appendix 2](#)). In the formulation of [Blonder *et al.* \(2011\)](#), the main limitation on the diffusion of water vapour was that through the airspaces and out of the stomata, while in this formulation, it is diffusion across the mesophyll tissue between vein and epidermis. Both formulations confuse the fact that water transport through leaves includes a bulk liquid flow phase, constrained by hydraulic conductance (quantified as K_{leaf}), and a vapour phase, constrained by diffusional conductance, mainly limited by the stomata (quantified as g_s). Further, xylem properties as well as the hydraulic pathways outside the xylem would determine the leaf hydraulic conductance (K_{leaf}) and thus influence A_{area} (see main text). In any case, whatever the conceptual errors in its derivation, this equation does not capture the influence of vein traits on A_{mass} . A sensitivity analysis like that in [Appendix 2](#) shows that this new equation is sensitive to leaf thickness and LMA , and negligibly affected by VLA (analysis not shown).

[Blonder *et al.*](#) further introduced two power laws: one expressing LT as a function of VLA , and the other expressing VLA as a function of leaf area. [Blonder *et al.*](#) assigned variable exponents to their power laws, stating that different

sets of individual plants or sets of species can show different exponents, and they may be positive, zero, or negative. Blonder *et al.* listed different scenarios that might result in different values of the exponents, for different sets of plants, allowing all possible directions and strengths of power laws relating LT to VLA , and VLA to leaf area. Blonder *et al.* justified these variable power law exponents with disparate mechanisms for alternative linkages among these traits, based on listing certain correlations reported in the literature, by speculating new mechanisms, or by including concerted convergences of traits that are independent functionally and developmentally but that have been reported to shift together along environmental gradients. Then, these power laws were substituted into the equations for LMA and A_{mass} , resulting in new equations predicting LMA and A_{mass} from VLA and leaf area. These equations were then simplified, removing many terms by assigning unity values to constants and leaf morphological variables. (Indeed, the terms in the equation for predicting LMA were removed such that in the final equation LMA was expressed as being equal to leaf thickness). Because such removing of terms in equations is not mathematically valid, and, in addition, typos or operation errors were found in the further derivations, we do not reproduce the equations here. In any case, the exponents are free parameters, which may vary according to datasets, and a number of variables and constants were removed, and thus, the final equations were no longer able to make quantitative predictions, or to be tested or falsified using empirical data. Blonder *et al.* did not test these equations against data, but considered qualitative outcomes if the power law exponents should vary. However, even the new equations were not able to explain even the direction of the relationships among LMA , A_{mass} , VLA , and leaf area in their selected structural equation model for their *Populus* data, finally leading Blonder *et al.* to question the selection of their structural equation model.

In fact, these equations have no clear mechanistic basis, given the inclusion of concerted convergences in addition to mechanistic linkages, and no predictive value due to the simplifications, and the unknown values for constants. Further, as for the original ‘vein origin’ hypothesis, the authors did not perform a sensitivity analysis. Thus, the extension suffers from the same flaws identified for the vein origin hypothesis in the main text. While a role for VLA in driving LES traits might seem apparent by writing an equation with VLA as a term, considering the relative sensitivities shows that even in this extended formulation, A_{mass} and LMA are negligibly influenced by VLA in this formulation (sensitivity analysis as in [Appendix 2](#), not shown). We reject the extended version of the ‘vein origin’ hypothesis, as not reflective of anatomy or mechanism and without predictive value. It is imperative that models are subjected to a sensitivity analysis before being used for interpreting mechanisms or for prediction.

References

- Aasamaa K, Sober A, Rahi M.** 2001. Leaf anatomical characteristics associated with shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes of leaf water status in temperate deciduous trees. *Australian Journal of Plant Physiology* **28**, 765–774.
- Ackerly DD, Reich PB.** 1999. Convergence and correlations among leaf size and function in seed plants: a comparative test using independent contrasts. *American Journal of Botany* **86**, 1272–1281.
- Arcand N, Kagawa AK, Sack L, Giambelluca TW.** 2008. Scaling of frond form in Hawaiian tree fern *Cibotium glaucum*: compliance with global trends and application for field estimation. *Biotropica* **40**, 686–691.
