Scaling relationships of leaf vein and areole traits versus leaf size for nine Magnoliaceae species differing in venation density

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Abstract
Premise: Across species, main leaf vein density scales inversely with leaf area (A). Yet, minor vein density manifests no clear relationship with respect to A, despite having the potential to provide important insights into the trade-off among the investments in leaf mechanical support, hydraulics, and light interception.

Methods: To examine this phenomenon, the leaves of nine Magnoliaceae leaves were sampled, and the scaling relationships among A and midrib length (ML), total vein length (TVL), total vein area (TVA), total areole area (TAA), and mean areole area (MAA) were determined. The scaling relationships between MAA and areole density (the number of areoles per unit leaf area) and between MAA and A were also analyzed.

Results: For five of the nine species, A was proportional to ML². For eight of the nine species, TVL and TVA were both proportional to A. The numerical values of the scaling exponents for TAA vs. A were between 1.0 and 1.07 for eight species; i.e., as expected, TAA was isometrically proportional to A. There was no correlation between MAA and A, but MAA scaled inversely with respect to areole density for each species.

Conclusions: The correlation between midrib “density” (i.e., ML/A) and A, and the lack of correlation between total leaf vein density and A result from the A∝ ML² scaling relationship and the proportional relationship between TVL and A, respectively. Leaves with the same size can have widely varying MAA. Thus, leaf size itself does not directly constrain leaf hydraulic efficiency and redundancy.

Keywords
areole density, leaf area, principle of similarity, reticulate venation, scaling relationship

The hierarchical reticulate venation network of most eudicot leaves is considered to be an evolutionary innovation that increases the mechanical and photosynthetic efficiency of leaves compared to leaves with parallel or open dichotomous venation patterns (Carvalho et al., 2018). The densities of major veins (which usually comprise the first-, second-, and third-order veins) are reported to decrease as leaf size increases (Scoffoni et al., 2011; Sack et al., 2012). However, less is known about the morphometrics of higher order veins. Nevertheless, over the course of evolution, more minor veins have appeared between the third-order veins thereby reducing the distance from veins to evaporation sites associated with stomata and increasing hydraulic conductance (Brodribb et al., 2010; Sack and Scoffoni, 2013). Different orders of veins form a large number of closed polygons (called areoles). The highest order of veins often ends freely within the smallest areoles, but the minimum mean distance of stomata from the nearest veins typically does not scale with overall areole size because of the existence of free-ending veinlets (Fiorin et al., 2016). For this reason, prior studies report that total leaf vein density (defined as the sum of major and minor veins per unit area) is not correlated with leaf size (Scoffoni et al., 2011; Sack et al., 2012). However, all of these trends are based on interspecific pooled data across phylogenetically diverse species. For example, Sack et al. (2012) studied the
scaling of the first-, second-, and third-order vein lengths with leaf size across 485 globally distributed species and found that the higher the order of veins, the weaker the scaling relationship between vein density and leaf size. In the case of leaves with the hierarchical reticulate venation pattern, different orders of veins are densely distributed in the lamina, resulting in an isometric (one-to-one) scaling relationship between total vein length and leaf area. However, it remains unclear as to whether the same trend holds at the intraspecific level in which a direct trade-off exists between the investments in mechanical support and photosynthetic tissues (Milla and Reich, 2007; Niklas et al., 2007). In addition, leaf size, shape, and vein density are affected significantly by environmental factors (e.g., temperature and precipitation) (Peppe et al., 2011; Zhu et al., 2011; Wright et al., 2017), which may lead to significant variations in leaf vein architecture across species (Sack and Scoffoni, 2013). It is important therefore to compare leaves differing in size from the same species from plants growing in similar as well as different environments. For example, some data indicate that the proportion of midrib area (and midrib length) to the whole leaf area of smaller leaves is larger than that of bigger leaves (Scoffoni et al., 2011; Sack et al., 2012), although this is not always the case (Niinemets et al., 2007a).

