ORIGINAL ARTICLE

Leaf vascular architecture in temperate dicotyledons: correlations and link to functional traits

Kiyosada Kawai[1,2] · Naoki Okada[3]

Abstract

*Main conclusion*s **Using 227 dicotyledonous species in temperate region, we found the relationships among densities of different-order veins, creating diversity of leaf vascular architectures.**

Abstract Dicotyledonous angiosperms commonly possess a hierarchical leaf vascular system, wherein veins of different orders have different functions. Minor vein spacing determines leaf hydraulic efficiency, whereas the major veins provide mechanical support. However, there is limited information on the coordination between these vein orders across species, limiting our understanding of how diversity in vein architecture is arrayed. We aimed to examine the (1) relationships between vein densities at two spatial scales (lower- vs. higher-order veins and among minor veins) and (2) relationships of vein densities with plant functional traits. We studied ten traits related to vein densities and three functional traits (leaf dry mass per area [LMA], leaf longevity [LL], and adult plant height [H_{adult}]) for 227 phylogenetically diverse plant species that occur in temperate regions and examined the vein–vein and vein–functional traits relationships across species. The densities of lower- and higher-order veins were positively correlated across species. The minor vein density was positively correlated with the densities of both areoles and free-ending veins, and vascular networks with higher minor vein density tended to have a lower ratio of free-ending veins to areoles across species. Neither densities of lower- nor higher-order veins were related to LMA and LL. On the other hand, the densities of veins and areoles tended to be positively correlated with H_{adult}. These results suggest that densities of different-order veins are developmentally coordinated across dicotyledonous angiosperms and form the independent axis in resource use strategies based on the leaf economics spectrum.

Keywords Areole · Free-ending vein · Leaf economics spectrum (LES) · Major vein · Minor vein · Phylogenetic signal

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Introduction

The leaf vasculature of dicotyledonous angiosperms is remarkably diverse across species and commonly possesses a hierarchal vein system, which consists of major (first- to third-order) and minor (higher than third-order) veins that have different functions (Hickey 1973; Roth-Nebelsick et al. 2001; Sack and Scoffoni 2013).

Minor veins are the major resistance in the xylem water pathway in the leaf, and their geometry strongly constrains the physiological properties of leaves (Sack and Scoffoni 2013). In particular, minor vein density (VD_{min}; or minor vein length per unit area) is positively correlated with leaf hydraulic conductance, stomatal conductance, and maximum photosynthetic capacity across a diverse range of species (Sack and Frole 2006; Brodribb et al. 2007; Kawai and Okada 2016; McElwain et al. 2016; Scoffoni et al. 2016).

Minor vein anatomy, such as the sizes of phloem loading cells and sieve elements, would determine the transport capacity of assimilates within leaves (Muller et al. 2014; Demmig-Adams et al. 2017). On the other hand, the abundance of major veins is related to leaf hydraulic vulnerability during drought in that increased major vein density may provide alternative pathways of water flow around embolised veins (Scoffoni et al. 2011). In addition, primary and secondary veins act as beams that support the weight of the leaf and provide resistance to mechanical loading (Niklas 1999; Kobayashi et al. 2000; Kawai and Okada 2016).

Despite a lot of documentation on the functions of different vein orders, the relationships among these vein traits are relatively unknown. This limits our understanding of how vein architecture is developmentally organized, and, thus, how the functions of veins are constrained across species. In the present study, we examined the relationships between vein densities at two spatial scales: (1) between lower- and higher-order veins and (2) among the minor veins. For the former, densities of lower- and higher-order veins would be determined independently of each other across species because of their different roles (e.g., Kawai and Okada 2016) and developmental mechanisms. The major veins first form during the 'slow' phase of leaf expansion, whereas the minor veins form later during the 'rapid' phase (Dengler and Kang 2001; Sack et al. 2012). Thus, major vein density is negatively correlated with leaf area, and minor-vein density can change independent of leaf area (Sack et al. 2012). For minor veins, we focused on densities of areoles (D_A) and free-ending veins (FEVs; D_{FEV}). Areoles are the smallest lamina area surrounded by veins, and FEVs are free-ending ultimate veins that often end within an areole (Ellis et al. 2009). Theoretically, a venation network with high D_{FEV}, a more open (less closed) venation, achieves the highest water supply rate for a given investment of veins (Banavar et al. 2000; Durand 2007; Corson 2010; Dodds 2010). On the other hand, a network in which veins reconnect with one another (high D_A) with low D_{FEV}, a more closed (less open) venation, will be selected when there is a risk of unstable water flow or damage by embolism or herbivory (Roth-Nebelsick et al. 2001; Durand 2006; Katifori et al. 2010). In addition, FEVs enable to reduce the distance between veins and stomata (Fiorin et al. 2016). However, variations in D_{FEV} and D_A and their relationships with leaf hydraulic conductance (indexed by VD_{min}) across many species remain unclear and limit our understanding of the interspecific variations in vascular transport strategies.

