

ZERO-SUM ALLOCATIONAL STRATEGIES DETERMINE THE ALLOMETRY OF SPECIFIC LEAF AREA¹

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- *Premise of the study:* Specific leaf area (SLA) is a critical component of the leaf economics spectrum, and many functional leaf traits have been empirically demonstrated to covary with SLA. However, a complete understanding of how change in leaf size influences SLA has not yet emerged.
- *Methods:* To help develop a more complete understanding of the determinants of variability in SLA, we present a covariation model of leaf allometry that predicts a zero-sum interdependence of leaf thickness, density, and surface area on leaf mass. We test the model's predictions on measurements of 900 leaves from 44 angiosperm species.
- *Key results:* We observe that “diminishing returns,” the negative allometry (slope < 1) of surface area versus mass, does not hold universally across species. Rather, the scaling of SLA is linked to the relative allocation to thickness and density. Specifically, diminishing returns are observed when leaves grow thicker, more than their density decreases, with increasing mass. Finally, we confirm model predictions that the allometric dependence of area, thickness, and density on mass can be well approximated by a zero-sum allocational process.
- *Conclusions:* Our work adds to the growing body of evidence that allometric covariation is a hallmark of the scaling behavior of complex plant and leaf traits. Moreover, because our model makes predictions based on allocational constraints, it provides a foundation to understand how deviations from zero-sum tradeoffs in allocation to leaf thickness, density, or area determine the allometry of SLA and, ultimately, underlie adaptive strategies within and across plant species.

Key words: allometry; leaf density; leaf mass per area; leaf thickness; LMA; photosynthesis; SLA; specific leaf area.

Recent studies of the form and functional traits of leaves have highlighted multiple scaling patterns that hold across plant species (Westoby et al., 2002; Poorter et al., 2009). These scaling patterns typically represent the relationship between some functional trait and a proxy for leaf size—for example, the scaling of leaf photosynthetic rate with leaf mass or the relationship between two functional traits (e.g., scaling of leaf life span with leaf mass per unit area (LMA)) (Reich et al., 1997; Wright et al., 2004) or scaling of net photosynthesis with leaf nitrogen content (Niinemets, 1999; Reich et al., 1999). The scaling of functional traits in leaves is of particular interest because, as the photosynthetic apparatus of higher plants, leaves constitute a major pathway for the flow of carbon in terrestrial landscapes (Schimel, 1995; Landsberg and Waring, 1997). Understanding the constraints on the scaling between leaf size and functional traits will improve our understanding of how leaves maintain a positive carbon balance and influence whole plant fitness, as well as inform physiological models of leaf growth.

Among the most influential and frequently measured traits is the ratio of leaf surface area to leaf mass (SLA) or, alternatively, its reciprocal, LMA (Roderick and Cochrane, 2002;

Westoby et al., 2002; Poorter et al., 2009). SLA represents the mass investment necessary to maintain a given surface area and has been shown to covary strongly with indicators of leaf performance such as leaf photosynthetic capacity per unit mass, life span, nitrogen and phosphorus content, mass-specific dark respiration rates (Reich et al., 1997; Wright et al., 2004), and, ultimately, plant growth rate (Lambers and Poorter, 1992), although several recent analyses of tropical forest trees suggest that this relationship may be weaker than previously suspected (Poorter et al., 2008; Wright et al., 2010). SLA is also used as a proxy for the relative ability of leaves to intercept light and photosynthesize. For example, for a given leaf nitrogen concentration, leaves with a higher SLA value have a higher net photosynthetic rate per unit mass (Reich et al., 1998). In addition, SLA varies with numerous environmental factors; for example, species in low-rainfall environments have thicker, denser tissue, on average—and, thus, lower SLA—than species in higher-rainfall environments (Cunningham et al., 1999; Fonseca et al., 2000; Westoby et al., 2002).

The relationship between leaf surface area, A_S (mm²), and leaf mass, M (g), is often expressed as an allometric relationship of the form

$$A_S = cM^\alpha \quad (1)$$

where α is a scaling exponent and c is a prefactor that may differ between species and whose units are mm²/g ^{α} . We use the term “prefactor” for the variable c in multiplicative relationships of the form $y = cx$ and use the term “intercept” for the variable d in algebraic relationships of the form $y = cx - d$. It has been noted that as leaves increase in mass, increases in surface area often fail to keep pace with the increases in mass, both

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within and across species (Milla and Reich, 2007; Niklas et al., 2007; Price and Enquist, 2007). Empirical estimates of α are frequently less than 1, known by convention as an example of a negative allometry (Niklas, 1994), in which area increases more slowly than mass. One hypothesized driver of this negative allometry is that as leaves grow, they must increase their investment in structural and/or defense tissue, a phenomenon characterized as one of “diminishing returns” (Niklas et al., 2007; Niklas and Cobb, 2008). Alternatively, an allometric covariation model argues that the geometry of the vessel network determines the value of α (and several other scaling relationships in leaves), which need not be less than 1 (Price and Enquist, 2007). These two hypotheses are not mutually exclusive, and it is likely that network supply constraints and allocation to structure and defense both influence the scaling of leaf form.