- Berling DJ, Franks PJ.** 2010. Plant science: the hidden cost of transpiration. *Nature* **464**, 495–496.
- Blonder B, Violle C, Bentley LP, Enquist BJ.** 2011. Venation networks and the origin of the leaf economics spectrum. *Ecology Letters* **14**, 91–100.
- Blonder B, Violle C, Enquist BJ.** 2013. Assessing the causes and scales of the leaf economics spectrum using venation networks in *Populus tremuloides*. *Journal of Ecology* **101**, 981–989.
- Boyce CK, Brodribb TJ, Feild TS, Zwieniecki MA.** 2009. Angiosperm leaf vein evolution was physiologically and environmentally transformative. *Proceedings of the Royal Society B* **276**, 1771–1776.
- Boyce CK, Zwieniecki MA.** 2012. Leaf fossil record suggests limited influence of atmospheric CO_2 on terrestrial productivity prior to angiosperm evolution. *Proceedings of the National Academy of Sciences, USA* **109**, 10403–10408.
- Brodribb TJ, Feild TS.** 2010. Leaf hydraulic evolution led a surge in leaf photosynthetic capacity during early angiosperm diversification. *Ecology Letters* **13**, 175–183.
- Brodribb TJ, Feild TS, Jordan GJ.** 2007. Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiology* **144**, 1890–1898.
- Brodribb TJ, Feild TS, Sack L.** 2010. Viewing leaf structure and evolution from a hydraulic perspective. *Functional Plant Biology* **37**, 488–498.
- Brodribb TJ, Jordan GJ.** 2011. Water supply and demand remain balanced during leaf acclimation of *Nothofagus cunninghamii* trees. *New Phytologist* **192**, 437–448.
- Brodribb TJ, Jordan GJ, Carpenter RJ.** 2013. Unified changes in cell size permit coordinated leaf evolution. *New Phytologist* **199**, 559–570.
- Buckley TN, Mott KA, Farquhar GD.** 2003. A hydromechanical and biochemical model of stomatal conductance. *Plant, Cell and Environment* **26**, 1767–1785.
- Buckley TN, Sack L, Gilbert ME.** 2011. The role of bundle sheath extensions and life form in stomatal responses to leaf water status. *Plant Physiology* **156**, 962–973.
- Castro-Diez P, Puyravaud JP, Cornelissen JHC.** 2000. Leaf structure and anatomy as related to leaf mass per area variation in seedlings of a wide range of woody plant species and types. *Oecologia* **124**, 476–486.
- Cochard H, Nardini A, Coll L.** 2004. Hydraulic architecture of leaf blades: where is the main resistance? *Plant, Cell and Environment* **27**, 1257–1267.
- Coomes DA, Sack L.** 2009. Response to comment on Coomes *et al.* ‘Scaling of xylem vessels and veins within the leaves of oak species’. *Biology Letters* **5**, 381–382.
- Cornelissen JHC, Lavorel S, Garnier E, *et al.*** 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany* **51**, 335–380.

- Cowan IR.** 1972. Electrical analog of evaporation from, and flow of water in plants. *Planta* **106**, 221–226.
- Cramer MD, Hawkins H-J, Verboom GA.** 2009. The importance of nutritional regulation of plant water flux. *Oecologia* **161**, 15–24.
- Cramer MD, Hoffmann V, Verboom GA.** 2008. Nutrient availability moderates transpiration in *Ehrharta calycina*. *New Phytologist* **179**, 1048–1057.
- de Boer HJ, Eppinga MB, Wassen MJ, Dekker SC.** 2012. A critical transition in leaf evolution facilitated the Cretaceous angiosperm revolution. *Nature Communications* **3**, 1221.
- Diaz S, Hodgson JG, Thompson K, et al.** 2004. The plant traits that drive ecosystems: evidence from three continents. *Journal of Vegetation Science* **15**, 295–304.
- Donovan LA, Maherali H, Caruso CM, Huber H, de Kroon H.** 2011. The evolution of the worldwide leaf economics spectrum. *Trends in Ecology and Evolution* **26**, 88–95.
- Dunbar-Co S, Sporck MJ, Sack L.** 2009. Leaf trait diversification and design in seven rare taxa of the Hawaiian *Plantago* radiation. *International Journal of Plant Sciences* **170**, 61–75.