There has been some debate on how veins influence the leaf economics spectrum (LES; Wright et al. 2004), which represents the trade-off between photosynthetic capacity and longevity of leaves (Blonder et al. 2011; Sack et al. 2013). Although previous studies have empirically shown the independence of minor veins from LES traits (e.g., leaf dry mass

per area [LMA], leaf longevity [LL], and leaf nitrogen concentration) (Sack and Frole 2006; Li et al. 2015; Kawai and Okada 2016; Kawai et al. 2017 but see Kawai and Okada 2018), the relationships between major veins and LES traits have been rarely examined. Because veins contain costly lignin (Lambers and Poorter 1992) and large veins occupy a large volume within a leaf (Sack et al. 2012), the abundance of major veins is expected to influence leaf construction cost (LMA) (McKown et al. 2010; Walls 2011; Sack et al. 2013), and thus leaf longevity (Wright et al. 2004; Poorter et al. 2009). In addition, we examined the relationships between vein densities and adult plant height, which represents different dimension of plant adaptive strategies from LES, such as the ability of the plant to capture light and respond to disturbance (Westoby 1998; Díaz et al. 2015). Because hydraulic conductivity at terminal twig increases with plant height (Olson et al. 2014), densities of higher-order veins are also expected to increase with plant height to accord with water supply and demand. In addition, because leaves in tall trees are likely to experience high evaporative demand (Sanches et al. 2010; Kenzo et al. 2015), such leaves would have high density of higher-order veins to compensate for increased water loss. Clarification of above points would contribute to the understanding of functional significances of veins as well as how diversity in plant form and functions is arrayed across species.

Here, we have compiled a new data set for 227 dicotyledonous angiosperm species from temperate regions to explore the rules underlying the variations in vein densities and their linkages to functional traits. Specifically, we tested the four following predictions across species: (1) despite their differences in functions and developing process, densities of lower- and higher-order veins are positively correlated because the formation of higher-order veins is influenced by the vascular patterns of prior-formed veins (lower-order veins). (2) Densities of areoles and free-ending veins can be variable for a given minor vein density, achieving various transport strategies in terms of safety and cost efficiency. (3) The high abundance (density) of lower-order vein is associated with high leaf dry mass per area and thus contributes to greater leaf longevity. (4) Densities of higher-order vein increase with plant height. We also generated the phylogenetic tree for studied species based on the previous study (Zanne et al. 2014) to determine trait phylogenetic signals and to examine how trees have evolutionarily developed above relationships.

Materials and methods

Data collection

For the calculation of vein densities, we used Yokoyama Akira Cleared Leaf Collection stored at The Museum of

Nature and Human, Hyogo, Japan (Handa 2015), in addition to our published data (Kawai and Okada 2018). The data included chemically cleared and stained whole leaves of 227 species that represent 126 genera and 56 families (Supplementary material Table S1). The families which occupy most of our data set are Rosaceae (25), Fagaceae (16), Oleaceae (13), Ericaceae (11), and Magnoliaceae (11) (numbers in parenthesis show number of species). For the stored collection, fully matured and healthy leaves were collected by Mr. Akira Yokoyama during 1979–1982 mostly from two botanical gardens in western Japan which locate in the temperate regions; Kyoto Botanical Gardens (35º03´ N, 135º45´ E, 70 m in altitude) and Kobe City Forest Botanical Garden (34º44´ N, 135º10´ E, 450 m in altitude). According to the most adjacent meteorological stations run by Japan Meteorological Agency, the mean annual temperature during 1981–2010 was 15.9 °C and 14.1 °C and the mean annual precipitation was 1491 mm 1216 mm at Kyoto Botanical Gardens and Kobe City Forest Botanical Garden, respectively. For the stored specimens, there are no descriptions on plant ontogenetic stage, irradiance and crown position of collected leaves, and number of individuals per species. Plant nomenclature followed the *Japanese name–scientific names index* (Yonekura and Kajita 2003) and The Plant List (ver. 1.1; available online at http://www.theplantlist.org/).

We assigned vein orders according to Hickey (1973) and calculated the density of each vein order, defined as the vein length for a given area. For primary and secondary vein densities (VD_1 and VD_2), we measured the total length per leaf and divided it by leaf lamina area (LA) using Fiji/ImageJ (Schindelin et al. 2012). Leaves that showed apparent deflection or tearing were not included in the measurement. Third-order vein density (VD_3) was calculated from a ca. 60 mm^2 square region around the lamina centre. The sum of vein densities from the first- to third-orders is termed as major vein density (VD_{maj}) (e.g., Sack and Scoffoni 2013).

Minor vein density (VD_{min}) was calculated from a ca. 2 mm^2 square region around the lamina centre using Win-Rhizo Pro (Regent Instruments Inc., Canada) at ca. ×5 magnification (Fig. 1). This software was designed to automatically recognize and calculate the geometric characteristics of fine roots. When the clearing or staining of vein samples was incomplete, we manually corrected the images using the pen tool of the software. Before application, we examined the validity of our method by comparing the results obtained by WinRhizo Pro with those obtained by the more common method of manually tracing veins with Fiji/ImageJ (e.g., Sack and Frole 2006). Because VD_{min} values of 21 species (ranging from 2.2 to 14.9 mm mm^{-2}) obtained using the two methods were strongly correlated ($P < 0.001$, $R^2 = 0.98$) and 95% confidence interval of the slope and intercept crossed 1 and 0, respectively, we judged that our method was reliable. Total vein density (VD) was calculated as the sum of

VD_{maj} and VD_{min}. As for the geometrical characteristics of the minor veins, we measured the density of FEVs (D_{FEV}) and the density of areoles (D_A) from a ca. 4–36 mm^2 region, depending on the species, in the area adjacent to that used for the minor vein analysis. The definitions of FEV and areole followed Ellis et al. (2009).

