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

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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 lightsaturated 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 ($A_{\text{mass}}$), and higher nitrogen concentration per mass ($N_{\text{mass}}$) and respiration rate per mass ($R_{\text{mass}}$), but shorter leaf lifespan ($L_{L}$), 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 (Lammers 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 $A_{\text{mass}}$ 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 ‘venin origin’ hypothesis.

This review critically examines the ‘venin origin’ hypothesis by evaluating its assumptions, derivations, and predictions. Because this evaluation did not support the ‘venin 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 ($K_{\text{leaf}}$) and $A_{\text{area}}$, (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 $A_{\text{mass}}$ 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

<table>
<thead>
<tr>
<th>Trait, variable, or feature</th>
<th>Symbol</th>
<th>Typical unit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leaf venation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total vein length per leaf area</td>
<td>VLA (or σ)</td>
<td>mm mm–2</td>
</tr>
<tr>
<td>Major vein length per leaf area</td>
<td>Major VLA</td>
<td>mm mm–2</td>
</tr>
<tr>
<td>Minor vein length per leaf area</td>
<td>Minor VLA</td>
<td>mm mm–2</td>
</tr>
<tr>
<td>Intervenial distance</td>
<td>l/VD (or d)</td>
<td>μm</td>
</tr>
<tr>
<td>Vein-to-epidermal distance</td>
<td>VED</td>
<td>μm</td>
</tr>
<tr>
<td>Number of vein areoles per leaf area</td>
<td>VAA (or ξ)</td>
<td>n mm–2</td>
</tr>
<tr>
<td>Maximum distance from vein to stomata</td>
<td>Dm</td>
<td>μm</td>
</tr>
<tr>
<td>Total vein length per mass</td>
<td>VLM</td>
<td>m g–1</td>
</tr>
<tr>
<td>Vein radius</td>
<td>r_v</td>
<td>μm</td>
</tr>
<tr>
<td>Free-ending veins</td>
<td>FEVs</td>
<td></td>
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<tr>
<td>Bundle sheath</td>
<td>BS</td>
<td></td>
</tr>
<tr>
<td>Bundle sheath extensions</td>
<td>BSEs</td>
<td></td>
</tr>
<tr>
<td>Mesophyll pathways for water flow</td>
<td>MPs</td>
<td></td>
</tr>
<tr>
<td><strong>Leaf structure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf thickness</td>
<td>LT</td>
<td>μm</td>
</tr>
<tr>
<td>Leaf mass density</td>
<td>LD</td>
<td>g cm–3</td>
</tr>
<tr>
<td><strong>Leaf economics spectrum and leaf composition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf mass per area</td>
<td>LMA</td>
<td>g m–2</td>
</tr>
<tr>
<td>Mass density of vein tissue</td>
<td>ρ_v</td>
<td>g cm–3</td>
</tr>
<tr>
<td>Mass density of non-vein leaf tissue</td>
<td>ρ_L</td>
<td>g cm–3</td>
</tr>
<tr>
<td>Light-saturated photosynthetic rate per leaf area</td>
<td>A_area</td>
<td>μmol m–2 s–1</td>
</tr>
<tr>
<td>Light-saturated photosynthetic rate per leaf mass</td>
<td>A_mass</td>
<td>mmol g–1 s–1</td>
</tr>
<tr>
<td>Nitrogen concentration per leaf area</td>
<td>N_area</td>
<td>g m–2</td>
</tr>
<tr>
<td>Nitrogen concentration per leaf mass</td>
<td>N_mass</td>
<td>g g–1</td>
</tr>
<tr>
<td>Leaf lifespan</td>
<td>LL</td>
<td>Months</td>
</tr>
<tr>
<td>Respiration rate per leaf area</td>
<td>R_area</td>
<td>μmol m–2 s–1</td>
</tr>
<tr>
<td>Respiration rate per leaf mass</td>
<td>R_mass</td>
<td>nmol g–1 s–1</td>
</tr>
<tr>
<td>Mesophyll conductance to CO₂ diffusion</td>
<td>g_m</td>
<td>mmol m–2 s–1</td>
</tr>
<tr>
<td><strong>Hydraulic physiology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf hydraulic conductance (leaf area basis)</td>
<td>K_leaf</td>
<td>mmol m–2 s–1 MPa–1</td>
</tr>
<tr>
<td>Leaf hydraulic conductance (leaf mass basis)</td>
<td>K_leaf,mass</td>
<td>mmol kg–1 s–1 MPa–1</td>
</tr>
<tr>
<td>Leaf xylem and outside-xylem hydraulic conductance</td>
<td>K_x and K_ox</td>
<td>mmol m–2 s–1 MPa–1</td>
</tr>
<tr>
<td><strong>Gas exchange physiology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant hydraulic conductance</td>
<td>K_plant</td>
<td>mmol m–2 s–1 MPa–1</td>
</tr>
<tr>
<td>Leaf water potential</td>
<td>Ψ_leaf</td>
<td>MPa</td>
</tr>
<tr>
<td>Leaf-to-soil water potential gradient</td>
<td>ΑΨ_leaf-to-soil</td>
<td>MPa</td>
</tr>
<tr>
<td><strong>Stomatal anatomy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatal density</td>
<td>n_s, SD</td>
<td>n mm–2</td>
</tr>
<tr>
<td>Area of stomatal pore</td>
<td>a_s, SS</td>
<td>μm²</td>
</tr>
<tr>
<td>Stomatal pore thickness</td>
<td>t_s</td>
<td>μm</td>
</tr>
<tr>
<td>Stomatal pore area index</td>
<td>SPI</td>
<td>%</td>
</tr>
<tr>
<td>Stomatal index</td>
<td>SI</td>
<td>cells cell–1</td>
</tr>
<tr>
<td><strong>Whole plant growth and biomass allocation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf mass fraction</td>
<td>LMF</td>
<td>g g–1</td>
</tr>
<tr>
<td>Leaf area ratio</td>
<td>LAR</td>
<td>m² g–1</td>
</tr>
<tr>
<td>Unit leaf rate</td>
<td>ULR</td>
<td>g m–2 day–1</td>
</tr>
</tbody>
</table>