Total leaf lamina surface area (denoted as A hereafter) is an important functional trait that has received considerable theoretical and practical attention (Wright et al., 2017; Shi et al., 2019a; Baird et al., 2021). However, few studies have focused on the fact that A is the sum of vein area and areole area. If total leaf vein area is proportional to A, the disproportionate scaling relationship between leaf mass and A, which has been referred to as “diminishing returns” (Niklas et al., 2007), can be easily explained, given that the mass of leaf veins and their bundle sheath extensions do not scale isometrically with the leaf-vein surface area. Any increase in leaf veins and their bundle sheath extensions must reduce the space that is available for mesophyll provided that A is conserved (Lambers and Poorter, 1992; Niinemets et al., 2007a; Brodribb et al., 2010; Baresh et al., 2019). Thus, it is important to explore the relationship between total leaf vein area and total leaf area. An additional point of interest is the spatial distribution of stomata, which play an important and obvious role in photosynthesis (de Boer et al., 2016). Across the Magnoliaceae, stomata are generally confined to the areole (Shi et al., 2021; Wang et al., 2021). Because the number of stomata within an areole is positively correlated with areole size (Fiorin et al., 2016), total areole area and mean areole area can reflect the number and density of stomata. Therefore, it is valuable to explore the relationships between total areole area and A, and between mean areole area and A.

To understand the foregoing complexities at the level of conspecific comparisons, we sampled 120–380 leaves from each of nine Magnoliaceae species (four deciduous Magnolia species and five evergreen Michelia species) and measured A, midrib length, total leaf vein length, total leaf vein area, the number of areoles per leaf, and individual areole area. These data were used to examine two morphometric attributes: (1) whether midrib length, total vein length, total vein area, total areole area, and mean areole area per leaf scale with (or are proportional to) A for each species and (2) whether mean areole area scales inversely with respect to areole density (i.e., the quotient of the number of areoles per leaf and leaf area) for each species.

Finally, we draw attention to two important potential insights that can emerge from this study. First, leaf vein density is widely reported to be positively correlated with photosynthetic rates per leaf dry mass (Sack et al., 2013). If leaf vein density is independent of A, total leaf surface area per plant (i.e., the sum of leaf surface areas for all leaves of a plant) is unrelated to leaf vein density, which means that the larger the total leaf area per plant, the greater the photosynthetic carbon acquisition of the plant. Second, if leaf vein area is proportional to A, the scaling relationship between the surface area of leaf veins (including their bundle sheath extensions) and A may help to explain why increases in leaf mass exceed gains in A (i.e., the phenomenon called diminishing returns).

**MATERIALS AND METHODS**

**Leaf sampling**

Leaves of nine species of Magnoliaceae were collected from Nanjing Botanical Garden, Chinese Academy of Sciences, and Nanjing Forestry University Xinzhuang Campus, Nanjing, China (Table 1). Fully expanded and fully developed leaves, at least 3 months old were sampled between July to September in 2019 and 2020. The sampling of leaves for any one species in 2019 and 2020. The sampling of leaves for any one species was completed well within 1 year, to avoid phenological changes in leaf architecture. However, the sampling of all the leaves from all of the species was not performed in the same year owing to the heavy workload required. For each species, 300 to 500 leaves from three to five mature trees were randomly sampled from the middle canopy without distinguishing between sun leaves and shade leaves (because the objective was to explore general trends rather than focusing on the influence of light on the characteristics of leaf veins and areoles, which is nevertheless worthy of future research). Only undamaged healthy leaves were sampled. Fresh leaves were wrapped in wet paper to minimize desiccation and brought to the laboratory within 2 h after sampling.

**Chemical treatment and image scanning of leaf veins**

The leaves were labelled by attaching a small paper tag to their petioles. Each leaf was subsequently placed into a nylon mesh bag and boiled in a 5–10% w/v NaOH solution for 30 min in an open stainless steel pot (ST24P1, Supor, Wuhan, China; diameter: 28 cm; capacity: 1.2 L). The boiled leaves were washed with running water, and a soft brush was used to carefully remove epidermal and mesophyll tissues. The remaining leaf
skeleton was then stained with a 0.5% w/v aqueous safranin solution and scanned at 600 dpi using an Epson scanner (V550, Epson Indonesia, Batam, Indonesia; see Figure 1 for representative images of the scanned leaves of the nine species). We initially sampled and boiled 300–500 leaves. Between 120 and 380 leaves were useable (with intact leaf veins). By estimate, there was on average less than a 5% loss of leaf veins for each of the useable leaves. However, this loss is estimated to not substantially influence the results reported here because of the very large sample size for each the species examined in this study.