The data on leaf dry mass per area (LMA, 106 species), leaf longevity (LL, 42 species), and adult plant height (H_{adult}, 95 species) were obtained from Ohsawa and Nitta (1997), Kikuzawa and Ackerly (1999), GLOPNET database (Wright et al. 2004), and Aiba et al. (2016) (For details, see Table S1). Because our vein data mostly came from Japanese flora and was collected in Japan, we cited studies conducted in Japan. For the graphical data, we digitalized figures to generate a numerical data set using Fiji/ImageJ. The data on leaf habit (deciduous vs. evergreen) was obtained from the books published by Satake et al. (1989a, b). Our study species were mostly deciduous (182 out of 227 species).

The calculation of LMA followed the standard protocol (Cornelissen et al. 2003); dry mass of whole leaf, including petioles and the rachis of a compound leaf was dived by the leaf area. For LMA, where traits were reported separately for sun leaves and shade leaves, data of sun leaves were used. The LL was estimated as the age of the branch having a half of initial leaves (leaf half-life) or estimated by a census of the number of leaves emerging and falling over a time interval, both of which are shown to provide similar results (Dungan et al. 2003). The H_{adult} was obtained from various floras of Japan (for detail, see Aiba et al. 2016).

Statistical analysis

We conducted all analyses in R (ver. 3.3.2; R core team 2016). When multiple images were available for the same species (23 of 227 species), we used the averaged values. Although our sample size for a given species was too small to consider intraspecific variations, we believe that our trait values could robustly represent species mean, enabling interspecific comparisons, because interspecific variations typically account for at least more than 70% of the variance in vein traits (e.g., Iida et al. 2016; Blonder et al. 2017). Because functional traits also show larger or similar interspecific variation compared with intraspecific variation (e.g., Messier et al. 2010; Asner and Martin 2011), we believe that we can test the species-level relationships among traits in a robust manner despite traits being measured at different sites.

The data on each trait, except for H_{adult}, was log_{10}-transformed to improve normality and heteroscedasticity before the analysis. First, we tested the relationships among the traits using Pearson's correlation coefficient (*r*). The lines were fitted by standard major axis (SMA) using

Fig. 1 Detection of minor veins of **a** *Fagus crenata*, **b** *Carpinus laxiflora*, **c** *Machilus thunbergii*, and **d** *Ilex pedunculosa* using WinRhizo Pro. Blue lines in each image showed traced veins. Note that some tiny veins were not detected. In this case, we manually added the missing veins before calculation of length. Bar represents 1 mm
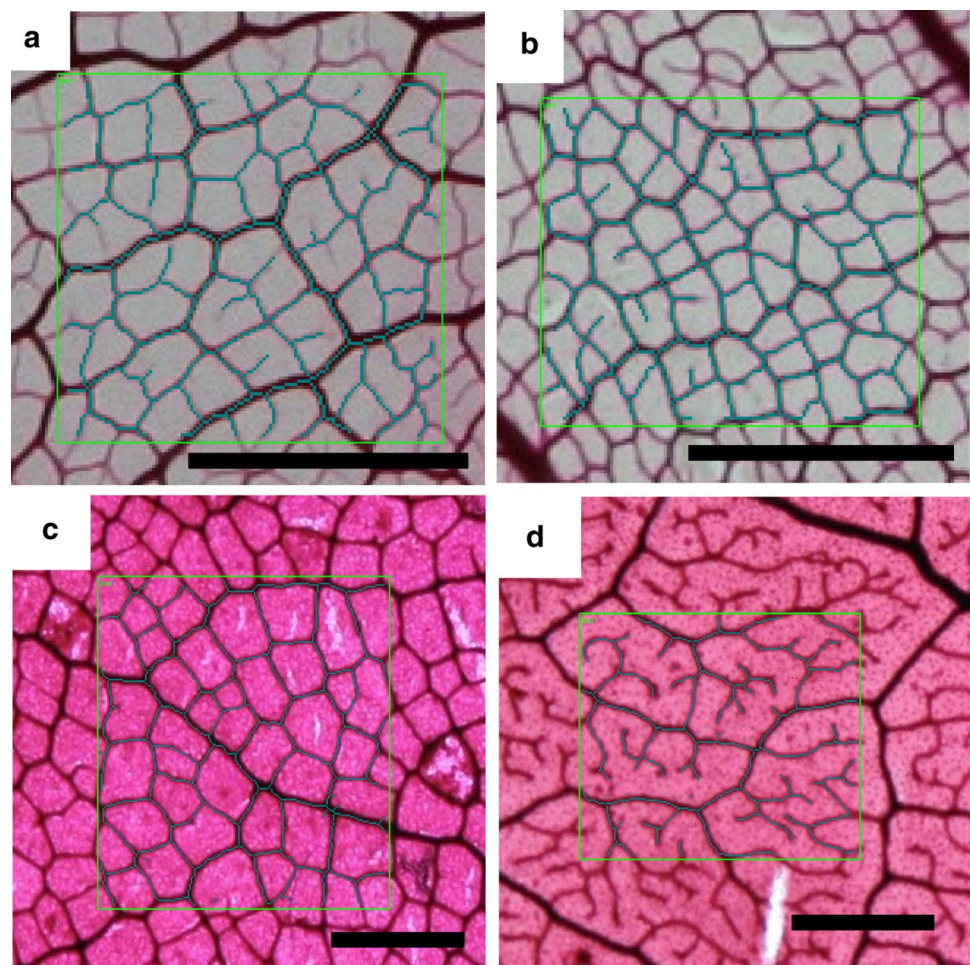
the 'smatr' package for R (Warton et al. 2012), and the allometric slope and intercept were calculated.