(Continued)
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 $K_{\text{leaf}}$, 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...
Influence of vein length per area on the leaf economic spectrum

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

![Image of a diagram illustrating the influence of vein traits on hydraulic, stomatal, and photosynthetic traits and whole-plant relative growth rate.](https://academic.oup.com/jxb/article-abstract/64/13/4053/438787)
Table 2. Comparison of sampling strategies to test correlations between traits, such as between VLA and other flux-related traits

<table>
<thead>
<tr>
<th>Sampling strategy (from broadest to narrowest scale)</th>
<th>Power and significance of strategy</th>
<th>Weakness and ambiguity of strategy</th>
<th>Correlation between VLA and other flux traits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Across diverse species (across multiple communities, across biomes, or a compiled global dataset)</td>
<td>High variation in all traits; generality and broad importance; potential ability to compare adaptation in different habitats</td>
<td>Correlations between any given traits may be affected by other trait variation and influenced by the wide range of phylogeny and habitats sampled</td>
<td>Weak for ( A_{\text{area}} ) (Blonder et al., 2011; Walls, 2011) No for ( K_{\text{act}} ) (Scoffoni et al., 2011)</td>
</tr>
<tr>
<td>Across diverse species within a given community</td>
<td>High variation in all traits; potential ability to compare adaptation in different habitats within the community</td>
<td>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</td>
<td>Yes for ( K_{\text{act}} ) * (Sack and Frolle, 2006)</td>
</tr>
<tr>
<td>Across a range of lineages, including a few species within each lineage</td>
<td>High variation in all traits; insight into contrasting biology of different lineages</td>
<td>Potentially confounding other factors that influence trait correlations across lineages, including sun/shade adaptation or other trait variation that would influence the correlation</td>
<td>Yes for ( K_{\text{act}} ), ( A_{\text{area}} ), and ( g_s ) (Brodribb et al., 2007; Boyce et al., 2009; Brodribb et al., 2010)</td>
</tr>
<tr>
<td>Across diverse species within a given lineage (or varieties or populations within a species)</td>
<td>High variation in certain traits; variation among given traits may be easily linked with variation in growth form or adaptive radiation across environments</td>
<td>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</td>
<td>Yes for stomatal pore area per leaf area (Dunbar-Co et al., 2009) Yes for ( A_{\text{area}} ) and ( g_s ) * (Feild et al., 2011b) No for ( K_{\text{act}} ) (Sommerville et al., 2012)</td>
</tr>
<tr>
<td>Across genotypes within a species</td>
<td>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)</td>
<td>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</td>
<td>Yes for ( n_s ) (Manuel Perez-Perez et al., 2011)</td>
</tr>
<tr>
<td>Across plants of a given species grown in different treatments or of different populations across environments, or across leaf types within a species</td>
<td>Gain insight into the way that individual and clusters of traits lead to plant adaptation and optimization to habitat or climate</td>
<td>Difficult to show that trait relationships are general, as they may have evolved or developed as species-specific responses to contrasting environments</td>
<td>Yes for ( A_{\text{area}} ), ( K_{\text{act}} ), ( g_s ), ( n_s ) (Brodribb and Jordan, 2011; Murphy et al., 2012)</td>
</tr>
</tbody>
</table>