- Edwards EJ.** 2006. Correlated evolution of stem and leaf hydraulic traits in *Pereskia* (Cactaceae). *New Phytologist* **172**, 479–489.
- Ehleringer JR, Clark C.** 1988. Evolution and adaptation in *Encelia* (Asteraceae). In: LD Gottlieb, SK Jain, eds, *Plant evolutionary biology*. New York: Chapman and Hall. pp. 221–248.
- Evans GC.** 1972. *The quantitative analysis of plant growth*. Oxford: Blackwell.
- Evans JR, Schortemeyer M, McFarlane N, Atkin OK.** 2000. Photosynthetic characteristics of ten *Acacia* species grown under ambient and elevated atmospheric CO₂. *Australian Journal of Plant Physiology* **27**, 13–25.
- Farquhar GD, von Caemmerer S, Berry JA.** 2001. Models of photosynthesis. *Plant Physiology* **125**, 42–45.
- Feild TS, Arens NC, Doyle JA, Dawson TE, Donoghue MJ.** 2004. Dark and disturbed: a new image of early angiosperm ecology. *Paleobiology* **30**, 82–107.
- Feild TS, Brodribb TJ, Iglesias A, Chatelet DS, Baresch A, Upchurch GR, Gomez B, Mohr BAR, Coiffard C, Kvacek J, Jaramillo C.** 2011a. Fossil evidence for Cretaceous escalation in angiosperm leaf vein evolution. *Proceedings of the National Academy of Sciences, USA* **108**, 8363–8366.
- Feild TS, Chatelet DS, Brodribb TJ.** 2009. Ancestral xerophobia: a hypothesis on the whole plant ecophysiology of early angiosperms. *Geobiology* **7**, 237–264.
- Feild TS, Upchurch GR Jr, Chatelet DS, Brodribb TJ, Grubbs KC, Samain M-S, Wanke S.** 2011b. Fossil evidence for low gas exchange capacities for Early Cretaceous angiosperm leaves. *Paleobiology* **37**, 195–213.
- Ferrio JP, Pou A, Florez-Sarasa I, Gessler A, Kodama N, Flexas J, Ribas-Carbo M.** 2012. The Pecllet effect on leaf water enrichment correlates with leaf hydraulic conductance and mesophyll conductance for CO₂. *Plant, Cell and Environment* **35**, 611–625.
- Field CB, Mooney HA.** 1986. The photosynthesis–nitrogen relationship in wild plants. In: TJ Givnish, ed, *On the economy of plant form and function*. Cambridge, UK: Cambridge University Press. pp. 25–55.
- Flexas J, Barbour MM, Brendel O, et al.** 2012. Mesophyll diffusion conductance to CO₂: an unappreciated central player in photosynthesis. *Plant Science* **193**, 70–84.
- Flexas J, Scoffoni C, Gago J, Sack L.** 2013. Leaf mesophyll conductance and leaf hydraulic conductance: an introduction to their measurement and coordination. *Journal of Experimental Botany* **64**, 3965–3981.
- Franks PJ.** 2004. Stomatal control and hydraulic conductance, with special reference to tall trees. *Tree Physiology* **24**, 865–878.
- Franks PJ, Farquhar GD.** 2007. The mechanical diversity of stomata and its significance in gas exchange control. *Plant Physiology* **143**, 78–87.
- Fu Q, Cheng L, Guo Y, Turgeon R.** 2011. Phloem loading strategies and water relations in trees and herbaceous plants. *Plant Physiology* **157**, 1518–1527.
- Garnier E, Laurent G.** 1994. Leaf anatomy, specific mass and water content in congeneric annual and perennial grass species. *New Phytologist* **128**, 725–736.
- Gilliham M, Dayod M, Hocking BJ, Xu B, Conn SJ, Kaiser BN, Leigh RA, Tyerman SD.** 2011. Calcium delivery and storage in plant leaves: exploring the link with water flow. *Journal of Experimental Botany* **62**, 2233–2250.
- Girardin P, Tollenaar M, Muldoon JF.** 1985. Effect of temporary N starvation on leaf photosynthetic rate and chlorophyll content of maize. *Canadian Journal of Plant Science* **65**, 491–500.