Leaf mass can be expressed as being equal to leaf area multiplied by leaf thickness, T (mm) multiplied by leaf density, ρ (g/mm^3), or $M = A_s T \rho$. Therefore, the finding of negative allometry in the scaling of SLA can be recast as $A_s = M/(T\rho)$, or

$$\frac{A_s}{M} = \frac{1}{T\rho} \quad (2)$$

Predictions from this and similar algebraic rearrangements were highlighted by Niklas et al. (2007) as the origin of “diminishing returns” in leaves. Similarly, Roderick and Cochrane (2002) used this relationship and referred to $1/(T\rho)$ as the “Hughes constant” after an earlier paper that described isometry between surface area and leaf water mass (Hughes et al., 1970). Specifically, if $\alpha = 1$, then $1/(T\rho)$ must be statistically independent of leaf size. Similarly, if $\alpha < 1$, then $1/(T\rho)$ will decrease with increasing leaf mass. This rearrangement of the scaling of SLA implies that the basis for “diminishing returns” involves the scaling of both leaf density and thickness.

Previously, it has been shown that both density and thickness can vary with changes in leaf mass (Witkowski and Lamont, 1991; Niinemets et al., 2007), and one study indicated that $1/(T\rho)$ is “more or less” constant within species (Roderick and Cochrane, 2002). Here, we develop an allometric covariation model of the interdependence of leaf area, thickness, and tissue density with changes in leaf mass. We show that increases in leaf mass necessarily involve allocational tradeoffs and that these tradeoffs give rise to intraspecific scaling relationships of leaf traits, including that of SLA (Jurik, 1986). We test the model on a large data set of intraspecific allometric relationships for angiosperm leaves. The data set spans more than six orders of magnitude in leaf mass and comprises 900 leaves from annual and perennial herbs, trees, shrubs, and vines from 44 species and 31 families, constituting a broad sampling of angiosperm diversity (see Materials and Methods).

We first examine the correlation between scaling exponents and intercepts to test the interdependence of allocational strategies across a range of intraspecific leaf sizes. Specific predictions of our tradeoff model are that the sum of the scaling exponents describing how area, thickness, and density change with mass equal 1; and that the product of the intercepts for those same relationships also equals 1—predictions that we confirm in our data set (see Scaling Models). Next, we evaluate whether α is typically less than 1 as has previously been suggested (the “diminishing returns” hypothesis) and find that diminishing returns are found in the majority of cases (26 of 44), though it is not universal in the leaf data set we have compiled.

Finally, we demonstrate that diminishing returns are typically observed when thickness increases with mass more than density decreases with mass, which suggests that allocational strategies determine the scaling of SLA.

Scaling Models—The scaling of SLA depends, in part, on the following variables: leaf area (A_s), leaf thickness (T), tissue density (ρ), and leaf mass (M). Leaf traits are often approximated as power-law functions of leaf size (Milla and Reich, 2007; Price and Enquist, 2007; Niklas et al., 2009), a finding that holds within our data (see Results). We take a general approach in developing a scaling model of leaf form by assuming that A_s , T , and ρ scale allometrically with M . Hence, in addition to Equation 1, thickness and density vary with mass as follows:

$$T = aM^\gamma \quad (3)$$

and

$$\rho = bM^\beta \quad (4)$$

where γ and β are scaling exponents and a and b are prefactors whose units are $\text{mm}/\text{g}^\gamma$ and $\text{g}^{1-\beta}/\text{mm}^3$, respectively. These exponents are not strictly independent, because the product of area, thickness, and density should be equal to mass. Substituting the righthand side of Equations 1, 3, and 4 in Equation 2 and rearranging leads to the following constraint among the exponents and prefactors:

$$\text{Log}(M) = \text{Log}(abc) + (\alpha + \beta + \gamma)\text{Log}(M) \quad (5)$$

where all logarithms in Equation 5 and throughout this manuscript are done in base-10 with no loss of generality. Therefore, $\alpha + \beta + \gamma = 1$, and $abc = 1$. As long as power-law relationships among these traits hold, then

$$\alpha = 1 - (\beta + \gamma) \quad (6)$$

Equation 6 is an example of an allometric constraint that should hold regardless of the particular value of scaling exponents (Price and Enquist, 2007; Price et al., 2007).

Allocational strategies are therefore a zero-sum process leading to constraints among intraspecific scaling exponents. A zero-sum process is one in which increases in the allocation to one trait takes away from allocation to other traits. For example, given a fixed amount of mass to allocate, increases in tissue density will necessarily lead to relative decreases in leaf area and/or thickness. Specifically, Equation 6 predicts that an interspecific regression of α against $\beta + \gamma$ should have a slope of -1 and intercept of 1. Therefore, a finding of negative allometry of SLA depends on the scaling of both thickness and density, as we explain next. Note that the type of interspecific relationships we describe here differ from many prior interspecific explorations of SLA. In prior work, the specific leaf area of mature leaves was compared between species. Here, we are primarily concerned with the change in intraspecific allometries (e.g., the intraspecific scaling of leaf surface area with leaf mass) as compared between species.