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 \( A_{\text{area}} \), \( N_{\text{area}} \), and \( R_{\text{area}} \), leading to a correlation of these area-based traits with \( LMA \) and also driving positive relationships among those traits, strengthened by their biochemical interdependence (Nüenemets, 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 \( A_{\text{mass}} \), \( N_{\text{mass}} \), and \( R_{\text{mass}} \) would 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 $A_{\text{mass}}$; this will occur even if $A_{\text{area}}$ were completely random. Such a negative relationship of $A_{\text{mass}}$ 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 veination-mediated economic strategies’ (Blonder et al., 2011). According to this hypothesis, LMA, $A_{\text{mass}}$, $N_{\text{mass}}$, and LL are directly and mechanistically determined by VLA and two other traits, the interveinal 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 $\sigma$, $d$, and $\xi$, 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 ‘mechanically 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 (Philppott, 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 Frolo, 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.

<table>
<thead>
<tr>
<th>Row</th>
<th>Assertion</th>
<th>Traits</th>
<th>Dataset, r-value, P-value (number of species)</th>
<th>Supported?</th>
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| 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) (Ficus 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):  
Acer: VLA and LMA; r=0.40, P=0.023 (32)  
Helianthus, Quercus, Hawaiian Plantago and Viola: 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) (Ficus 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 in 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_{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 independendly 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 Helianthus when mean annual precipitation is partialled out |
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 only 17±0.2% of the total vein volume per leaf area and 2±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 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’).

**Table 3.** Continued

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<th>Traits</th>
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<td>( LL ) and ( VLA ), partialling out ( LMA ): ( r_{\text{partial}}=-0.62, P&lt;0.05 ) (14)</td>
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<td>( LL ) and ( IVD ), partialling out ( LMA ): ( r_{\text{partial}}=0.61, P&lt;0.05 ) (14)</td>
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<td>Heilanthus: ( LL ) and ( VLA ): ( r=-0.90, P=0.002 ) (8)</td>
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<td>( LL ) and ( LMA ): ( r=-0.79, P=0.020 ) (8)</td>
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<td>( LL ) and ( VLA ), partialling out ( LMA ): ( r_{\text{partial}}=-0.85, P=0.02 ) (8)</td>
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<td>( LL ) and ( VLA ), partialling out ( MAP ): ( r_{\text{partial}}=-0.24, P=0.31 ) (8)</td>
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**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 \( K_{\text{leaf}} \) 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 \( K_{\text{leaf}} \). 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. Chatellet, 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.
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 $K_{sel}$?’). 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 $IVD$ (Brodribb 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 $VLD$ 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; Brodribb 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
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 $LMA$, $LL$, $A_{\text{mass}}$, and $N_{\text{mass}}$ 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 $A_{\text{mass}}$, 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 $A_{\text{mass}}$ 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