**Acquisition of leaf vein and areole data**

The software LEAF GUI developed using the MATLAB platform (Price et al., 2011) was used to obtain leaf area, total leaf vein length, total leaf vein area, the number of areoles per leaf, and individual areole area from cleared leaf images. The total areole area was calculated by summing the areas of all of the individual areole areas per leaf. The original RGB images were transformed into black and white binary images, and the adaptive threshold and global threshold were set in LEAF GUI to obtain ultraclear leaf vein images. The thresholds were determined empirically for each leaf image. LEAF GUI does not provide midrib length. Thus, the R script developed by Shi et al. (2018) and Su et al. (2019) was used to obtain leaf length, which was defined as the distance from the leaf base to leaf apex regardless of lamina curvature, and leaf width, which was defined as the maximum distance between two orthogonal points of the leaf edge forming a straight line perpendicular to the leaf length axis. Leaf length was used as the substitute of midrib length, although for curved leaves, the numeric values of these traits can moderately differ. Given that most leaves had almost perfect bilateral symmetry, the difference between midrib and lamina lengths was judged to be negligible (Figure 1). The raw data used here were uploaded to the Dryad Digital Repository (Shi et al., 2022).

**Data analyses**

Tukey’s honestly significant difference (HSD) test at a 0.05 significance level (Hsu, 1996) was used to test for significant differences in leaf area, ratio of leaf width to length, total leaf vein length, total areole area, total leaf vein density and mean areole area among the nine Magnoliaceae species. A power-law relationship was used to describe the scaling relationship between any two variables of interest, i.e.,

\[ Y = \beta X^\alpha, \]

where \( Y \) and \( X \) are the interdependent variables of interest, \( \beta \) is the normalized scaling factor, and \( \alpha \) is the scaling exponent to be determined using regression protocols after the logarithmic transformation of the two variables to stabilize their variance; i.e., \( y = y + \alpha x \), where \( y = \ln Y \), \( x = \ln X \), and \( y = \ln \beta \). The ordinary least-squares method (OLS; Sack et al., 2012) was used to fit a linear equation to determine the scaling exponents of the bivariate plots of midrib length (ML), total leaf vein length (TVL), total leaf vein area (TVA), total areole area (TAA) vs. leaf area (A), and the 95% confidence intervals (CIs) of the scaling exponents. The OLS protocols were also used to fit the scaling relationship between mean areole area and the number of areoles per unit leaf area rather than the standard major axis regression protocols because a higher dimensional measure of size (e.g., area) generally manifests a more statistically robust scaling relationship between its variance and mean than a lower dimensional measure of size (e.g., length) (Giometto et al., 2015; Shi et al., 2019b).

The statistical software R (version 3.6.1; R Core Team, 2019) was used to carry out all calculations and to fit the model and draw the figures.

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**Table 1** Leaf sampling information of the nine Magnoliaceae species.

| Species code | Latin name                  | Type       | Locality | Latitude and longitude | Sampling time | No. of leaves |
|--------------|------------------------------|------------|----------|------------------------|---------------|--------------|
| 1            | *Magnolia amoena* Cheng      | Deciduous  | NBG      | 32°3′27″N, 118°49′56″E | 31 Jul. 2020  | 185          |
| 2            | *Magnolia denudata* Desr.    | Deciduous  | NBG      | 32°4′43″N, 118°48′33″E | 13 Sep. 2019  | 133          |
| 3            | *Magnolia soulangeana* Soul.-Bod. | Deciduous  | NBG      | 32°3′29″N, 118°49′55″E | 30 Jul. 2019  | 290          |
| 4            | *Magnolia tomentosa* Thunb.  | Deciduous  | NBG      | 32°3′28″N, 118°49′55″E | 30 Jul. 2019  | 190          |
| 5            | *Michelia cavaleriei* var. platypetala (Hand.-Mazz.) N.H. Xia | Evergreen | NFU      | 32°4′48″N, 118°48′30″E | 26 Aug. 2020  | 257          |
| 6            | *Michelia chapensis* Dandy   | Evergreen  | NBG      | 32°3′28″N, 118°49′55″E | 25 Jul. 2020  | 298          |
| 7            | *Michelia compressa* (Maxim.) Sarg. Gard. et For. | Evergreen | NBG      | 32°3′28″N, 118°49′55″E | 30 Jul. 2019  | 232          |
| 8            | *Michelia figo* (Lour.) Spreng. | Evergreen | NFU      | 32°4′46″N, 118°48′28″E | 29 Jul. 2020  | 123          |
| 9            | *Michelia maudiae* Dunn      | Evergreen  | NFU      | 32°4′45″N, 118°48′25″E | 31 Jul. 2020  | 371          |