When SLA decreases with leaf mass, $\alpha < 1$, which conforms to the “diminishing returns” hypothesis. A scaling exponent of $\alpha < 1$ implies that $\beta + \gamma > 0$ (see Equation 6), which can occur only if $\gamma > -\beta$. Such a condition can hold in one of three ways,

depending on the sign of the allometry, either positive or negative, of thickness and density: (1) thickness increases with mass faster than density decreases with mass ($\gamma > 0$, $\beta < 0$); (2) thickness decreases with mass slower than density increases with mass ($\gamma < 0$, $\beta > 0$); or (3) thickness and density are invariant or both increase with mass ($\gamma \geq 0$, $\beta \geq 0$). In our empirical analyses it is overwhelmingly the first case that is observed (i.e., γ is positive and β is negative). Likewise, if SLA increases with leaf mass ("increasing returns"; i.e., $\alpha > 1$), this implies that $\beta + \gamma < 0$, which can occur only if $\gamma < -\beta$. Ancillary scaling predictions may also be made using this framework. For example, a finding of invariant SLA with mass implies that $(\beta + \gamma) = 0$ (i.e., changes in thickness are in balance with changes in tissue density with mass).

MATERIALS AND METHODS

The relationships described in Equations 1–6 hold precisely only if one uses data from either fresh leaves or dried leaves consistently throughout. However, it is common in studies such as these to report relationships based on dry mass and, in the same study, bulk tissue density (dry mass/fresh volume) using surface area and leaf thickness measured on fresh leaves. This is done primarily because the ratio of dry mass to fresh leaf surface area is proportional to dry-mass investment per unit leaf area deployed (Wright et al., 2004). It is difficult to measure the surface area of dried leaves because of the contraction of the leaf upon desiccation, which makes the assumption of a relatively flat surface invalid. Similarly, it is difficult to measure the thickness of dried leaves with calipers, primarily because the dried leaves break easily. Therefore, to remain internally consistent throughout, we present parallel analyses on both fresh and dry leaves. We report scaling relationships based on fresh leaf mass and fresh tissue density (fresh mass/fresh volume). We also report scaling relationships based on dry leaf mass and bulk tissue density (dry mass/fresh volume), but note that surface area and thickness were measured on fresh leaves.

We collected leaves representing 900 individual leaves from 44 species; most species were represented by 20 individuals each, with as large a range of leaf sizes within a species as could be found (Appendices S1 and S2; see Supplemental Data at <http://www.amjbot.org/cgi/content/full/ajb.1000118/DC1>). Leaves exposed to full sun were collected during the summer of 2007 from plants in the greater Atlanta region (33°75'N, -84°38'W). Species were selected for collection on the basis of local availability and whether their leaves would fit on a 9.5 × 14 inch scanner. For each fresh leaf, lamina thickness was measured with digital calipers (increment = 0.01 mm) at two locations, one near the base and a second near the leaf apex (Roderick and Cochrane, 2002). Measurements were made close to the leaf margin, between secondary veins whenever possible, so as to represent increases in thickness that were not due to major veins. Fresh leaves were digitally scanned at 600 dpi, and surface area (A_s , mm²) was measured with image analysis software (Scion Image Beta 4.0.2; www.scioncorp.com).

To measure leaf tissue density (bulk and fresh; g/mm³), leaf disks were obtained from each leaf, using a hole-punch at the sites where lamina thickness was measured (Cornelissen et al., 2003). The hole-punch had a constant area (~30.9 mm²), which was multiplied by thickness to obtain volume. The mass of each leaf disk was measured, and then mass was divided by volume to estimate density. The average of these two density measures (two disks) was used. Nine of the species we collected had leaves that were too small to collect leaf disks (i.e., the punch diameter was greater than leaf width). For these species, tissue density was estimated as the ratio of mass to whole leaf volume (the product of surface area and thickness). For four of the species, tissue density samples were mechanically degraded during storage and unusable; thus, dry density values are not reported for those species (Appendix S2). All leaves and density samples were then dried to constant mass in a drying oven at 60°C.

The drawback of the current approach is that leaf density as measured does not include contributions from veins. However, we chose this approach to determine tissue density over alternative approaches based on Archimedes' principle (Raskin, 1983) to achieve the concordance between thickness and density measures. Moreover, density measures based on Archimedes' principle are not without complications, primarily the influence of small bubbles on water displacement potentially affecting volume measures.

Bivariate scaling relationships for the power-law relationships described in Equations 1, 3, and 4 were analyzed by fitting standardized major axis (SMA)

regression lines to Log scaled variables. When there is likely to be equivalent measurement error in both the X and Y variables, SMA regression techniques allow for a better estimate of a line fitting both variables than ordinary least-squares regression (OLS; Sokal and Rohlf, 1995; Warton et al., 2006). SMA regression statistics for intraspecific relationships (Appendix S1) were calculated using the statistical program SMATR (Falster et al., 2003). To test whether the mean SLA and α were different between annual and perennial species, we used a simple one-way analysis of variance.