To determine whether phylogenetic correction was necessary for our correlation analyses, we quantified the phylogenetic signal of all traits. Blomberg's K statistic was calculated for each trait using the 'phylosignal' package for R (ver. 1.1; Keck et al. 2016). Larger K values indicate a greater phylogenetic conservatism for a given trait (Blomberg et al. 2003). A K value of 1 indicates that the traits evolved under Brownian motion, and $K < 1$ indicates random or divergent trait evolution more than that expected under the Brownian motion model. A K value of more than 1 indicates more conservative patterns of trait evolution than that expected under the Brownian motion. The significance was assessed by comparing the observed K statistic with the distribution of K statistics obtained by 10,000 permutations of trait values across the tips of the tree. PHYLOMATIC (ver. 3) (Webb and Donoghue 2005; available online at http://www.phylodiversity.net/phylomatic/) was used to create the phylogenetic trees of the studied species on the basis of the megatree by Zanne et al. (2014). We excluded *Vitis ficifolia* and two garden species (*Cerasus* × *yedoensis* and *Magnolia* × *watsonii*) from the phylogenetic analysis because

of the absence in a phylogenetic tree and uncertain phylogenetic positions, respectively. Multichotomies in trees were randomly resolved to dichotomy by the 'multi2di' function in the 'ape' package for R (Paradis et al. 2004). Throughout the analyses, the significance level (α) was set as 0.05, and Bonferroni correction was applied for multiple testing.

Because the vein and functional traits, except D_A, did not show significant phylogenetic signals (Table 1), we used the phylogenetic comparative method only to the pairwise correlations including D_A. We evaluated the effects of the phylogenetic relationships using generalized least squares (GLS) regressions with the Ornstein–Uhlenbeck evolution model in 'nlme' package for R (Pinheiro et al. 2018). Specifically, we used the function 'gls' with the 'correlation' argument set corMartins (1, tree), where tree refers to a phylogenetic tree.

Results

Two hundred and twenty-seven temperate plant species showed remarkable variations in vascular architecture, especially in D_A and D_{FEV} (Tables 1 and S1). Minor

Table 1 Traits studied, the number of species measured per trait, minimum species mean, the mean of species means, the maximum species mean, and Blomberg's K statistic

Trait	Symbol	n	Units	Mean	Min	Max	Blomberg's K
Primary vein density	VD_1	213	mm mm^{-2}	0.06	0.01	0.34	0.07
Secondary vein density	VD_2	211	mm mm^{-2}	0.26	0.07	1.10	0.07
Primary and secondary vein density	$VD_{1.2}$	211	mm mm^{-2}	0.33	0.08	1.26	0.07
Tertiary vein density	VD_3	227	mm mm^{-2}	0.67	0.19	3.21	0.06
Major vein density	VD_{maj}	209	mm mm^{-2}	1.00	0.32	3.93	0.07
Minor vein density	VD_{min}	216	mm mm^{-2}	7.59	2.41	15.3	0.07
Total vein density	VLA	202	mm mm^{-2}	8.64	2.98	18.0	0.07
Density of free-ending veins	D_{FEV}	216	mm^{-2}	11.4	0.55	52.8	0.06
Density of areoles	D_A	216	mm^{-2}	9.42	0.11	59.0	**_0.12_**
Leaf lamina area	LA	213	cm^2	17.5	0.54	81.2	0.07
Leaf dry mass per area	LMA	106	g m^{-2}	83.9	34.7	204	0.06
Leaf longevity[a]	LL	42[a]	month	15.5	3.29	45.3	0.11
Adult plant height	H_{adult}	95	m	12.8	2.50	30.0	0.09

All traits, except H_{adult}, were log$_{10}$-transformed before analysis. For each species' data, see Table S1. Values of significant phylogenetic signals detected here by the permutation test are represented in underlined bold ($P < 0.01$) after Bonferroni correction

[a]Twenty-two deciduous and twenty evergreen species

and major vein densities (VD_{min} and VD_{maj}) ranged from 2.4 mm mm^{-2} in *Magnolia compressa* to 15.2 mm mm^{-2} in *Rosa hirtula* and from 0.3 mm mm^{-2} in *Dendropanax trifidus* to 3.9 mm mm^{-2} in *Amorpha fruticosa*, respectively. These ranges spanned much of the global range in angiosperms [1–25 mm mm^{-2} (Boyce et al. 2009) and 0.25–4.86 mm mm^{-2}, respectively (Sack et al. 2012)]. We found several significant relationships (1) among veins at two spatial scales (lower- vs. higher-order veins and among the minor veins) and (2) between the vein and functional traits (Figs. 2, 3. 4, Tables 2, 3). All vein and functional traits, except D_A, lacked phylogenetic signal; these traits were more labile than expected from a Brownian motion model of trait evolution (Table 1). In addition, phylogenetic regression analyses for the pairwise correlations including D_A showed results similar to those of non-phylogenetic correlative analyses (Table 4). This indicates that the observed relationships were attributable to multiple instances of coordinated evolution rather than changes in traits within a few lineages.