NBG: Nanjing Botanical Garden, Chinese Academy of Sciences; NFU: Nanjing Forestry University Xinzhuang Campus. The shortest geodesic distance between two sampling sites is 3.4 km.
RESULTS

A large variation in leaf size, leaf shape, total leaf vein length, and total areole area was observed among the nine Magnoliaceae species (Figure 2A–D). *Magnolia denudata* had the largest leaves and leaf width/length ratio; *Michelia compressa* had the smallest leaves; and *Michelia cavaleriei* var. *platypetala* had the smallest width/length ratio (Figure 2A, B). The means of leaf area, total leaf vein length, and total areole area ranked similarly across species (Figure 2A, C, D). In contrast, species ranking based on total leaf vein density (Figure 2E) and mean areole area (Figure 2F) differed from that based on leaf size and total vein length. *Magnolia tomentosa* had the largest leaf vein density and *Michelia chapensis* the smallest leaf vein density (Figure 2E), whereas *M. chapensis* had the largest mean...
FIGURE 2  (See caption on next page)
areole area (Figure 2F). The average (±SE) ratios of total leaf vein area to leaf area for the nine species ranged from 0.2037 ± 0.0242 (Michelia compressa) to 0.3376 ± 0.0356 (Magnolia amoena) (Figure 2G); a converse trend approximately held true the ratios of total areole area to leaf area for the nine species (Figure 2H).

The scaling exponents of the leaf area vs. midrib length relationships of five of the nine species (i.e., M. amoena, M. denudata, M. soulangiana, Michelia cavaleriei var. platypetala, and M. chapensis) had 95% CIs that included 0.5, i.e., leaf area was proportional to midrib length squared for these five species (Appendix S1: Figure S1; Table 2). Apart from Michelia maudiae, the lower bounds of the 95% CIs of the midrib length vs. leaf area scaling exponents of the remaining three species also approximated 0.5.

Total leaf vein length and total leaf vein area were both proportional to leaf area for eight of the nine species. That is, with the exception of M. compressa, the 95% CIs of eight of the nine scaling exponents of total leaf vein length vs. leaf area included 1, and with the exception of M. figo, the 95% CIs of eight of the nine scaling exponents of total leaf vein area vs. leaf area included 1 (Appendix S1: Figures S2 and S3; Table 2). With the exception of Magnolia denudata, the 95% CIs of the scaling exponents of total areole area vs. leaf area for the remaining eight species did not include 1 (Appendix S1: Figures S4; Table 2). However, the lower bounds of the 95% CIs were approximately equal to 1. The estimated scaling exponents ranged from 1.0 to 1.1. Given that a proportional relationship between total leaf vein area and leaf area has been demonstrated, regression analyses indicated that total areole area is approximately proportional to leaf area. For each species, the goodness of fit to the data (r²) for total areole area vs. leaf area was better than those for total leaf vein length and leaf vein area vs. leaf area (Appendix S1: Figures S2–S4; Table 2).

Mean areole area was negatively correlated with the number of areoles per leaf (Table 2), although the correlations were relatively weak in most species (r² < 0.8 for eight species; Table 2). There were robust scaling relationships between mean areole area and areole density (number of areoles per unit leaf area), and r² was > 0.99 in eight of the nine species (Appendix S1: Figure S5; Table 2). However, mean areole area was not correlated with leaf area for any of the species (all P values > 0.05; Table 3).