The zero-sum theory predicts two constrained relationships among allometric prefactors (Log (abc) = 0) and scaling exponents ($\alpha + \beta + \gamma = 1$). However, there is error in the intraspecific slope estimates for α , β , and γ and in the prefactor estimates for a , b , and c . We used a bootstrap approach to account for this error in testing the general validity of the zero-sum allocational strategy. The results for the SMA regression statistics for intraspecific relationships contain 95% confidence intervals (CIs). These were used to generate a normal distribution surrounding each slope and intercept. We then drew one value at random from each of these distributions for the 44 species and calculated the intercept product (abc) and the exponent sum ($\alpha + \beta + \gamma$). For the product of intercepts, we evaluated the null hypothesis, H_0 : Log (abc) = 0 (see Equation 5). For the exponent sum, we evaluated the null hypothesis, H_0 : ($\alpha + \beta + \gamma$) = 1 (see Equation 5). We calculated our p values using a two-sided hypothesis test directly on the resampled data.

Next, to evaluate the hypothesis that the exponent from Equation 1, α , is negatively related to the sum of the thickness and density scaling exponents, $\beta + \gamma$, we again used a bootstrap approach. The results for the SMA regression statistics for intraspecific relationships contain 95% CIs. These were used to generate a normal distribution surrounding each slope estimate. We drew one value at random from normal distributions for the exponents of 44 species generated from the SMA regression statistics. We then fit an SMA regression line between α and $\beta + \gamma$. This procedure was repeated 10000 times to yield distributions for the slope estimates for Equation 6.

RESULTS

Power-law scaling—Using a data set of 900 leaves from 44 species, we find that the relationship between surface area and mass (Equation 1) in angiosperm leaves is very well approximated by a power law. The average coefficient of determination (R^2) for this relationship across all species examined is 0.99 (fresh leaves) and 0.95 (dry leaves) (Appendices S1 and S2). For fresh leaves, 18 of the 44 species (41%) had values for α (Equation 1) greater than 1, leaving 26 (59%) with values less than 1 (Figs. 1, 3, and 4; Appendices S1 and S2). Thus, for a majority of species SLA decreased as mass increased. Eleven of the 44 (25%) species had 95% CIs for α that included 1. Across all species, mean α was 0.995 (95% CIs 0.98–1.01, $R^2 = 0.95$, $N = 900$), which is indistinguishable from 1.

For dried leaves, 18 of the 44 (41%) species had values for α (Equation 1) greater than 1, leaving 26 (59%) with values less than 1. Although these are the same proportions found in the fresh leaves, they are not the same species; some species switched from fresh-estimated α greater than 1 to less than 1 when using dry-estimated α (and vice versa) (Appendix S1). Twenty-three of the 44 dried species had confidence intervals for α that included 1. The interspecific regression value of α for dried leaves was 0.970 (95% CIs 0.95–0.98, $R^2 = 0.093$, $N = 900$), which is slightly less than 1.

Next, bivariate plots of thickness or density versus mass reveal that these relationships are usually linear on a logarithmic scale. However, many of the intraspecific slope values for b and g are not different from zero, with low correlation coefficients (Appendices S1 and S2). This should not be interpreted as lack of support for our model. In fact, our scaling model predicts that the exponents β and γ will approach zero (Figs. 3 and 4) whenever the sum of α and one of the other exponents (γ or β) approaches 1. For example, if $\alpha + \beta = 1$ then $\gamma = 0$. Moreover, low R^2