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**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.
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\textsubscript{mass}, and A\textsubscript{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\textsubscript{mass}, and N\textsubscript{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 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.
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):

\[
K_{leaf} = \left( K_x^{-1} + K_{ox}^{-1} \right)^{-1} \quad \text{Equation 1}
\]

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 $K_{\text{leaf}}$?). Consistent with this influence of $VLA$ on leaf hydraulic capacity, across tropical rainforest angiosperm tree species, $K_{\text{sa}}$ and $K_{\text{leaf}}$ correlated with $VLA$ (Sack and Frake, 2006), and $K_{\text{leaf}}$ 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 $K_{\text{leaf}}$ (Fig. 2), and in several species sets, no relationship was observed between $K_{\text{leaf}}$ 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 $K_{\text{leaf}}$ 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 $K_{\text{leaf}}$ scales up to the whole plant hydraulic conductance ($K_{\text{plant}}$). According to the Ohm’s law analogy, $K_{\text{plant}}$ determines leaf water potential ($\Psi_{\text{leaf}}$) 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 $\Psi_{\text{soil}}$ 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 ($g_{\text{s}}$) under moderate wind speeds, when the boundary layer conductance ($g_{\text{b}}$) is sufficiently high:

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

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

The $g_{\text{s}}$ is a function of the maximum stomatal conductance ($g_{\text{max}}$) and the degree that stomata close as $\Psi_{\text{leaf}}$ declines. The $g_{\text{max}}$ 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 $g_{\text{s}}$ 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, $g_{\text{s}}$ does not depend directly on bulk $\Psi_{\text{leaf}}$ but rather on the water potential at or near the guard cells or epidermis ($\Psi_{\text{gce}}$), 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 $g_{\text{s}}$ to humidity near the stomatal pore (Peak and Mott, 2011).

Ultimately, the $g_{\text{s}}$, influenced by the vein and hydraulic traits as described above, is a strong determinant of photosynthetic rate per leaf area ($A_{\text{area}}$). The $A_{\text{area}}$ depends on the chloroplastic CO$_2$ concentration ($C_s$) and biochemical parameters including the electron transport rate ($J_{\text{max}}$) and the maximum carboxylation capacity of Rubisco ($V_{\text{max}}$), and, at a given ambient CO$_2$ concentration ($C_a$), $C_s$ is dynamically determined by $g_{\text{s}}$ and mesophyll conductance ($g_{\text{m}}$; 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 ($N_{\text{area}}$), as does respiration rate ($R_{\text{area}}$) (Evans et al., 2000). Shifts in these parameters can thus alter the coordination among $A_{\text{area}}$ and hydraulic and venation traits.

Declining water potentials throughout the plant system lead to reductions of $K_s$ and $K_{\text{sa}}$ 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 $g_{\text{s}}$ (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 $\text{LMA}$, 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 ($N_{\text{mass}}, R_{\text{mass}}$ and $A_{\text{mass}}$, respectively). Additionally, mechanically, the $\text{LMA}$ tends to increase leaf lifespan ($L_L$; Wright et al., 2004). The linkages among area-based and mass-based traits that are interconvertible by $\text{LMA}$ 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. $A_{\text{area}}$ 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 $\text{LMA}$ (Evans, 1972; Poorter et al., 2009, 2012).
Supporting this synthesis of the importance of vein traits in determining $K_{\text{leaf}}$ and its importance in determining $g_*$ and $A_{\text{area}}$, 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 $K_{\text{leaf}}$ 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 $g_*$ with $K_{\text{leaf}}$ across diverse species (Feild et al., 2011b), or of $g_*$ 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 Frolé, 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) has also developed further, as the coordination arises due to genetic and developmental linkages 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 stomatal traits 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 $\Psi_{\text{leaf}}$, $\Psi_{\text{soil}}$, 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 $g_*$ relative to $K_{\text{leaf}}$ 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 $A_{\text{area}}$ depends not only on $g_*$, but also on $J_{\text{max}}$ and $V_{\text{max}}$, which are related to nitrogen allocation, the modulation of these parameters can shift the coordination of $A_{\text{area}}$ 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 $A_{\text{area}}$ 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 $A_{\text{area}}$ (Fu et al., 2011; Nikinmaa et al., 2013) and that, in some species, $VLA$ may provide additional phloem delivery capacity and increase $A_{\text{area}}$, 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 $A_{\text{area}}$, 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 $K_{\text{leaf}}$?**