For the pooled data of the nine Magnoliaceae species, midrib length, total leaf vein length, area, and total areole area scaled positively with leaf area (Figure 3A–D). In addition, mean areole area scaled inversely with the number per leaf and with the number of areoles per unit leaf area (Figure 3E, F).

**DISCUSSION**

**Midrib length and leaf area**

Leaf veins provide the mechanical and hydraulic systems required for foliar water and photosynthetic transport (Roth-Nebelsick et al., 2001; Brodribb et al., 2010). There are three main types of leaf venation patterns: parallel venation, open dichotomous venation, and hierarchical reticulate venation. Among these, the latter has the highest architectural and hydraulic complexity (Carvalho et al., 2018) and was thus the focus of this study.

The data presented here manifest some clear interrelated trends, despite quantitative differences among the leaves of the nine species selected for study. For five of the nine species, total leaf area scales proportionally as the square of midrib length (i.e., if \( A \propto M^L \)). For eight of the nine species, total vein length and total vein area are both proportional to total leaf area (i.e., TVL \( \propto A \) and TVA \( \propto A \)), indicating that total vein length and total vein area scales isometrically with respect to one another (i.e., TVL \( \propto TVA \)). Although the numerical values of the scaling exponents for total areole area vs. total leaf area are between 1.0 and 1.07 (which exceeds unity only slightly), given that the total vein area constitutes a relatively large fraction of leaf area for the studied nine Magnoliaceae species (17–37%), total areole area (i.e., A – TVA) scales isometrically with respect to total leaf area (i.e., TAA \( \propto A \)). There is no correlation between mean areole area and total leaf area. Thus, mean areole area is inversely proportional to the number of areoles per unit leaf area for each of the nine species. Total vein density is independent of leaf area given that there is a proportional relationship between total vein length and total leaf area (i.e., if \( A \propto TVL \), it follows that TVL/A tends to be a constant at the species level). Collectively, the data indicate that mean areole area is constrained by the number of areoles per unit leaf area. The potential significance of these findings is discussed in detail and in light of previous studies in the following sections.

**Vein density and leaf area**

Leaf vein density and mesophyll thickness are postulated to affect leaf hydraulic efficiency (Asaamaa et al., 2001; Sack and Frole, 2006) and can also alter leaf mechanical robustness (Niinemets et al., 2007b). Brodribb et al. (2005, 2007) proposed that the distance between minor leaf veins and mesophyll cells to evaporation sites controls leaf hydraulic conductance. To reduce this distance and thus

**FIGURE 2** Boxplots of (A) leaf area, (B) ratio of leaf width to length, (C) total leaf vein length, (D) total areole area, (E) total leaf vein density, (F) mean areole area, (G), ratio of total leaf vein area to leaf area, and (H) ratio of total areole area to leaf area. Species codes, 1–9, are provided in Table 1. The letters on the whiskers represent the significance of the differences between any two species. The values at the top of whiskers represent the coefficients of variation (%) in each species. The horizontal solid line represents the median, and the red asterisk presents the mean. The whiskers extend to the most extreme data point, which is no more than 1.5 times the interquartile range from the box.
In order to increase leaf hydraulic conductance, minor veins must be densely distributed throughout the lamina, which should, at least in theory, result in an approximately isometric scaling relationship between total vein length and total lamina area. If true, total vein density should be independent of total lamina area. This prediction is confirmed by the data presented here (Appendix S1: Figure S2; Table 2).

### TABLE 2  Fitted scaling relationships among leaf size, leaf vein, and areole traits for each studied species on a log-log scale.

| Species code | ML vs. A | r² | TVL vs. A | r² |
|--------------|----------|----|-----------|----|
| 1            | 0.512 (0.495, 0.530) | 0.946 | 1.039 (0.973, 1.102) | 0.832 |
| 2            | 0.497 (0.474, 0.520) | 0.918 | 0.999 (0.929, 1.065) | 0.893 |
| 3            | 0.513 (0.500, 0.525) | 0.967 | 0.994 (0.962, 1.027) | 0.920 |
| 4            | 0.528 (0.508, 0.548) | 0.956 | 1.027 (0.968, 1.084) | 0.876 |
| 5            | 0.530 (0.499, 0.558) | 0.836 | 0.969 (0.936, 1.003) | 0.917 |
| 6            | 0.513 (0.497, 0.528) | 0.951 | 1.003 (0.959, 1.048) | 0.846 |
| 7            | 0.532 (0.510, 0.553) | 0.923 | 1.075 (1.025, 1.131) | 0.893 |
| 8            | 0.539 (0.500, 0.583) | 0.836 | 0.953 (0.863, 1.039) | 0.771 |
| 9            | 0.538 (0.516, 0.557) | 0.874 | 0.992 (0.967, 1.017) | 0.949 |