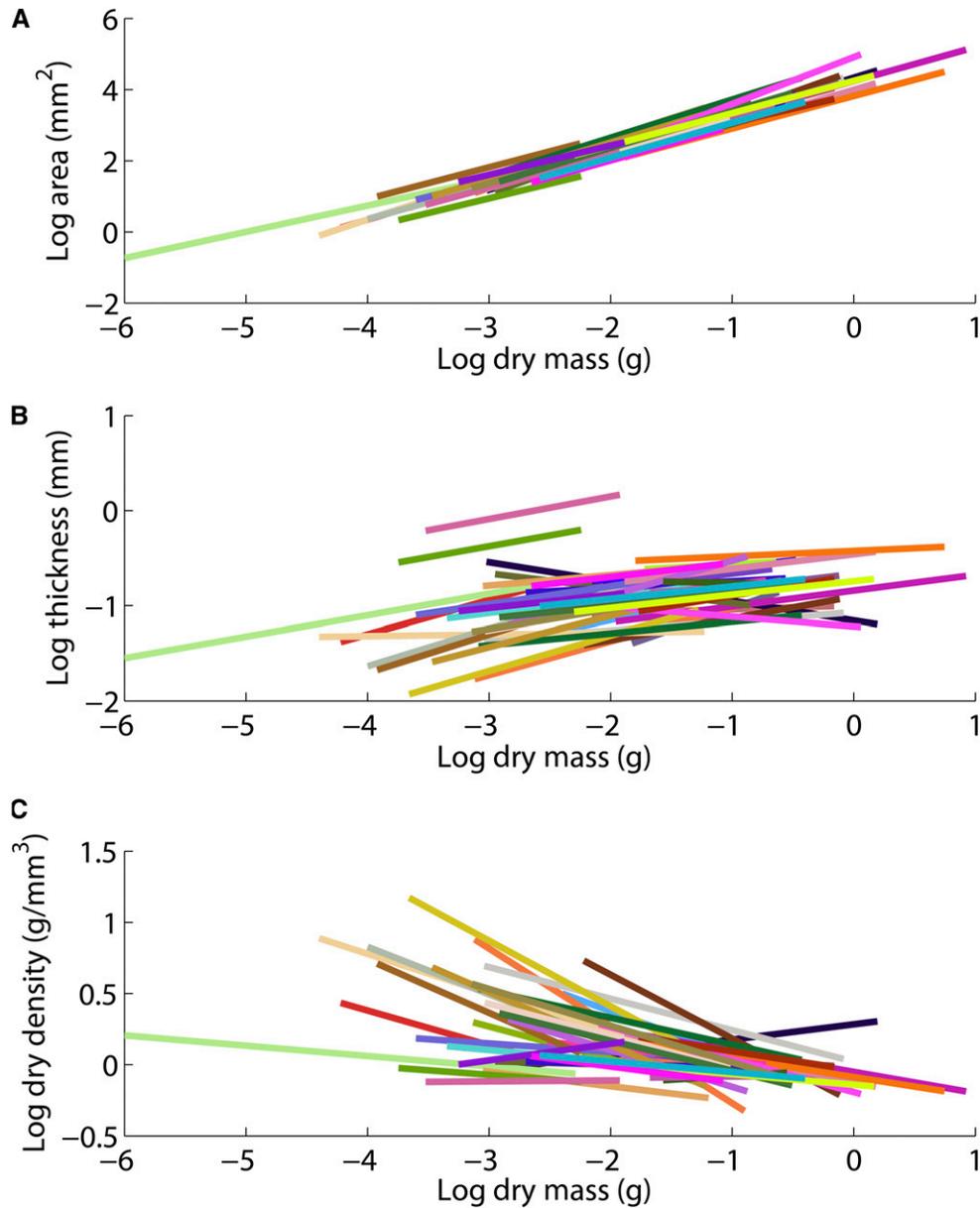


Fig. 1. Regression slopes of (A) surface area versus dry mass, (B) thickness versus dry mass, and (C) density versus dry mass for the 44 species we examined. Each color corresponds to a particular species. In the interest of figure clarity we have not presented the actual data points (details on regressions are available in Appendix S2). Note the low slope variability in panel A, in contrast with higher variability in B and C. In addition, as noted in the text and reported in Appendix S1, the slopes in B and C are often approximately opposite.

values are expected when slopes approach zero, because R^2 denotes the fraction of variance in the ordinate (y -unit) explained by the abscissa (x -unit). When slopes approach zero, the variation in the ordinate is independent of the abscissa (Warton et al., 2006).

Allometric covariation—To test the predictions of our model, we explored the zero-sum relationship among the intraspecific allometric exponents and intercepts described above. First, the distribution of summed exponents ($\alpha + \beta + \gamma$) is centered around 1 (Fig. 2A). The mean of the distribution of resampled sums yields a p value $\ll 0.0001$, so we cannot reject the hypothesis that exponents are constrained, on average, as predicted

from our zero-sum theory. In addition, as predicted, the distribution of the Log of intercept products (abc) is centered around zero (Fig. 2B). The mean of the distribution of resampled products yields a p value $\ll 0.0001$, which suggests that we cannot reject the hypothesis that intercepts are constrained, on average, as predicted.

For the intraspecific regression data, for fresh leaves, the average value for the sum of α , β , and γ was 0.98 (95% CI 0.96–1.00; Appendix S1) and, thus, not statistically different from 1, and the average value for the product of abc is 1.01 (95% CI 0.83–1.19), which supports our model assumptions. The average value for β was -0.19 (95% CI -0.24 to -0.13), and the average value for γ was 0.20 (95% CI 0.15–0.26).

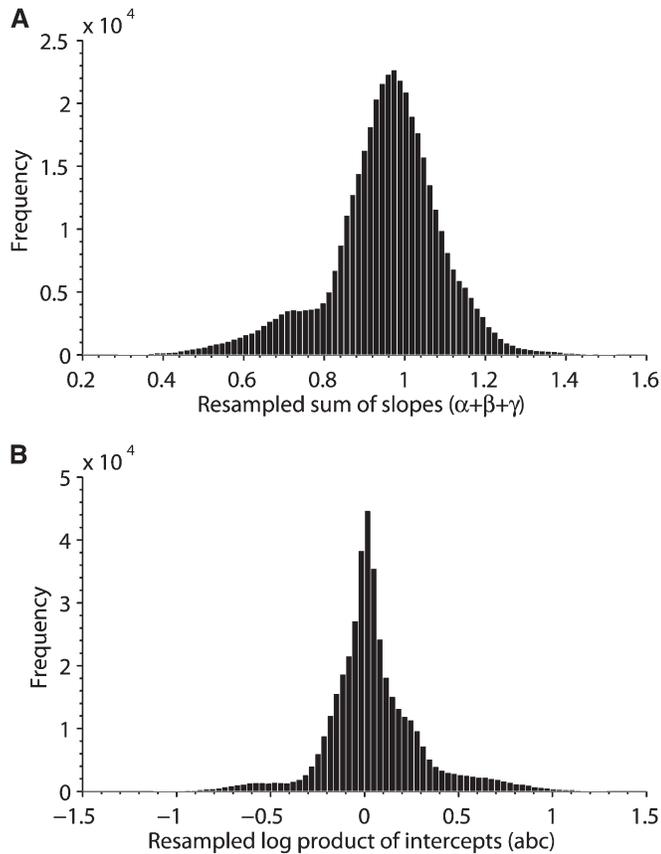


Fig. 2. Consistent with Equation 5, (A) the bootstrapped distribution of summed exponents ($\alpha + \beta + \gamma$) is centered around 1, and (B) the distribution of the Log of intercept products (abc) is centered around zero. This indicates that the covariation of exponents and intercepts predicted by the zero-sum model is robust, on average, even when accounting for error in species-level regression coefficient estimates.