According to our conceptual model, the $VLA$ affects higher-level traits via its influence on $K_{\text{leaf}}$. 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 $K_{\text{ox}}$ because it corresponds to a larger number of xylem flow pathways in parallel. A higher $VLA$ can also increase $K_{\text{ox}}$ 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 $K_{\text{ox}}$ 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 characterizations would also influence the flow pathway. For example, all else being equal, a thicker leaf would have a higher $K_{\text{ox}}$, 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 $K_{\text{ox}}$, as water would travel a longer distance. In one formulation of the anatomical basis for water flow pathways, assuming water evaporation near the stomata, $K_{\text{ox}}$ should relate negatively to the mean distance that water moves across the mesophyll from the vein to the epidermis ($D_{\text{ox}}$), 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 $D_{\text{ox}}$ as a proxy for $K_{\text{ox}}$ or $K_{\text{leaf}}$ and as a primary mechanism for the influence of $VLA$ on $A_{\text{area}}$ is a valuable working hypothesis, proposed in one study which found strong negative correlation of both $K_{\text{leaf}}$ and $A_{\text{area}}$ with $D_{\text{ox}}$ 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’ subverting 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 Brodribb 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 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 (Brodribb and Jordan, 2011).

Indeed, the exact influence of $VLA$ on $K_{leaf}$ 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; Brodribb 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

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**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±SE, 82±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±SE, 18±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 LMA 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 \( P_{GRR} \). \( A_{mass} \) is the key trait to scale leaf gas exchange to whole plant carbon fluxes.

![Fig. 8. Importance of leaf vein length per area in determining](https://academic.oup.com/jxb/article-abstract/64/13/4053/438787/4069)

(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:

\[
(A) A_{area}=1.28/VLA-1.54; (B) A_{mass}=5.60/VLA^{1.33}.
\]

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

\[
A_{\text{mass}} = A_{\text{area}} / \text{LMA}
\]  
Equation 5

Analysis of our global database showed for the first time a correlation of \( A_{\text{mass}} \) with \( VLA \) (Fig. 8B). Notably, \( A_{\text{mass}} \) was driven by its negative correlation with \( \text{LMA} \) (Fig. 9A) and its positive correlation with \( A_{\text{area}} \) (\( R^2 \) 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 \( A_{\text{mass}} \) is calculated from those variables (Equation 5, also previously discussed in the section ‘Mechanistic trait linkages versus concerted convergence’). A model predicting \( A_{\text{mass}} \) from \( \text{LMA} \) and \( VLA \) thus could explain more of the variance than one based on \( VLA \) alone (Fig. 10). While the relationships of \( A_{\text{area}} \) and \( A_{\text{mass}} \) 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 \( K_{\text{leaf}} \), and species vary in the coordination of \( K_{\text{leaf}} \) with \( A_{\text{area}} \), 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 \( \Psi_{\text{leaf}} \) and thus provide the hydraulic conductance necessary for high \( A_{\text{area}} \) and thus high \( A_{\text{mass}} \).