Relationships between densities of lower- and higher-order veins

Consistent with our prediction (1), the densities of lower- and higher-order veins were positively correlated, except for the relationship between VD_1 and VD_{min} (Fig. 2, Tables 2, 3). The slope of each allometric equation was positive and less than 1, except for the relationship between VD_2 and VD_3 (Table 2). In addition, the slope values decreased as the differences in vein order increased. A similar trend was observed for the strength of the correlation, for example, the
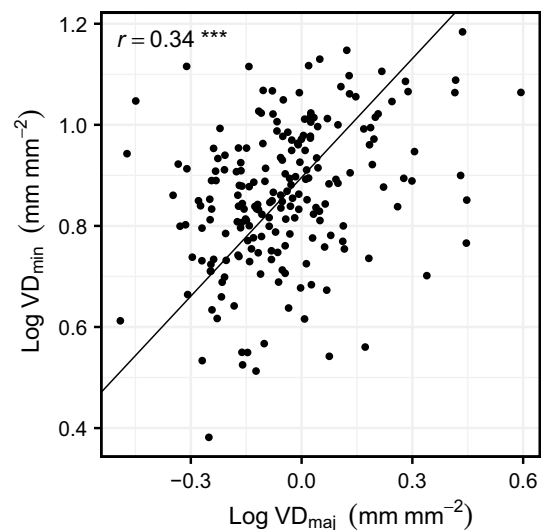

Fig. 2 Relationship between major (VD_{maj}) and minor vein density (VD_{min}) across 202 angiosperm species. The line was fitted by standard major axis (log $VD_{min} = 0.90 + 0.78$log VD_{maj}). ***$P < 0.001$ after Bonferroni correction

strength of correlations between VD_{min} and the density of lower-order veins slightly increased as the order in lower-order veins increased ($r^2 = 0.03$–0.10, Table 2). This indicates that densities of closer orders of veins (e.g., primary and secondary veins) are more tightly coordinated.

Relationships among minor veins

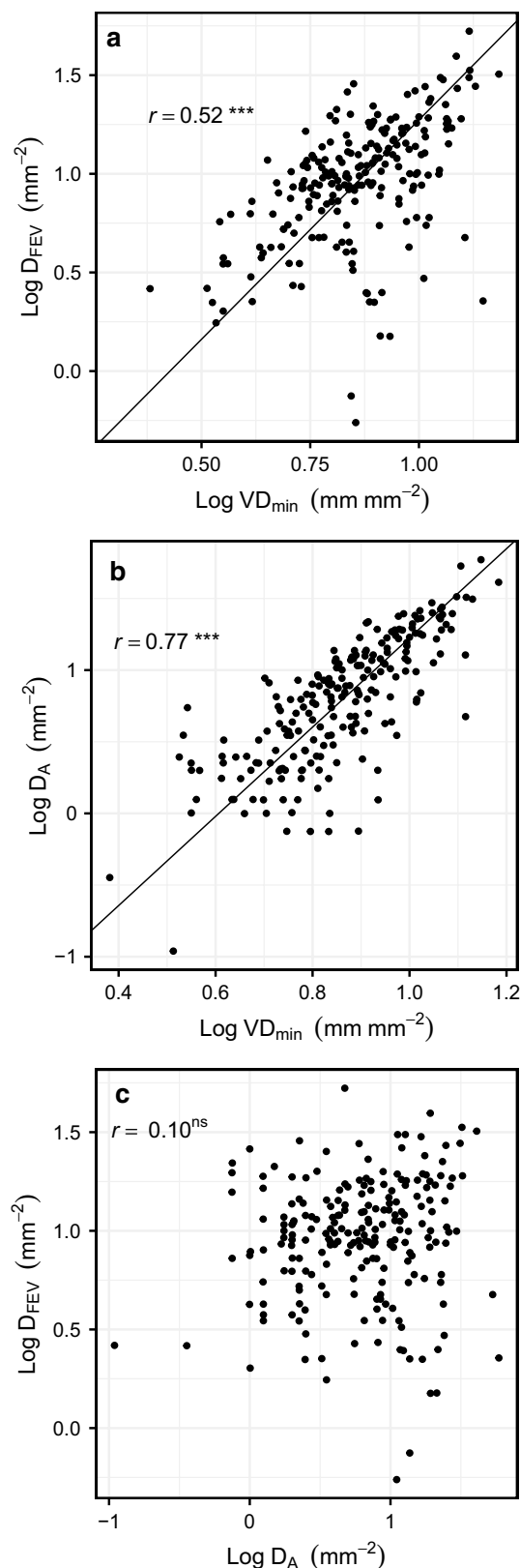The minor vein density was positively correlated with the densities of both FEVs (D_{FEV}; $r^2 = 0.27$) and areoles (D_A;

Fig. 3 Relationships between **a** densities of free-ending (D_{FEV}) and minor veins (VD_{min}), **b** density of areole (D_A) and VD_{min}, and **c** D_{FEV} and D_A across 216 angiosperm species. The lines were fitted using standardized major axes (log $D_{FEV} = -0.95 + 2.22$ log VD_{min}; log $D_A = -1.89 + 3.11$ log VD_{min}). ***$P < 0.001$; ^{ns} not significant after Bonferroni correction

$r^2 = 0.60$) across 216 species (Fig. 3a, b). On the other hand, D_A and D_{FEV} were not correlated across species ($P = 0.15$; Fig. 3c). The slope in log D_A–log VD_{min} was steeper than that in log D_{FEV}–log VD_{min} (mean = 3.11, 95% CIs = 2.86–3.39 vs. mean = 2.22, 95% CIs = 1.98–2.50, respectively). The comparison of the two lines indicates that when minor vein densities exceed 11.3 mm mm^{-2} (the point of intersection), areoles are present at greater densities than FEVs. Conversely, when minor vein densities fall below 11.3 mm mm^{-2}, FEVs are more abundant than areoles. Thus, contrary to our prediction (2), high VD_{min} was associated with more areoles and less FEVs.