| Species code | TVA vs. A | r² | TAA vs. A | r² |
|--------------|-----------|----|-----------|----|
| 1            | 0.970 (0.930, 1.011) | 0.916 | 1.068 (1.042, 1.095) | 0.970 |
| 2            | 1.032 (0.966, 1.090) | 0.914 | 1.007 (0.985, 1.065) | 0.987 |
| 3            | 1.022 (0.981, 1.066) | 0.890 | 1.028 (1.016, 1.038) | 0.993 |
| 4            | 1.035 (0.984, 1.081) | 0.874 | 1.041 (1.029, 1.054) | 0.991 |
| 5            | 0.977 (0.933, 1.019) | 0.877 | 1.032 (1.018, 1.047) | 0.987 |
| 6            | 1.010 (0.984, 1.038) | 0.949 | 1.024 (1.011, 1.037) | 0.988 |
| 7            | 1.028 (0.970, 1.090) | 0.854 | 1.067 (1.051, 1.080) | 0.990 |
| 8            | 0.896 (0.806, 0.991) | 0.712 | 1.067 (1.025, 1.107) | 0.948 |
| 9            | 1.006 (0.975, 1.035) | 0.909 | 1.027 (1.016, 1.038) | 0.986 |

| Species code | MAA vs. the number of areoles | r² | MAA vs. areole density | r² |
|--------------|-------------------------------|----|------------------------|----|
| 1            | -0.847 (−0.897, −0.801)       | 0.837 | -0.965 (−0.979, −0.951) | 0.994 |
| 2            | -0.635 (−0.739, −0.55)        | 0.312 | -1.142 (−1.157, −1.124) | 0.996 |
| 3            | -0.764 (−0.825, −0.707)       | 0.381 | -1.075 (−1.081, −1.069) | 0.998 |
| 4            | -0.755 (−0.831, −0.683)       | 0.578 | -1.054 (−1.065, −1.042) | 0.995 |
| 5            | -0.719 (−0.794, −0.653)       | 0.194 | -1.126 (−1.138, −1.115) | 0.994 |
| 6            | -0.841 (−0.892, −0.789)       | 0.701 | -1.00 (−1.010, −0.993)  | 0.995 |
| 7            | -0.781 (−0.853, −0.715)       | 0.407 | -1.057 (−1.073, −1.043) | 0.992 |
| 8            | -1.001 (−1.127, −0.886)       | 0.479 | -1.181 (−1.198, −1.165) | 0.994 |
| 9            | -0.666 (−0.742, −0.603)       | 0.241 | -1.087 (−1.125, −1.052) | 0.985 |

ML: midrib length (cm); A: leaf area (cm²); TVL: total vein length (cm); TVA: total vein area (cm²); TAA: total areole area (cm²); MAA: mean areole area (cm²); the number of areoles: the total of areoles per leaf; areole density: the number of areoles per unit leaf area; 95% CI: the 95% confidence intervals of a slope. The slopes were calculated using ordinary least-squares protocols with the except of those in the MAA vs. the number of areoles scaling relationships that used standard major axis protocols. The significance of a slope was determined by observing whether the 95% CI included 0. The scaling relationship was judged to be significant if the 95% CIs of a slope did not include 1. The species codes are the same as those in Table 1. The degree to which the data points deviate from a regression line can be visually evaluated in Figs. S1–S5 in Appendix S1.

