American Journal of Botany

American Journal of Botany 100(10): 1936–1948. 2013.

SPECIAL INVITED PAPER

Allometry of cells and tissues within leaves[1]

Grace P. John, Christine Scoffoni, and Lawren Sack[2]

Department of Ecology and Evolutionary Biology, University of California, Los Angeles, Los Angeles, California 90095-1606, USA

- *Premise of the study:* Allometric relationships among the dimensions of leaf cells, cell walls, and tissues, and whole-leaf thickness and area are likely to have key implications for leaf construction and function, but have remained virtually untested, despite the explosion of interest in allometric analysis of numerous plant properties at larger scales.
- *Methods:* Using leaf transverse cross sections and light microscopy, we measured leaf dimensions, tissue thicknesses, mesophyll and xylem cell sizes, and cell wall thicknesses for 14 diverse angiosperm species of wet and dry habitats and tested hypothesized allometric relationships based on geometric scaling due to development and/or function.
- *Key results:* We found strong novel allometries relating the dimensions of cells, cell walls, tissues, and gross leaf form. Cell sizes and cell wall thicknesses tended to scale isometrically across mesophyll tissues within the leaf, such that species with large cells or thick cell walls in one tissue had these also in the other tissues; however, leaf vein xylem conduit sizes were independent of those of other cell types. We also found strong geometric scaling of cell wall thicknesses with cell sizes throughout the mesophyll, but not in the leaf vein xylem. Further, leaf thickness scaled with cell sizes, cell wall thicknesses and the thicknesses of component mesophyll tissues, but leaf area was independent of anatomical traits across species.
- *Conclusions:* These novel allometries suggest design rules operating at the smallest scales of leaf construction and the possibility of applying these relationships to better characterizing the basis for differences among species in leaf form and functional traits.

Key words: allometry; bundle sheath; development; leaf traits; mesophyll; scaling.

For over 100 years, plant scientists have studied the construction of tissues and of whole plants on the basis of cell properties. For example, in Katherine Esau's classic *Plant Anatomy* (Esau, 1965), each tissue was described first in terms of cells and cell walls, before discussion of how the larger scale structures are developed. However, there has been little quantitative study of the properties of plant cells in diverse species. In particular, although the allometric approach to understanding variation of organ sizes and functions with plant size has yielded insights at a wide range of scales, from the early work of Pearsall (Pearsall, 1927) and Huxley (1932 [reprint 1993]) to more recent work on the mechanical design of organs and whole plants (Niklas, 1992, 1994), there has been no previous quantification of allometric relationships among the dimensions of cells, cell walls, and tissues within leaves, even in the most well-known texts that describe allometry in plants (e.g., Niklas, 1992, 1994; Brown and West, 2000).

[1]Manuscript received 20 November 2012; revision accepted 13 August 2013.

A portion of this work was presented at the Katherine Esau Research Symposium, "Integrating Plant Structure with Function, Development and Evolution," March 29, 2012, University of California, Davis.

The authors thank M. Culiffo, A. Patel, J. Smith, and C. Vuong for logistical assistance and V. Savage for helpful discussion. This work was supported by National Science Foundation grants IOS-0753233 and IOS-1147292 to L.S. A portion of this work was presented at the Katherine Esau Research Symposium, "Integrating Plant Structure with Function, Development and Evolution," 29 March 2012, University of California, Davis.

[2]Author for correspondence: (e-mail: lawrensack@ucla.edu); phone: 310-825-6525; fax: 310-825-9433

doi:10.3732/ajb.1200608

Our aim was to test a series of key hypotheses for fundamental allometries in leaf cells and tissues. Our hypotheses were developed based on the general expectation that any two anatomical traits x and y will show power law relationships, i.e., in the form of $y = ax^b$, linearized using log-transformed data to log $y = \log a + b \log x$, where a and b are constants; b is known as the allometric slope, and log a is the allometric intercept. We tested expectations of geometric or "dimensional" scaling, i.e., that traits representing lengths (L), areas (A) or volumes (V) will scale together as expected from geometry; thus, $L \propto L^1$, $L \propto A^{1/2}$, $A \propto V^{2/3}$ (Niklas, 1992, 1994). Geometric scaling is to be expected if across species that vary in cell and leaf size, the dimensions of all components increase proportionately. That would be the case, for example, if there exists a developmental coordination of cell expansion rates and/or times for all the cells in the different leaf tissues and if the thickening of cell walls is coordinated with cell expansion (Granier and Tardieu, 1998; Donnelly et al., 1999; Granier et al., 2000; Albersheim et al., 2011; Pantin et al., 2012). Additionally, geometric scaling might be expected if the relative dimensions of cells within and among different tissues need to be maintained within an approximate range of proportions for mechanical reasons, e.g., if cells with larger lumens require proportionately thicker cell walls for support or for other biological functions, e.g., if it is optimal for a leaf to have substantial contribution and thus similar sizes of both mesophyll cell types for light capture, photosynthesis and/or water balance (Haberlandt, 1914; Pyankov and Kondrachuk, 2003).