For dried leaves, the average value for the sum of α , β , and γ was 1.00 (95% CIs 0.93–1.07) and, thus, not statistically different from 1. The average value for the product of abc was 1.32 (95% CIs 1.03–1.61). The average value for β was -0.21 (95% CIs -0.29 to -0.14), and the average value for γ was 0.21 (95% CIs 0.15 – 0.26).

Although the zero-sum expectations were consistent with the bootstrapped distributions shown above (and in Fig. 2), in theory this sum could have held even if the particular relationship among its individual exponents did not. Therefore, we next evaluated covariation among the exponents. Consistent with the predictions of Equation 6, across all leaves and species as the sum of β and γ increases, α decreases (Fig. 3). The SMA regression slope (α vs. $\beta + \gamma$) for the fresh leaves is -0.713 (95% CIs -0.836 to -0.609 , $R^2 = 0.74$), and the intercept is 0.976 (95% CIs 0.959 – 0.993). The SMA regression slope for the dried leaves is -0.693 (95% CIs -0.861 to -0.558 , $R^2 = 0.508$), and the intercept is 1.00 (95% CIs 0.978 – 1.03). The mean bootstrapped estimate of the slope of α versus $\beta + \gamma$ for the fresh leaves is -0.71 , with a standard deviation of 0.052 . For dried leaves the slope is -0.74 , with a standard deviation of 0.07 . Note that although the regression slope is negative, as expected, its confidence intervals do not include -1 , which implies that details of the zero-sum allometric covariation model

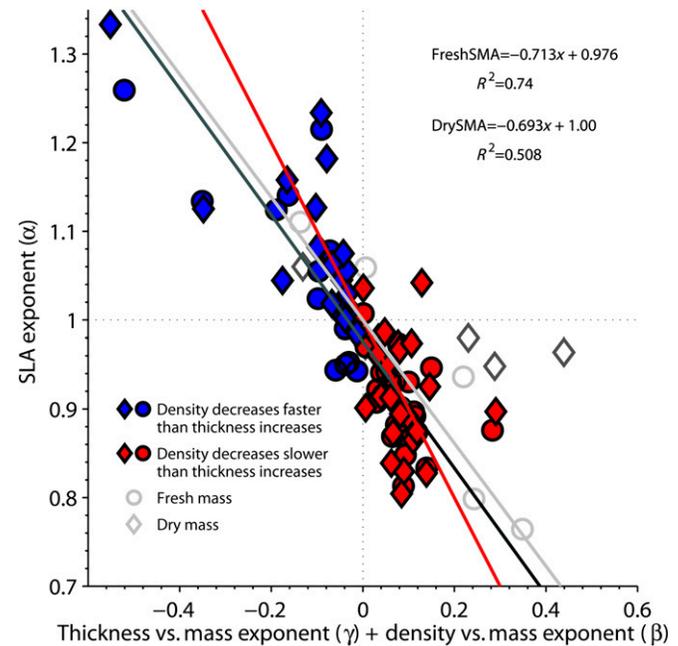


Fig. 3. Exponent of the relationship of surface area and mass relationship (α ; Equation 1) as a function of the sum of the density-versus-mass and thickness-versus-mass exponents (β and γ ; Equation 6) for each of the 44 species. When the sum of β and γ is close to zero (dashed vertical line), α is close to one (dashed horizontal line). Additionally, when the sum of β and γ is greater than zero, density is decreasing slower than thickness is increasing. Similarly, when the sum of β and γ is less than zero, thickness is increasing slower than density is decreasing. The black line and equation are from SMA regression. The red line is the model prediction. Open circles represent the few cases in which both β and γ were positive or negative. Note the greater variance explained for the fresh-leaf data.

presented here may need modification to incorporate heterogeneity or other covariation in leaf structure.

Having established the negative relationship between α and $\beta + \gamma$, we next evaluated the relationship between β and γ themselves. Again, consistent with the negative relationship predicted by Equation 6, as β increases, γ decreases (Fig. 4). The SMA regression slope for the fresh leaves is -0.986 (95% CIs -1.23 to -0.786 , $R^2 = 0.465$), and the intercept is 0.019 (95% CIs -0.043 to 0.08). The SMA regression slope for the dried leaves is -0.981 (95% CIs -1.24 to -0.775 , $R^2 = 0.418$), and the intercept is 0.022 (95% CIs -0.043 to 0.088). The mean bootstrapped estimate of the slope of γ versus β for the fresh leaves is -0.99 , with a standard deviation of 0.06 . For dried leaves the slope is -0.97 , with a standard deviation of 0.07 . The value of γ versus β also determines the relative allometry of SLA. In Figure 4, we label those combinations of exponents that should, according to Equation 6, predict diminishing returns or increasing returns. Note that the empirical measurements of the allometry of SLA almost uniformly agree with theory, where red-filled symbols denote an intraspecific measurement of $\alpha < 1$ and blue-filled symbols denote an intraspecific measurement of $\alpha > 1$.