Total areole area and total leaf area

Different orders of veins contribute to the complex lamina “skeleton” with minor veins arranged over the lamina interconnecting the major veins and forming closed areoles. Such a reticulate venation pattern provides a significant hydraulic redundancy by
TABLE 3 The tested results for the correlations between mean areole area and leaf area of the nine Magnoliaceae species on a log-log scale

| Species code | y        | SD of y | a        | SD of a | r        | P       |
|--------------|----------|---------|----------|---------|----------|---------|
| 1            | -4.2771  | 0.5382  | -0.2858  | 0.1679  | -0.1248  | 0.0904  |
| 2            | -4.7187  | 0.3201  | -0.1163  | 0.0738  | -0.1365  | 0.1173  |
| 3            | -5.3226  | 0.2312  | 0.0288   | 0.0638  | 0.0265   | 0.6526  |
| 4            | -5.1472  | 0.2443  | -0.1655  | 0.0927  | -0.1291  | 0.0759  |
| 5            | -5.2537  | 0.2252  | 0.0448   | 0.0576  | 0.0487   | 0.4372  |
| 6            | -3.7107  | 0.3535  | -0.0429  | 0.0957  | -0.2621  | 0.6540  |
| 7            | -5.3648  | 0.1957  | 0.0496   | 0.0771  | 0.0423   | 0.5212  |
| 8            | -5.9753  | 0.4084  | 0.2369   | 0.1363  | 0.1560   | 0.0848  |
| 9            | -5.1762  | 0.1639  | -0.0191  | 0.0457  | -0.0218  | 0.6756  |

Here, y and a are the estimated intercept and slope, respectively; SD represents the standard deviation; r is the correlation coefficient; P is used to test whether the slope is significantly different from 0 at 0.05 significance level. All slopes are not significant since the P values are larger than 0.05.

providing alternative pathways in a vascular system (Strang, 1986; Roth-Nebelsick et al., 2001). The shape and size of individual areoles are determined by the topological structure of leaf veins, and the number of stomata within each areole is positively associated with the areole contour (i.e., the length of the looping vein contour, which can be regarded as an indicator of areole size) (Fiorin et al., 2016). The whole leaf area is the sum of total vein area and total areole area, thereby mirroring the biomass investment in photosynthetic tissues because the total areole area correlates with the area of mesophyll cells wherein photosynthesis is carried out, i.e., the larger the investment in veins and their bundle sheath extensions (Sack and Scoffoni, 2013), the lower the space available for mesophyll tissues (Lambers and Poorter, 1992; Niinemets et al., 2007a, b; Brodribb et al., 2010; Baresh et al., 2019). Additionally, for any constant cell shape, tissues composed of smaller cells have higher surface areas relative to total tissue volume compared to tissues composed of larger cells (Theroux-Rancourt et al., 2021; Borsuk et al., 2022). Thus, although areole size is an important variable of interest because it defines the amount of space available for mesophyll, cell size is also an important parameter. Smaller cells (perhaps partially enabled by smaller genomes [see Šimová and Herben, 2012; Simonin and Roddy, 2018; Roddy et al., 2020]) allow for larger mesophyll surface areas, which can offset reductions in areole size.

Mean areole area and areole density

Areole density quantifies the number of areoles per unit leaf area, which is highly correlated with the density of minor veins. Previous studies indicate that mesophyll resistance to water transport is higher than xylem resistance (Aasamaa et al., 2001, 2005; Brodribb et al., 2007), such that increasing vein area and decreasing mean areole area can enhance within-leaf water transport and thereby benefit photosynthesis. Research also indicates that increasing areole density increases hydraulic redundancy and can maintain the water supply when some veins are damaged (Nardini et al., 2001; Sack et al., 2008). Thus, a high areole density in theory can help to offset the negative effects of herbivory damage of leaves with hierarchical reticulate venation patterns (Boyce et al., 2009; Brodribb and Field, 2010). Our data indicate that mean areole area is not correlated with total leaf area for any of the nine species examined in this study. However, there is a strong inverse scaling relationship between mean areole area (MAA = TAA/n, where n denotes the number of areoles) and total areole density (n/A, where A denotes total leaf area the equals the sum of TAA and TVA). Given the proportional relationship between TAA and total leaf area (A), it follows that MAA is proportional to A/n, which is the reciprocal of total areole density. Relative to the number of areoles, the areole density has a stronger converse scaling relationship with mean areole area (Figure 3E vs. 3F). Put differently, it appears that the absolute number of areoles on a leaf tends to increase as leaf area increases, whereas areole density appears to be independent of total leaf area. The independence of areole density with respect to total leaf area helps to explain why foliage photosynthetic efficiency does not necessarily correlate with leaf size. In the context of physiological advantage, photosynthetic efficiency can be increased independent of size by decreasing the distance between the access of mesophyll tissues to water in the xylem and the access of photosynthates to phloem.