The non-linearity of the relationships (i.e. saturation of the effect on \( A_{\text{area}} \) and \( A_{\text{mass}} \) 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 \((A_{\text{mass}})\) and leaf mass per area \((\text{LMA})\) 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 \( \text{LMA} \) (Fig. 3C), \( A_{\text{mass}} \) correlated positively with \( VLM \). Lines were fitted using standard major axes: (A) \( A_{\text{mass}} = 29.64 \text{LMA}^{-1.24} \); (B) \( A_{\text{mass}} = -0.92 \text{VLM} + 1.11 \). 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 \((A_{\text{mass}})\) in addition to leaf mass per area \((\text{LMA})\). (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 \( \log(\text{LMA}) \): \( \log(A_{\text{mass}}) = 3.52 - 0.722 \log(\text{LMA}) \) \((R^2=0.34, P<0.001; n=119)\). (b) \( \log(A_{\text{mass}}) \) data from a global database for angiosperm species plotted against values predicted from a model based on \( \log(\text{LMA}) \) and \( \log(\text{VLA}) \): \( \log(A_{\text{mass}}) = 3.10 - 0.727 \log(\text{LMA}) + 0.378 \log(\text{VLA}) \) \((R^2=0.42, P<0.001; n=119)\). 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 $VLA$, $K_{leaf}$, $n$, $SPI$, 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 (Ackery 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 $K_{leaf, mass} = K_{leaf}/LMA$ (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 $K_{leaf, mass}$ 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 Zwie Niecki, 2012). In some applications, $A_{area}$ has been reconstructed from single vein traits (i.e. $IVD$ or $VLA$; Brodribb and Feild, 2010; Boyce and Zwie Niecki, 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 et al., 2012).
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 causative 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 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.

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**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:

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

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

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

and

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

where \( r_v \), \( \rho_t \), and \( \rho_L \) 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 \( r_v \), \( \rho_t \), \( \rho_L \), \( a_s \), \( n_s \), 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,
\[ LMA = \pi r_v^2 VLA \left( \rho_v - \rho_L \right) + \frac{2 \rho_L IVD}{k_o} \]  

Equation 4

where \( r_v, \rho_v, \) and \( \rho_L \) 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 \( r_v, \rho_v, \) and \( \rho_L \), constants were used, based on the literature, of \( 2 \times 10^{-5} \) m, \( 1 \times 10^3 \) g m\(^{-3} \), and \( 3 \times 10^6 \) g m\(^{-3} \) respectively. By the definition of \( k_o \), the second term simplifies to \( LT \times \rho_L \), and thus

\[ LMA = \pi r_v^2 VLA \left( \rho_v - \rho_L \right) + \left( LT \times \rho_L \right) \]  

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 inaccurancy 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 4 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 \( LT \times \text{total leaf density} \)). If a constant is used for \( \rho_L \) 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.: 

\[ \delta LMA/\delta VLA = \pi r_v^2 \left( \rho_v - \rho_L \right) = \pi \times \left( 2 \times 10^{-5} \right) \] 

\[ \left( 1 \times 10^3 - 3 \times 10^3 \right) g m^{-3} = 8.8 \times 10^{-4} \]  

Equation 4b

\[ \delta LMA/\delta LT = \rho_L = 3 \times 10^5 \]  

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,

\[ A_{mass} = \frac{c_o \left( 1 - h \right) WUE}{\pi r_v^2 VLA \left( \rho_v - \rho_L \right) + \frac{2 \rho_L IVD}{k_o}} \times \frac{1}{\pi D \times VLA + \frac{a_o n_v t_O}{D}} \times \frac{1}{2 \log \frac{IVD}{k_o}} + \frac{a_o n_v}{t_O + \sqrt{\frac{a_o}{\pi}}} \]  

Equation 6

where \( c_o, h, WUE, D, a_o, n_v, \) and \( t_O \) 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 \( a_o, n_v, \) and \( t_O, \) 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,

\[ A_{mass} = \frac{WUE}{LMA} \times \frac{c_o \left( 1 - h \right)}{\frac{1}{\pi D \times VLA} + \frac{1}{D} \times \frac{a_o n_v}{t_O + \sqrt{\frac{a_o}{\pi}}}} \]  