Our model also predicts that when α is approximately equal to 1, γ and β will both be zero or will be approximately equal with opposite signs. For fresh leaves, 40 of 44 species (91%) had γ (density vs. mass exponents) values greater than zero. Similarly, 39 of 44 species (89%) had β (thickness vs. mass exponents) values less than zero. For 39 of 44 species (89%), the signs of β and γ were

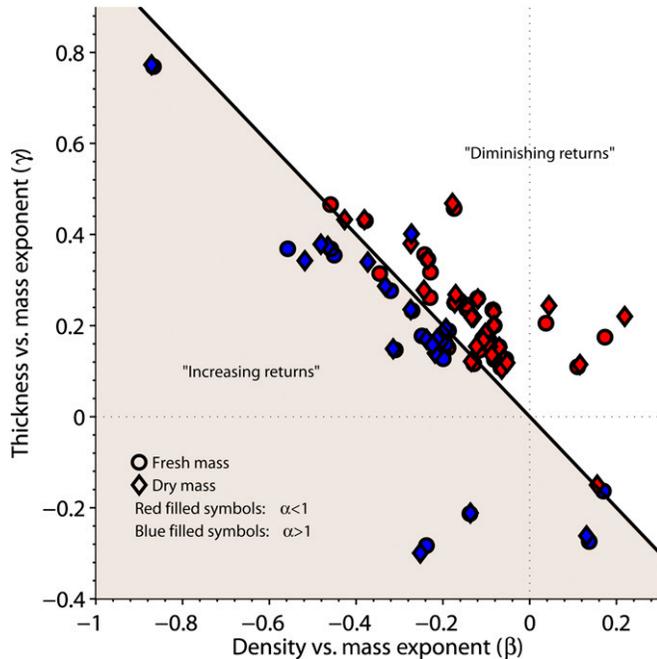


Fig. 4. Thickness-versus-mass exponent as a function of the density-versus-mass exponent (Equations 3 and 4) for each of the 44 species. Note that rearranging Equation 6 predicts that $\gamma = 1 - (\alpha + \beta)$. When α is 1, $\gamma = -\beta$ (mean $\alpha_{\text{FRESH}} = 0.97$, mean $\alpha_{\text{DRY}} = 0.99$). The line $\gamma = -\beta$ separates the covariation space into regions of “diminishing returns” (upper unshaded region) and “increasing returns” (lower shaded region). Red symbols are those for which the value of measured α is less than 1. Blue symbols are those for which the value of measured α is greater than 1.

opposite. For dried leaves, 40 of 44 species (91%) had γ (Equation 4) values greater than zero. Similarly, 36 of 41 species (89%) had β (Equation 3) values less than zero. For 36 of 41 species (89%) the signs of β and γ were opposite.

To evaluate the covariation between allocation to thickness and density predicted by Equation 6, we regressed Log leaf thickness against Log tissue density. For fresh leaves, 41 of 44 species (93%) had slopes that were less than zero, indicating that as thickness increases within species, density decreases. Notably, the three values for slopes that were greater than zero were not statistically different from zero (Appendices S1 and S2). For dried leaves, 37 of 41 species (90%) had values for the thickness-versus-tissue-density slope that were less than zero. For three species, dry density increased with thickness (S1).

Fresh and Dried Leaves—The correlation coefficients for regressions based on fresh mass and fresh density explain more variation than those based on dry density and dry mass (Figs. 3 and 4, Appendices S1 and S2). While in some cases this difference is small (e.g., Fig. 3), the observed differences may be attributable to the issue of internal consistency mentioned at the beginning of the Methods section. This has the potential to make accurate prediction difficult. This is particularly problematic with respect to SLA because the ratio of fresh leaf surface area to dry leaf mass necessarily introduces such an inconsistency.

Annuals vs. Perennials—We observe significant ($p < 0.001$) differences in mean SLA between the annuals (= 269.5)

and perennials (= 220.5) in our data. However, we find no difference between mean α or c between annuals and perennials ($p = 0.84$ and $p = 0.15$ respectively).

DISCUSSION

The interplay between leaf surface area, mass, thickness, and density has been an active area of inquiry for decades (Witkowski and Lamont, 1991; Niinemets, 1999), yet relatively few studies exist in which all these traits were measured simultaneously (Garnier et al., 1999; Poorter et al., 2009). The link between morphological and functional traits is of particular importance in understanding how leaves assimilate carbon for plants and contribute to overall growth (McMillen and McClendon, 1983; Lambers and Poorter, 1992). Here, we have demonstrated that a central leaf trait, SLA, does not universally exhibit diminishing returns. Further, we showed that the scaling of SLA is well approximated as a zero-sum process (where a gain in allocation to one trait is offset by an equal loss to another trait or combination of traits) that ultimately depends on differential allocation to leaf area, thickness, and density.

A majority of the species (59%) had values for the SLA scaling exponent, α , less than 1. This is the pattern characterized as “diminishing returns” by Niklas (Niklas et al., 2007). However, 41% of species had values >1 , which indicates that the “diminishing returns” phenomenon does not hold universally, which is consistent with results from other recent work (Milla and Reich, 2007; Price and Enquist, 2007).