- Givnish TJ.** 1987. Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. *New Phytologist* **106** (Suppl), 131–160.
- Givnish TJ, Pires JC, Graham SW, et al.** 2005. Repeated evolution of net venation and fleshy fruits among monocots in shaded habitats confirms *a priori* predictions: evidence from an *ndhF* phylogeny. *Proceedings of the Royal Society B* **272**, 1481–1490.
- Grubb PJ.** 1998. A reassessment of the strategies of plants which cope with shortages of resources. *Perspectives in Plant Ecology, Evolution and Systematics* **1**, 3–31.
- Guyot G, Scoffoni C, Sack L.** 2012. Combined impacts of irradiance and dehydration on leaf hydraulic conductance: insights into vulnerability and stomatal control. *Plant, Cell and Environment* **35**, 857–871.
- Hao GY, Sack L, Wang AY, Cao KF, Goldstein G.** 2010. Differentiation of leaf water flux and drought tolerance traits in hemiepiphytic and non-hemiepiphytic *Ficus* tree species. *Functional Ecology* **24**, 731–740.
- Heberling JM, Fridley JD.** 2012. Biogeographic constraints on the world-wide leaf economics spectrum. *Global Ecology and Biogeography* **21**, 1137–1146.
- Hubbard RM, Ryan MG, Stiller V, Sperry JS.** 2001. Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant, Cell and Environment* **24**, 113–121.
- Jasienski M, Bazzaz FA.** 1999. The fallacy of ratios and the testability of models in biology. *Oikos* **84**, 321–326.
- John GP, Scoffoni C, Sack L.** 2013. Allometry of cells and tissues within leaves. *American Journal of Botany*.

- Jordan GJ.** 2011. A critical framework for the assessment of biological palaeoproxies: predicting past climate and levels of atmospheric CO₂ from fossil leaves. *New Phytologist* **192**, 29–44.
- Kattge J, Diaz S, Lavorel S, et al.** 2011. TRY—a global database of plant traits. *Global Change Biology* **17**, 2905–2935.
- Kerton M, Newbury HJ, Hand D, Pritchard J.** 2009. Accumulation of calcium in the centre of leaves of coriander (*Coriandrum sativum* L.) is due to an uncoupling of water and ion transport. *Journal of Experimental Botany* **60**, 227–235.
- Lambers H, Poorter H.** 1992. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Advances in Ecological Research* **23**, 187–261.
- Lloyd J, Bloomfield K, Deomingues TF, Farquhar GD.** 2013. Photosynthetically relevant foliar traits correlating better on a mass vs an area basis: of ecophysiological relevance or just a case of mathematical imperatives and statistical quicksand? *New Phytologist* **199**, 311–321.
- Manuel Perez-Perez J, Rubio-Diaz S, Dhondt S, Hernandez-Romero D, Sanchez-Soriano J, Beemster GTS, Rosa Ponce M, Micol JL.** 2011. Whole organ, venation and epidermal cell morphological variations are correlated in the leaves of *Arabidopsis* mutants. *Plant, Cell and Environment* **34**, 2200–2211.
- Mason CM, McGaughey SE, Donovan LA.** 2013. Ontogeny strongly and differentially alters leaf economic and other key traits in three diverse *Helianthus* species. *Journal of Experimental Botany* **64**, 4091–4101.
- McGill BJ, Enquist BJ, Weiher E, Westoby M.** 2006. Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution* **21**, 178–185.
- McKown AD, Cochard H, Sack L.** 2010. Decoding leaf hydraulics with a spatially explicit model: principles of venation architecture and implications for its evolution. *American Naturalist* **175**, 447–460.
- Meidner H.** 1983. Our understanding of plant water relations. *Journal of Experimental Botany* **34**, 1606–1618.
- Méndez-Alonzo R, Ewers FW, Sack L.** 2013. Ecological variation in leaf biomechanics and its scaling with tissue structure across three mediterranean-climate plant communities *Functional Ecology* **27**, 544–554.
- Milla R, Reich PB.** 2007. The scaling of leaf area and mass: the cost of light interception increases with leaf size. *Proceedings of the Royal Society B* **274**, 2109–2114.