The effect(s) of leaf shape on scaling relationships

An as yet unexplored issue is the potential effects of leaf shape on the scaling relationships reported here. Leaf shape has been demonstrated to be closely related to leaf venation patterns (Runions et al., 2005, 2017). Yet, leaf shape does not appear to affect total vein density (and thus photosynthetic efficiency). To test whether leaf shape does in fact affect total leaf vein density, we used the leaf ellipticalness index (EI; see Li et al., 2021) to quantify leaf shape, i.e., \( EI = \frac{4A}{\pi LW} \), where A, L, and W denote leaf area, length, and width, respectively (data not shown). If EI approximately equals 1, leaf shape is approximately elliptical. Using the Pearson’s product moment correlation coefficient test with a 0.05 significance level, we found that eight of the nine species manifested no significant correlation between the ellipticalness index and leaf vein density (\( P > 0.10 \)). The exception was Michelia compressa (\( r = -0.21, \)
**FIGURE 3** Fitted scaling relationships (A) between midrib length and leaf area, (B) between total leaf vein length and leaf area, (C) between total leaf vein area and leaf area, (D) between total areole area and leaf area, (E) between the mean areole area and the number of areoles per leaf, and (F) between the mean areole area and areole density (i.e., the number of areoles per unit leaf area) for the pooled data of the nine Magnoliaceae species. The open circles are the observations (species color codes as in Figure 2); the solid line is the regression line; CI represents the 95% confidence intervals of the slope (i.e., the scaling exponent); $r^2$ is the coefficient of determination; $n$ is the sample size for the pooled data, i.e., the total number of leaves of all the nine Magnoliaceae species.

$P = 0.0014 < 0.01$). This exception is difficult to explain because the leaf shape of this species does not appear to manifest a difference with other *Michelia* species (see Figure 1). The explanation of this singular exception is beyond the scope of the present work. However, on the basis of the data from the remaining eight species, it is fair to say that leaf shape had no demonstrable effect on the conclusions reported here.
CONCLUSIONS

Leaf area tends to be proportional to the square of midrib length for the majority of the Magnoliaceae species examined in this study, but it does not scale with total leaf vein density. Leaf area is also proportional to total leaf-vein length, total leaf-vein area, and total areole area, but does not scale with mean areole area per leaf. Mean areole area inversely scales with the number of areoles per unit leaf area, as a consequence of the proportional relationship between total leaf areole area and leaf area, whereas mean areole area has no correlation with leaf area. The more areoles per leaf, the smaller the mean areole area. The proportional relationships between leaf length squared and leaf area and that between total leaf vein area and leaf area help to explain the inverse scaling of midrib density vs. leaf area, and the lack of a correlation between leaf vein density (or minor vein density regardless of major vein density) and leaf area across species. Although hierarchical reticulate venation networks may have become more complex over time, increases in total leaf vein length appears to keep pace with changes in leaf size measured as a proportional function of lamina length and width.

AUTHOR CONTRIBUTIONS

K.J.N., Ü.N., and P.S. designed the experiment, analyzed the data, wrote the manuscript, and contributed equally to this work; Q.M., M.L., Y.L., and K.Y. carried out the experiment; Y.L. and K.Y. participated in the writing of the Discussion section. All authors have read and approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository at https://doi.org/10.5061/dryad.8cz8w9gs (Shi et al., 2022).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1.

Figure S1. Fitted scaling relationships between midrib length and leaf area for each of the individual species.

Figure S2. Fitted scaling relationships between total leaf vein length and leaf area for each of the individual species.

Figure S3. Fitted scaling relationships between the total leaf vein area and leaf area for each of the individual species.

Figure S4. Fitted scaling relationships between the total areole area and leaf area for each of the individual species.

Figure S5. Fitted scaling relationships between the mean areole area and areole density (i.e., the number of areoles per unit leaf area) for each of the individual species.

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