Relationships between vein and functional traits

Contrary to our prediction (3), the densities of lower-order veins ($VD_{1.2}$ and VD_{maj}) were not correlated with LMA and LL across species (Fig. 4a, b and Table 3). In Fig. 4b, the data were divided into two groups because of the large difference in LL between deciduous (4.9 ± 0.9 months, expressed mean $\pm$ 1SD, $n = 22$) and evergreen species (27.2 ± 8.8 months, $n = 20$). Consistent with previous studies (e.g., Sack et al. 2013; Li et al. 2015), minor vein (VD_{min}) was not related to LMA (Fig. 4c) and LL (Table 3).

Contrary to our prediction (4), VD_{min} was not correlated with H_{adult} (Table 3). On the other hand, total vein density (VD) and D_A were positively correlated with H_{adult} (Fig. 4d and Table 3).

Discussion

Using 227 phylogenetically diverse species, we examined the relationships among vein densities and between vein and functional traits. The principal novel findings of this study are (1) the linkage between densities of lower- and higher-order veins; (2) the relationship between minor vein density and its morphology (dense vein is more closed); and (3) weak positive relationship between adult plant height and vein density. We also found that, for all vein and functional traits, except D_A, the phylogenetic signal was weak and not significant (Table 1). These results suggest that most vein traits, including the densities of major and minor veins, are evolutionarily labile and potentially being able to change even within closer lineages, as demonstrated in some plant groups (Givnish et al. 2005; Blonder et al. 2016).

Relationships among vein densities

We found positive relationships between the densities of lower- and higher-order veins across 202 temperate dicotyledonous species (Fig. 2, Table 2, and Table 3), which is consistent with our prediction. This suggests that, although
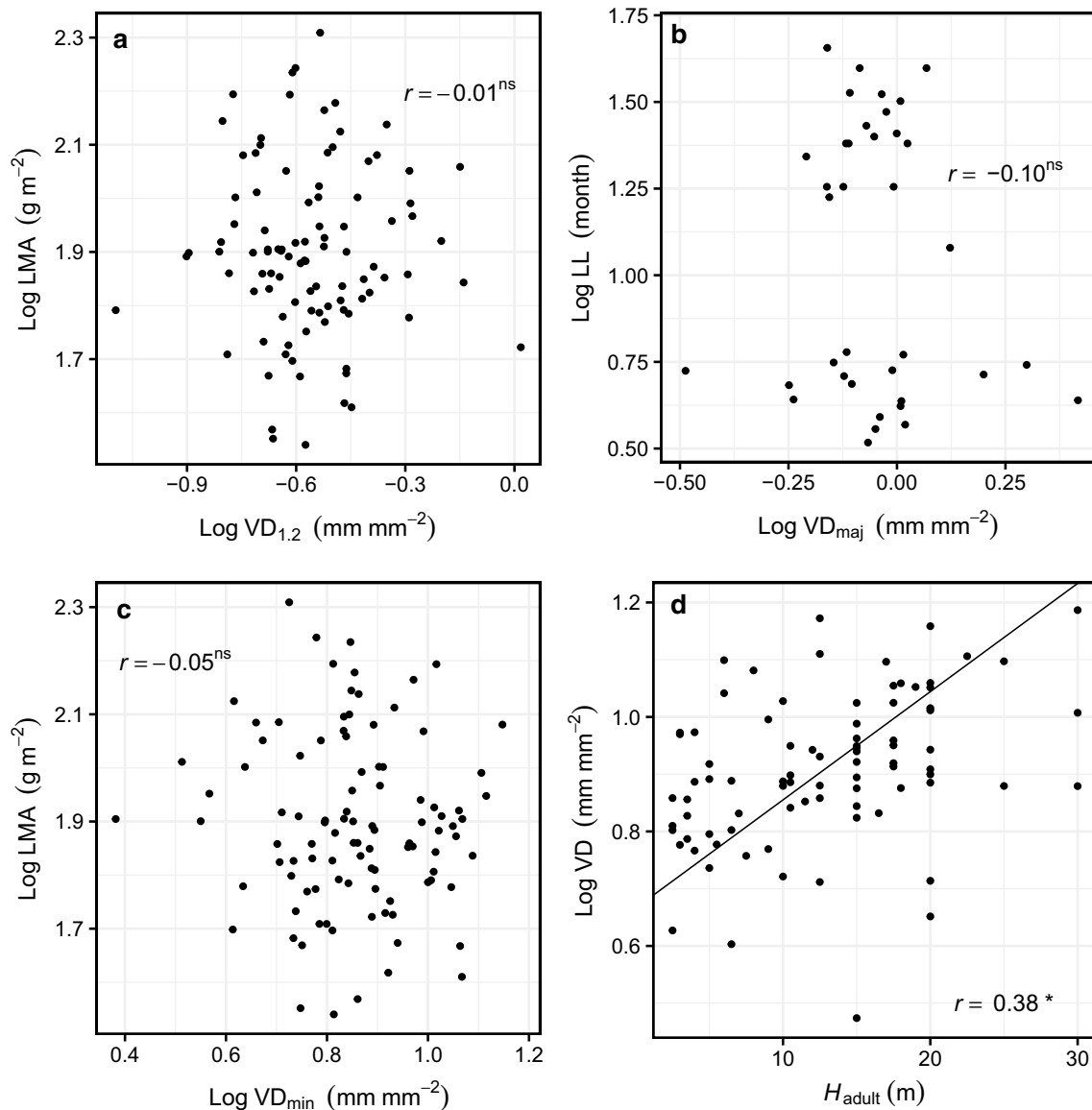
Fig. 4 Relationships between the **a** density of primary and secondary veins ($VD_{1.2}$) and leaf dry mass per area (LMA) ($n = 97$), **b** density of major veins (VD_{maj}) and leaf longevity (LL) ($n = 37$), **c** density of minor veins (VD_{min}) and LMA ($n = 100$), and **d** adult plant height (H_{adult}) and total vein density (VD) ($n = 90$). The line was fitted using standardized major axes (log $VD = 0.67 + 0.02\ H_{adult}$). ^{ns} not significant; $^*P < 0.05$ after Bonferroni correction