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 \( C_d \left( 1 - h \right) g \) (leaf conductance to water vapour), with \( g \) then decomposed into \( g_o, g_x \) (xylem hydraulic conductance), and \( g_y \) (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 apoplastic, 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 $g_7$ 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 $g_s$ is negligible (an invalid assumption, given that $g_s$ 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 $g_s$ and $g_v$ should be of the same order (although in terms of diffusion conductances, $g_v$ 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.:

$$\delta A_{mass} / \delta VLA = \frac{2 \pi \times \ln(10) \times a^2 \pi^2 D \times (1 - h) \times (\ln(T) - \ln(t_r)) \times \ln(2)}{LMA \times \left[ \frac{\sqrt{\pi}}{\sqrt{2}} \times \left[ (\ln(T) - \ln(t_r)) - \ln(2) \right] \times VLA + \ln(10) \right]} \left[ \frac{n \times \frac{a \pi}{\sqrt{2}}}{ \ln(D)} \right]$$

Equation 6b

$$\delta A_{mass} / \delta LT = \frac{2 \pi \times \ln(10) \times a^2 \pi^2 D \times (1 - h) \times VLA \times WUE}{LMA \times T \times \left[ \frac{\sqrt{\pi}}{\sqrt{2}} \times \left[ (\ln(T) - \ln(t_r)) - \ln(2) \right] \times VLA + \ln(10) \right]} \left[ \frac{n \times \frac{a \pi}{\sqrt{2}}}{ \ln(D)} \right]$$

Equation 6c

$$\delta A_{mass} / \delta LMA = \frac{-c_5(1 - h) \times WUE}{LMA^2 \times \left[ \frac{2 \pi D \times (\ln(T) - \ln(t_r)) - \ln(2)}{\ln(10)} \right] + \left[ \frac{a \pi \times n}{\sqrt{2}} \right]}$$

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 ± SE for $\delta A_{mass} / \delta VLA$ was $2.49 \times 10^{-1} ± 3.16 \times 10^{-14}$, while that for $\delta A_{mass} / \delta T$ was $7.73 \times 10^{-5} ± 9.06 \times 10^{-8}$ and that for $\delta A_{mass} / \delta LMA$ was $-6.72 \times 10^{-1} ± 6.47 \times 10^{-10}$. Thus $\delta A_{mass} / \delta T >> \delta A_{mass} / \delta LMA >> \delta A_{mass} / \delta VLA$, 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 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

$$N_{mass} = k_2 A_{mass} + \frac{k_3}{k_0} \left( \frac{IVD - k_0 \pi r_v^2}{LMA \times \left( T - \pi r_v^2 \times VLA \right)} \right)$$

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 $k_o$ 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,

$$N_{mass} = k_2 A_{mass} + \frac{1}{LMA} \left( \frac{T - \pi r_v^2 \times VLA}{IVD} \right)$$

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.:

$$\delta N_{mass} / \delta A_{mass} = k_2 = 1 \times 10^3$$

Equation 7b

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

Equation 7c

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

Equation 7d

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

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±SE for \( \Delta LMA / \Delta VLA \) was \(-1.20 \times 10^{-8} \pm 1.09 \times 10^{-9} \), while that for \( \Delta N_{mass} / \Delta LMA \) was \(-2.48 \times 10^{-8} \pm 5.04 \times 10^{-6} \) and that for \( \Delta N_{mass} / \Delta VLA \) was 9.57±0.865. Thus \( \Delta N_{mass} / \Delta A_{mass} >> \Delta N_{mass} / \Delta T >> \Delta N_{mass} / \Delta LMA \) >> \( \Delta N_{mass} / \Delta VLA \), and in this scheme, \( N_{mass} \) is effectively a function of \( A_{mass} \), and \( T \), 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 \(-0.01 \) 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 0.11). 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 \( A_{mass} \)-\( LMA \) 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:

\[
A_{mass} = \frac{A_s}{(LT + 4VLA)^{2/3}} \times \frac{1}{LMA} \tag{Equation 2 in Blonder et al., 2013}
\]

The new term on the left was based on the assumption that photosynthesis per leaf area is equal to a constant \( A_s \) 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 \( g \)). 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 operational 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 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 as in sensitivity analysis as in $LMA$ writing an equation with sensitivity analysis 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.

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