- Murphy MRC, Jordan GJ, Brodribb TJ.** 2012. Differential leaf expansion can enable hydraulic acclimation to sun and shade. *Plant, Cell and Environment* **35**, 1407–1418.
- Nardini A, Peda G, La Rocca N.** 2012. Trade-offs between leaf hydraulic capacity and drought vulnerability: morpho-anatomical bases, carbon costs and ecological consequences. *New Phytologist* **196**, 788–798.
- Nee S, Colegrave N, West SA, Grafen A.** 2005. The illusion of invariant quantities in life histories. *Science* **309**, 1236–1239.
- Niinemets Ü.** 1999a. Components of leaf dry mass per area—thickness and density—alter leaf photosynthetic capacity in reverse directions in woody plants. *New Phytologist* **144**, 35–47.
- Niinemets Ü.** 1999b. Research review. Components of leaf dry mass per area—thickness and density—alter leaf photosynthetic capacity in reverse directions in woody plants. *New Phytologist* **144**, 35–47.
- Niinemets Ü, Kull O, Tenhunen JD.** 2004. Within-canopy variation in the rate of development of photosynthetic capacity is proportional to integrated quantum flux density in temperate deciduous trees. *Plant, Cell and Environment* **27**, 293–313.
- Niinemets Ü, Portsmouth A, Tena D, Tobias M, Matesanz S, Valladares F.** 2007a. Do we underestimate the importance of leaf size in plant economics? Disproportional scaling of support costs within the spectrum of leaf physiognomy. *Annals of Botany* **100**, 283–303.
- Niinemets Ü, Portsmouth A, Tobias M.** 2007b. Leaf shape and venation pattern alter the support investments within leaf lamina in temperate species: a neglected source of leaf physiological differentiation? *Functional Ecology* **21**, 28–40.
- Niinemets Ü, Sack L.** 2006. Structural determinants of leaf light harvesting capacity and photosynthetic potentials. *Progress in Botany* **67**, 385–419.
- Nikinmaa E, Holttä T, Hari P, Kolari P, Makela A, Sevanto S, Vesala T.** 2013. Assimilate transport in phloem sets conditions for leaf gas exchange. *Plant, Cell and Environment* **36**, 655–669.
- Niklas KJ, Cobb ED, Niinemets U, Reich PB, Sellin A, Shipley B, Wright IJ.** 2007. ‘Diminishing returns’ in the scaling of functional leaf traits across and within species groups. *Proceedings of the National Academy of Sciences, USA* **104**, 8891–8896.
- Noblin X, Mahadevan L, Coomaraswamy IA, Weitz DA, Holbrook NM, Zwieniecki MA.** 2008. Optimal vein density in artificial and real leaves. *Proceedings of the National Academy of Sciences, USA* **105**, 9140–9144.
- Onoda Y, Westoby M, Adler PB, et al.** 2011. Global patterns of leaf mechanical properties. *Ecology Letters* **14**, 301–312.
- Osnas JLD, Lichstein JW, Reich PB, Pacala SW.** 2013. Global leaf trait relationships: mass, area, and the leaf economics spectrum. *Science* **340**, 741–744.
- Peak D, Mott KA.** 2011. A new, vapour-phase mechanism for stomatal responses to humidity and temperature. *Plant, Cell and Environment* **34**, 162–178.
- Peppe DJ, Royer DL, Cariglino B, et al.** 2011. Sensitivity of leaf size and shape to climate: global patterns and paleoclimatic applications. *New Phytologist* **190**, 724–739.
- Perez-Harguindeguy N, Diaz S, Garnier E, et al.** 2013. New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* **61**, 167–234.
- Philpott J.** 1953. A blade tissue study of leaves of 47 species of *Ficus*. *Botanical Gazette* **115**, 15–35.
- Philpott J.** 1956. Blade tissue organization of foliage leaves of some Carolina shrub-bog species as compared with their Appalachian mountain affinities. *Botanical Gazette* **118**, 88–105.
- Poorter H, Niinemets U, Poorter L, Wright IJ, Villar R.** 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist* **182**, 565–588.
- Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L.** 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist* **193**, 30–50.

- Price CA, Enquist BJ.** 2007. Scaling mass and morphology in leaves: an extension of the WBE model. *Ecology* **88**, 1132–1141.