the functions and developmental timing are different between lower- and higher-order veins (Sack et al. 2012; Sack and Scoffoni 2013; Kawai and Okada 2016), a common developmental process exists in the formation of different vein orders. A possible process is cell expansion during leaf development, which decreases the densities of both lower- (Sack et al. 2012) and higher-order veins (Brodribb et al. 2013; Carins Murphy et al. 2016). Because the distance between neighbouring veins becomes greater for both lower- and higher-order veins during cell expansion, their densities would be positively correlated. We also found that the strength and allometric slope of this coordination

decreased as the difference in vein order increased (Table 2). This may be caused by the different timing of vein differentiation among the vein orders (e.g., Dengler and Kang 2001; Pantin et al. 2012; Sack et al. 2012). If the densities of different-order veins decrease during cell expansion, vein densities of more similar orders formed more closely in timing would be correlated more strongly. In addition, because veins formed earlier (lower-order veins) would be more affected by cell expansion than the veins formed later (higher-order veins), the allometric slope would be below 1 and become smaller with increasing difference in vein order. Future studies should test whether cell expansion interplays

Table 2 Parameters of fitted allometries for relationships between the density of lower- and higher-order veins

variable x	variable y	n	R^2	b	$\log a$
VD_1	VD_2	211	0.58***	0.81, **0.89**, 0.97	0.40. **0.50**, 0.60
	VD_3	211	0.13***	0.76, **0.86**, 0.98	0.72, **0.87**, 1.01
	VD_{min}	203	0.03[ns]	–	–
VD_2	VD_3	211	0.36***	0.87, **0.97**, 1.08	0.31, **0.38**, 0.45
	VD_{min}	203	0.09**	0.62, **0.70**, 0.81	1.24, **1.30**, 1.37
VD_3	VD_{min}	216	0.10***	0.65, **0.74**, 0.84	1.00, **1.02**, 1.06
VD_{maj}	VD_{min}	202	0.12***	0.69, **0.78**, 0.89	0.83, **0.90**, 0.92

For each relationship, a linearized power law ($\log y = \log a + b \log x$) was fitted by standard major axis (SMA) to log-transformed data. For each relationship, slope b and intercept $\log a$ are presented in bold in between lower and upper 95% confidence limits. Trait abbreviations as in Table 1. **$P < 0.01$, ***$P < 0.001$, [ns]not significant after Bonferroni correction

the positive correlation between the densities of lower- and higher-order veins.

In minor veins, we found the positive link between VD_{min} and both D_{FEV} and D_A across 216 species, although the latter relationship was stronger (Fig. 3a, b). In contrast to the intraspecific pattern, where areole size (inverse of D_A) and D_{FEV} are positively correlated (Fiorin et al. 2016), D_A and D_{FEV} were independent across species (Fig. 3c). This discrepancy may be due to variations in species-specific development patterns of minor veins (e.g., Foster 1952; Pray 1959, 1962; Hara 1962) that reflect the hydraulic and

photosynthetic requirements of species (Fiorin et al. 2016). These species-specific vein patterns would be driven by different distributional patterns of auxin and auxin transport proteins among the species (Sachs 1981; Nelson and Dengler 1997; Fujita and Mochizuki 2006) or elastic mismatch between the epidermis and mesophyll (Laguna et al. 2008).

We also found that denser veins were composed of more areoles and fewer FEVs (high $D_A : D_{FEV}$) on the basis of the comparison of the slopes of D_{FEV} and D_A against VD_{min}. This would mean that the minor vein network would become more closed when the venation is dense. Such venation is theoretically selected when there is a risk for unstable water flow or damage by embolism or herbivory at the expense of cost efficiency in leaf hydraulics (Banavar et al. 2000; Roth-Nebelsick et al. 2001; Durand 2007; Corson 2010; Dodds 2010; Katifori et al. 2010). Therefore, dense minor vein systems with higher leaf hydraulic conductance and rate of photosynthesis (e.g., Brodribb et al. 2007; Sack et al. 2013) appear to favour relatively safer transport within the leaf in our sample of species. This, in turn, presents a hypothesis that abiotic/biotic stresses that disturb leaf water flux can be a selective pressure for the evolution of high VD_{min} in dicotyledon angiosperms. In contrast, venation with low VD_{min} consists of many FEVs, implying that a species with potentially low productivity achieves high cost efficiency at the expense of safety.