We tested 10 hypotheses for such allometries across species:

(1) *Cell sizes in different tissues scale together isometrically*. Thus, across species, the cell cross-sectional area (CCA) in

tissues 1 and 2 would be proportional: $CCA_1 \propto CCA_2^1$. Previous studies found that the sizes of epidermal cells of different types (i.e., pavement cells and stomatal guard cells) were correlated across species of diverse families (Beaulieu et al., 2008), and across species within Proteaceae (Brodribb et al., 2013). Further, previous studies found that mesophyll and epidermis cell sizes were correlated across genotypes of *Arabidopsis thaliana* (Pérez-Pérez et al., 2011), and mesophyll, epidermal cell, and minor vein xylem conduit sizes were correlated across species within Proteaceae (Brodribb et al., 2013). However, there has been no study to our knowledge of the wide range of leaf cells or analyses of their allometric relationships.

(2) *Cell wall thicknesses in different tissues scale together isometrically*. Thus, across species, the cell wall thickness (CWT) in tissues 1 and 2 would be proportional: $CWT_1 \propto CWT_2^1$.

(3) *Cell wall thickness scales geometrically with cell cross-sectional area within each tissue*. Thus, across species, for a given tissue, the cell wall thickness (CWT), a length dimension, would scale with the cell cross-sectional area (CCA): $CWT \propto CCA^{0.5}$. In the one previous study that examined cell wall thicknesses in different leaf tissues, for eight forage species, trends were not analyzed; our analysis of the reported values indeed indicated that species with larger cells had thicker cell walls in the mesophyll, though not in the xylem (Moghaddam and Wilman, 1998).

Next, we tested whether *leaf thickness scales* (4) *geometrically with cell cross-sectional area in each tissue*, (5) *isometrically with cell wall thickness in each tissue*, and (6) *isometrically with the thicknesses of tissues*, i.e., upper cuticle, upper epidermis, palisade mesophyll, spongy mesophyll, lower epidermis, and lower cuticle. Thus, across species, given that the thickness of the whole leaf (LT) and of any tissue (TT) are length dimensions, $LT \propto CCA^{0.5}$, $LT \propto CWT^1$, and $LT \propto TT^1$. Several previous studies were consistent with hypotheses (4), (5), and (6). Previous studies showed a correlation of LT with mesophyll cell size across 94 alpine desert species (Pyankov et al., 1999) and of LT with mesophyll, epidermal, and minor vein xylem conduit cell sizes across species within Proteaceae (Brodribb et al., 2013). Several studies found that LT was correlated with the thickness of one or more of the component tissues in various species sets (e.g., Garnier and Laurent, 1994; Roderick et al., 1999; Sack and Frole, 2006), though previous studies did not analyze allometric relationships.

Additionally, we tested for a relationship of *leaf area with* (7) *cell cross-sectional area*, (8) *cell wall thickness*, and (9) *tissue thicknesses*. We expected that leaf size would be independent of cell sizes and cell wall and tissue thicknesses across species, given the much stronger variation observed in leaf sizes than in cell sizes and cell wall and tissue thicknesses across species. Supporting hypothesis (7), across 94 alpine desert species, and across species within Proteaceae, leaf area was uncorrelated with cell volume or leaf thickness (Pyankov et al., 1999; Brodribb et al., 2013).

Finally, (10) we hypothesized that the above relationships among cell size, cell wall thickness, and leaf dimensions would hold strongly for mesophyll cell types, but weakly or not at all *for xylem cells*, which are especially distinct in developmental origin and in function.

To test for fundamental allometries, 14 species from 12 families were selected for their morphological and physiological diversity, including trees, shrubs, a liana, and herbs, originating from wet or dry habitats. Our aim was to investigate fundamental allometries with reference to hypotheses based on expectations from development, mechanics, and biological function, thus providing a conceptual framework for future studies of broader species sets and traits.

MATERIALS AND METHODS

***Study species and sampling*—**We sampled plants of 14 species that were cultivated with irrigation. Twelve species were sampled in and around the campus of the University of California, Los Angeles, one (*Quercus agrifolia*) at Will Rogers State Park, and one grown in a greenhouse (*Helianthus annuus* var. Sunspot; Botanical Interests, Broomfield, Colorado, USA) (Table 1). Leaves from *H. annuus* were collected from greenhouse plants grown from seeds in 3.6-L pots (average minimum, mean, and maximum values for temperature, 21.1°C, 23.2°C, and 26.0°C; for humidity, 44%, 51%, and 59%). Sunflowers were irrigated every 2 d, with 200 to 250 $mL \cdot L^{-1}$ 20 : 20 : 20 NPK; the light availability measured at midday on a sunny day was up to 550 μmol photons$\cdot m^{-2} \cdot s^{-1}$ and on average 300 μmol photons$\cdot m^{-2} \cdot s^{-1}$ (LI-250 light meter; LI-COR, Lincoln, Nebraska, USA). These species were chosen to capture strong phylogenetic and functional diversity and to provide a first indication of broad trends, representing 12 families, five life forms, and native to at least eight geographic locations varying strongly in wet and dry habitat preferences (Table 1). Mature, sun-exposed leaves were sampled from three individuals per species from September 2011 to April 2012. To avoid tissue shrinkage, leaves were rehydrated overnight before fixing for tissue anatomy. Branches were cut from woody species or whole shoots from herbs, and these were brought to the

Table 1. Species tested for allometries of leaf cells and tissues, family, origin, leaf habit, life form, habitat preference, and mean ± SE for leaf area and thickness.