We have also demonstrated that a suite of leaf traits exhibit interspecific allometric interdependence. The allometric covariation model we developed (Equations 5 and 6) predicts that as leaves increase in size, a negative correlation between allocation to thickness or density ultimately determines whether the increase in surface area exceeds, keeps pace with, or lags behind the increase in mass. As illustrated in Figure 4, for those species in which thickness increases faster than density decreases ($\gamma > -\beta$; $\gamma > 0$, $\beta < 0$), the value of their exponent for the scaling of surface area and mass is generally <1 . Alternatively, for those species in which thickness increases slower than density decreases ($\gamma < -\beta$, $\gamma > 0$, $\beta < 0$), the value of the SLA scaling exponent α is generally >1 . This relationship also ultimately determines whether species increase or decrease in SLA with mass. Given the positive correlation between SLA and mass-specific photosynthetic capacity (Reich et al., 1998; Wright et al., 2004), this suggests that some species become more efficient at photosynthesis, whereas others become less efficient as leaf size increases, and that this efficiency is likely related to ontogenetic allocational strategies. However, in the absence of simultaneous morphological and functional data, the link between photosynthetic efficiency and allocational strategy remains speculative at present.

As described by Equation 6, the scaling of surface area, thickness, and density with mass is essentially a zero-sum process. It is interesting to note that the species values of β and γ were often approximately opposite, and across species these values are negatively correlated: the interspecific means for β and γ were roughly opposite in both the fresh- and dry-leaf analyses. This is also demonstrated in that the slope of the SMA regression between β and γ is not statistically different from -1 . Moreover, as the sum of β and γ departs from zero, α is much different from 1 (Fig. 3), and the mean sum of all three (α , β , and γ) is not statistically different from 1 whether based on

fresh- or dry-mass regressions (Appendix S1). This indicates that as leaves modify any one of these allometric traits in response to selective pressures the other traits must change accordingly (i.e., $\alpha + \beta + \gamma \approx 1$), and $abc \approx 1$. Identifying the selective pressures that lead to differentiation along the axes of allometric covariation in Figures 3 and 4 will further our understanding of the links between environment, the development of leaf form, and traits that contribute to photosynthetic efficiency (Poorter et al., 2009).

The physiology of leaves obviously differs in important and meaningful ways from the simple geometric abstraction of a homogeneous leaf we have presented here. Indeed, not all of our model predictions were met exactly. For example, the allometric covariation slopes in Figure 3 differ from the simple expectation of -1 (see Equation 6). This may simply be due to variability in the particular collection of species we examined, or to departure from power-law behavior. Although visual inspection and the high correlation coefficients we observe suggest that a power law is an appropriate choice here, we cannot rule out the possibility that a more parameterized model involving a nonlinear term could explain more residual variation while accounting for the increase in model complexity. Alternatively, this systematic departure may arise because of violations of the assumption of leaf homogeneity. For the purposes of the present study, we have made the common simplifying assumption that subsampling can serve as a proxy for thickness and density measures. However, leaf tissue is a complex composite of many different types (Garnier et al., 1999) that may vary in their fractional contributions over ontogeny. For example, a thickening of cell walls and increasing chloroplast concentration likely occur during leaf ontogeny (Poorter et al., 2009). Previous work in some collections of species has shown that the midrib and petiole contribute an increasing proportion of total leaf biomass (Niinemets et al., 2007). Further, the densities of veins and lamina can vary, with primary veins typically having denser tissue than lamina, particularly as leaves increase in size and rely on primary and secondary veins for structural support. Assuming that area was unaffected, both cell-wall thickening and increasing fraction of biomass dedicated to structural support via veins would serve to increase SLA in ways not accounted for by our model. Thus, a more complete theoretical and empirical accounting of the scaling of leaf mass, area, thickness, and density would break down the contributions from these different components (Roderick et al., 1999). Nonetheless, it is striking how much of the observed variation in SLA can be explained using a zero-sum model of allocation.

The negative relationship between SLA and leaf life span has been well documented (Wright et al., 2004) and contributes to one of the central axes of plant niche differentiation, the slow-growth-to-fast-growth continuum (Grime, 1979; Poorter et al., 2009). Although we observe significant differences in mean SLA between the annuals and perennials in our data, we find no difference between mean α or c between annuals and perennials. This suggests that the group-level differences are due to a shift along a common axis (i.e., that of leaf mass), but not of allocation.

In closing, the model we have presented here is internally consistent, with no free parameters, and allows us to predict the covariation and normalization of the relationships among key allometric traits that underpin the leaf economic spectrum. In particular, our model (1) predicts the covariation of thickness, density, mass, and area and how these traits combine to influence the allometry of SLA; (2) highlights a zero-sum relation-

ship underpinning the SLA scaling; and (3) provides an intuitive framework for the theoretical segregation of leaves with increasing and decreasing returns in surface area for their investment in mass. This work adds to a growing body of work highlighting allometric covariation as a hallmark of plant functional trait diversity. A deeper understanding of the environmental conditions that select for various combinations of allometric traits promises to further our understanding of plant trait covariation in general, and to expand our understanding of the key traits that influence whole-plant carbon balance.

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