Relationships between vein and functional traits

Our second aim was to test the relationships between leaf vein and functional traits. Contrary to our prediction, the densities of lower-order veins were not related to LMA and LL across the species (Fig. 4a, b, Table 3). For the former, leaf size may interplay in this independence: leaves with

Table 3 Pairwise Pearson correlations (r) between 13 traits across species

	VD_1	VD_2	$VD_{1.2}$	VD_3	VD_{maj}	VD_{min}	VD	D_{FEV}	D_A	LA	LMA	LL
VD_2	**0.76**											
$VD_{1.2}$	**0.85**	**0.99**										
VD_3	**0.36**	**0.60**	**0.57**									
VD_{maj}	**0.58**	**0.80**	**0.79**	**0.95**								
VD_{min}	0.16	**0.30**	**0.28**	**0.31**	**0.34**							
VD	**0.24**	**0.40**	**0.38**	**0.46**	**0.48**	**0.98**						
D_{FEV}	0.18	**0.29**	**0.28**	0.17	0.23	**0.52**	**0.52**					
D_A	0.12	0.22	0.21	**0.33**	**0.32**	**0.77**	**0.77**	0.10				
LA	**−0.85**	**−0.81**	**−0.85**	**−0.42**	**−0.62**	−0.19	**−0.28**	**−0.24**	−0.12			
LMA	0.02	0.00	−0.01	0.05	0.03	−0.06	−0.08	**−0.37**	0.02	−0.02		
LL	0.19	0.04	0.06	−0.17	−0.10	−0.37	**−0.51**	**−0.49**	−0.20	−0.21	**0.83**	
H_{adult}	−0.13	−0.08	−0.09	0.24	0.14	0.30	**0.38**	−0.12	**0.38**	0.14	0.26	−0.29

All traits except H_{adult} are $\log_{10}$-transformed before analysis. Trait abbreviations as in Table 1. Significance of correlation is indicated by bold font ($P < 0.05$) and underlined bold ($P < 0.01$) after Bonferroni correction

Table 4 Results of generalized least squares regressions with phylogenetic structure

Traits				
Independent	Dependent	Slope	P	DF
D_A	VD_3	0.14	**	211
	VD_{maj}	0.13	**	197
	VD_{min}	0.25	**	211
	VD	0.23	**	197
	H_{adult}	7.24	**	88

Only significant relationships that included D_A in Table 3 were tested, because other traits did not show significant phylogenetic signals (Table 1, for detail, see Materials and methods in text)

All traits except H_{adult} are $\log_{10}$-transformed before analysis. Trait abbreviations as in Table 1. DF degrees of freedom. **$P < 0.01$ after Bonferroni correction

higher densities of lower-order veins are generally associated with small leaf area (Table 3, Sack et al. 2012). Because the diameter of lower-order veins in such small leaves is smaller than that of large leaves (Sack et al. 2012), the effect of an increase in the density of lower-order veins on leaf dry mass may be offset by a decrease in vein diameter. Indeed, Niinemets et al. (2007) demonstrated that the dry mass fraction in primary veins largely depends on leaf size, suggesting that the relationship between densities of lower-order veins and leaf dry mass investment vary among leaf size. Therefore, the positive link of lower-order veins with LMA and leaf mechanical properties observed across eight Fagaceae species by Kawai and Okada (2016) may not be generally applicable to wide taxonomic groups and may be caused by the negative correlation between leaf area and LMA. To obtain deeper insights in the relationship between lower-order veins and leaf structural and mechanical properties, future studies should examine the other traits of lower-order veins, such as volume, mass, geometry, or anatomical features and analyse species with similar leaf sizes.

Minor vein density was also not correlated with LMA and LL (Fig. 4c, Table 3), which is consistent with the results of previous studies (Sack et al. 2013; Li et al. 2015; Kawai et al. 2017). These results suggest that the densities of both major and minor veins do not influence leaf construction cost and LL and that vein and associated water use strategies form an independent axis from the LES. On the other hand, similarly to lower-order veins, minor vein traits not considered here may influence LMA and LL. For example, minor vein diameter is reported to be positively correlated with LMA among 11 temperate woody species (Kawai and Okada 2018) and 25 Fagaceae species in East Asia (Kawai and Okada 2019).

Different from our prediction, density of total veins (VD) but not minor vein (VD_{min}) was positively correlated with H_{adult} (Fig. 4d, Table 3). This may be because VD was more tightly related to leaf hydraulic conductance than VD_{min}. The positive VD – H_{adult} relationship suggests that increase in hydraulic efficiency at twigs with plant height (Olson et al. 2014) is coupled with that at leaves, possibly contributing to high photosynthetic rate even at the greater hydraulic path length (e.g., Kenzo et al. 2015). In addition, we found that D_A was positively correlated with H_{adult}. Leaves with high D_A would be better suited to environments with a high evaporative demand owing to the reduced risk for hydraulic failure under desiccating conditions because of redundancy in the vascular architecture (Roth-Nebelsick et al. 2001; Durand 2006; Katifori et al. 2010). In contrast, under shaded conditions, low D_A may be beneficial because this geometry reduces the vein construction cost for a given water transport activity (Banavar et al. 2000; Durand 2007; Corson 2010; Dodds 2010).

Conclusions

Using 227 temperate species, our study demonstrated the (1) positive scaling relationships between different-order veins and (2) that venation becomes more closed in dense veins, and (3) provided further evidence for the independence of the densities of major and minor veins from LMA and leaf longevity. The results suggest that densities of different-order veins are developmentally and functionally coordinated across dicotyledonous species in temperate regions. This coordination forms an independent axis in the overall trait variation from the productivity–durability trade-off (LES). Future studies should focus on the developmental and functional mechanisms underlying the observed relationships. In addition, consideration of the traits other than density (e.g., vascular tissue mass per area and size and/or number of vessels per vein) may be necessary to link the leaf vein with leaf economic traits.

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