- Pyankov VI, Kondratchuk AV, Shipley B.** 1999. Leaf structure and specific leaf mass: the alpine desert plants of the Eastern Pamirs, Tadjikistan. *New Phytologist* **143**, 131–142.
- Quero JL, Villar R, Maranon T, Zamora R.** 2006. Interactions of drought and shade effects on seedlings of four *Quercus* species: physiological and structural leaf responses. *New Phytologist* **170**, 819–833.
- Reich PB, Walters MB, Ellsworth DS.** 1997. From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences, USA* **94**, 13730–13734.
- Reich PB, Wright IJ, Lusk CH.** 2007. Predicting leaf physiology from simple plant and climate attributes: a global GLOPNET analysis. *Ecological Applications* **17**, 1982–1988.
- Roderick ML, Berry SL, Noble IR, Farquhar GD.** 1999. A theoretical approach to linking the composition and morphology with the function of leaves. *Functional Ecology* **13**, 683–695.
- Royer DL, Miller IM, Peppe DJ, Hickey LJ.** 2010. Leaf economic traits from fossils support a weedy habit for early angiosperms. *American Journal of Botany* **97**, 438–445.
- Sack L, Cowan PD, Jaikumar N, Holbrook NM.** 2003. The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species. *Plant, Cell and Environment* **26**, 1343–1356.
- Sack L, Dietrich EM, Streeter CM, Sanchez-Gomez D, Holbrook NM.** 2008. Leaf palmate venation and vascular redundancy confer tolerance of hydraulic disruption. *Proceedings of the National Academy of Sciences, USA* **105**, 1567–1572.
- Sack L, Frole K.** 2006. Leaf structural diversity is related to hydraulic capacity in tropical rain forest trees. *Ecology* **87**, 483–491.
- Sack L, Holbrook NM.** 2006. Leaf hydraulics. *Annual Review of Plant Biology* **57**, 361–381.
- Sack L, Scoffoni C.** 2013. Leaf venation: structure, function, development, evolution, ecology and applications in the past, present and future. *New Phytologist* **198**, 983–1000.
- Sack L, Scoffoni C, McKown AD, Frole K, Rawls M, Havran JC, Tran H, Tran T.** 2012. Developmentally based scaling of leaf venation architecture explains global ecological patterns. *Nature Communications* **3**, 837.
- Sack L, Streeter CM, Holbrook NM.** 2004. Hydraulic analysis of water flow through leaves of sugar maple and red oak. *Plant Physiology* **134**, 1824–1833.
- Sack L, Tyree MT.** 2005. Leaf hydraulics and its implications in plant structure and function. In: NM Holbrook, MA Zwieniecki, eds, *Vascular transport in plants*. Oxford: Elsevier/Academic Press.
- Sack L, Tyree MT, Holbrook NM.** 2005. Leaf hydraulic architecture correlates with regeneration irradiance in tropical rainforest trees. *New Phytologist* **167**, 403–413.
- Saltelli A, Chan K, Scott EM.** 2009. *Sensitivity analysis*. New York: Wiley.
- Scoffoni C, McKown AD, Rawls M, Sack L.** 2012. Dynamics of leaf hydraulic conductance with water status: quantification and analysis of species differences under steady-state. *Journal of Experimental Botany* **63**, 643–658.
- Scoffoni C, Rawls M, McKown A, Cochard H, Sack L.** 2011. Decline of leaf hydraulic conductance with dehydration: relationship to leaf size and venation architecture. *Plant Physiology* **156**, 832–843.
- Shabala S, Schimanski LJ, Koutoulis A.** 2002. Heterogeneity in bean leaf mesophyll tissue and ion flux profiles: leaf electrophysiological characteristics correlate with the anatomical structure. *Annals of Botany* **89**, 221–226.
- Sheriff DW.** 1977. Evaporation sites and distillation in leaves. *Annals of Botany* **41**, 1081–and.
- Shipley B, Lechowicz MJ, Wright I, Reich PB.** 2006. Fundamental trade-offs generating the worldwide leaf economics spectrum. *Ecology* **87**, 535–541.
- Simonin KA, Limm EB, Dawson TE.** 2012. Hydraulic conductance of leaves correlates with leaf lifespan: implications for lifetime carbon gain. *New Phytologist* **193**, 939–947.
- Small E.** 1972. Photosynthetic rates in relation to nitrogen recycling as an adaptation to nutrient deficiency in peat bog plants. *Canadian Journal of Botany* **50**, 2227–2233.
- Sommerville KE, Sack L, Ball MC.** 2012. Hydraulic conductance of *Acacia phyllodes* (foliage) is driven by primary nerve (vein) conductance and density. *Plant, Cell and Environment* **35**, 158–168.
- RDC Team.** 2007. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Tyree MT, Zimmermann MH.** 2002. *Xylem structure and the ascent of sap*. Berlin: Springer.
- Uhl D, Mosbrugger V.** 1999. Leaf venation density as a climate and environmental proxy: a critical review and new data. *Palaeogeography Palaeoclimatology Palaeoecology* **149**, 15–26.
- Van Arendonk JJCM, Poorter H.** 1994. The chemical composition and anatomical structure of leaves of grass species differing in relative growth rate. *Plant, Cell and Environment* **17**, 963–970.
- Villar R, Maranon T, Quero JL, Panadero P, Arenas F, Lambers H.** 2005. Variation in relative growth rate of 20 *Aegilops* species (Poaceae) in the field: the importance of net assimilation rate or specific leaf area depends on the time scale. *Plant and Soil* **272**, 11–27.
- Villar R, Veneklaas EJ, Jordano P, Lambers H.** 1998. Relative growth rate and biomass allocation in 20 *Aegilops* (Poaceae) species. *New Phytologist* **140**, 425–437.
- Violle C, Navas M-L, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E.** 2007. Let the concept of trait be functional! *Oikos* **116**, 882–892.
- Walls RL.** 2011. Angiosperm leaf vein patterns are linked to leaf functions in a global scale data set. *American Journal of Botany* **98**, 244–253.
- Walters MB, Reich PB.** 1999. Low-light carbon balance and shade tolerance in the seedlings of woody plants: do winter deciduous and broad-leaved evergreen species differ? *New Phytologist* **143**, 143–154.
- Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ.** 2002. Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics* **33**, 125–159.

- Westoby M, Reich PB, Wright IJ.** 2013. Understanding ecological variation across species: area-based vs mass-based expression of leaf traits. *New Phytologist* **199**, 322–323.
- Witkowski ETF, Lamont BB.** 1991. Leaf specific mass confounds leaf density and thickness. *Oecologia* **88**, 486–493.
- Wright IJ, Reich PB, Cornelissen JHC, et al.** 2005a. Assessing the generality of global leaf trait relationships. *New Phytologist* **166**, 485–496.
- Wright IJ, Reich PB, Cornelissen JHC, et al.** 2005b. Modulation of leaf economic traits and trait relationships by climate. *Global Ecology and Biogeography* **14**, 411–421.
- Wright IJ, Reich PB, Westoby M, et al.** 2004. The worldwide leaf economics spectrum. *Nature* **428**, 821–827.
- Wright IJ, Westoby M.** 2002. Leaves at low versus high rainfall: coordination of structure, lifespan and physiology. *New Phytologist* **155**, 403–416.
- Wylie RB.** 1939. Relations between tissue organization and vein distribution in dicotyledon leaves. *American Journal of Botany* **26**, 219–225.
- Wylie RB.** 1946. Relations between tissue organization and vascularization in leaves of certain tropical and subtropical dicotyledons. *American Journal of Botany* **33**, 721–726.
- Wylie RB.** 1951. Principles of foliar organization shown by sun-shade leaves from ten species of deciduous dicotyledonous trees. *American Journal of Botany* **38**, 355–361.
- Wylie RB.** 1952. The bundle sheath extension in leaves of dicotyledons. *American Journal of Botany* **39**, 645–651.
- Wylie RB.** 1954. Leaf organization of some woody dicotyledons from New Zealand. *American Journal of Botany* **41**, 186–191.
- Zhang S-B, Guan Z-J, Sun M, Zhang J-J, Cao K-F, Hu H.** 2012. Evolutionary association of stomatal traits with leaf vein density in *Paphiopedilum*, Orchidaceae. *PLoS one* **7**, e40080.