Species	Family	Origin	Leaf habit	Life form	Habitat preference (wet/dry)	Leaf area (cm^2)	Leaf thickness (μm)
Bauhinia galpinii	Fabaceae	Africa	Evergreen	Tree	w	33.1 ± 4.34	91.3 ± 5.43
Camellia sasanqua	Theaceae	Japan	Evergreen	Shrub	w	14.0 ± 1.45	432 ± 11.6
Cercocarpus betuloides	Rosaceae	California, Mexico	Evergreen	Shrub	d	7.00 ± 2.08	261 ± 25.5
Comarostaphylis diversifolia	Ericaceae	California, Mexico	Evergreen	Shrub	d	11.1 ± 0.353	327 ± 28.6
Helianthus annuus	Asteraceae	Across North America	Deciduous	Annual herb	w	95.0 ± 8.86	183 ± 23.6
Hedera canariensis	Araliacaeae	Canary Islands	Evergreen	Liana	d	107 ± 15.3	312 ± 16.3
Heteromeles arbutifolia	Rosaceae	California, Mexico	Evergreen	Shrub	d	30.5 ± 3.66	395 ± 24.6
Lantana camara	Verbenaceae	Pantropical	Deciduous	Perennial shrub	w	31.6 ± 1.58	217 ± 4.28
Magnolia grandiflora	Magnoliaceae	Southern United States	Evergreen	Tree	w	118 ± 19.7	527 ± 22.2
Platanus racemosa	Platanaceae	California, Mexico	Deciduous	Tree	w	67.2 ± 37.6	200 ± 6.17
Quercus agrifolia	Fagaceae	California, Mexico	Evergreen	Tree	d	35.1 ± 10.1	293 ± 23.8
Raphiolepis indica	Rosaceae	Southern China, India	Evergreen	Shrub	w	137 ± 90.4	529 ± 12.2
Romneya coulterii	Papaveraceae	California, Mexico	Deciduous	Perennial herb	d	24.0 ± 9.66	374 ± 30.4
Salvia canariensis	Lamiaceae	Canary Islands	Deciduous	Perennial herb	d	19.1 ± 3.35	181 ± 23.9

laboratory with moist paper towel in black plastic bags, and recut underwater in the laboratory by at least two nodes, before rehydrating overnight with stem under water, covered with dark plastic bags. Leaves were then cut from each stem and stored in FAA solution (37% formaldehyde–glacial acidic acid–95% ethanol in deionized water at 10 : 5 : 50: : 35).

***Anatomical sample preparation and measurements*—**For anatomical analyses, one leaf was sampled from each of the three individual plants per species for cross-sectioning. A 1 × 0.5 cm rectangular subsample was cut from each leaf center and gradually infiltrated with mixtures of low viscosity acrylic resin (L. R. White; London Resin Co., UK) of increasing strength in ethanol, under vacuum, over the course of a week. Fully infiltrated samples were set in resin in gelatin capsules in an oven at 55°C overnight. Samples were sectioned in the transverse plane at 1 μm thickness using glass knives (cut using a LKB 7800 KnifeMaker; LKB Produkter; Bromma, Sweden) in a rotary microtome (Leica Ultracut E, Reichert-Jung, California, USA), placed on slides and stained with 0.01% toluidine blue in 1% sodium borate (w/v). Slides were imaged with a 20× or 40× objective using a light microscope (Leica Lietz DMRB; Leica Microsystems) and camera utilizing SPOT advanced imaging software (SPOT Imaging Solutions; Diagnostic Instruments; Sterling Heights, Michigan, USA).

We relied on transverse cross sections to determine the dimensions of cells, cell walls, and tissues within the leaf (following e.g., Oguchi et al., 2005; Pasquet-Kok et al., 2010; Tosens et al., 2012). Cross-sectional cell area was used as an index of cell size to test allometric relationships. Cell cross-sectional area would provide an accurate reflection of cell volume for cells that have azimuthal symmetry (i.e., that are symmetrical with rotation around the vertical axis), including palisade cells and spongy mesophyll cells, which are typically modeled as capsules and spheres respectively (Turrell, 1936; Nobel et al., 1975; Nobel, 1976, 2009). However, for epidermal cells, bundle sheath cells, and xylem cells, which might not necessarily have azimuthal symmetry (i.e., their cross-sectional area in the vertical plane may not be proportional to their cross section in the horizontal plane), the transverse cross-sectional cell area may not necessarily be a strong proxy for cell volume, and ideally, paradermal sections might allow a complete determination of cell size for those cell types. Thus, we qualify our conclusions relating to these cell types in light of our use of transverse cross-sectional cell areas (see Discussion).

Tissue thicknesses, mesophyll cell area, and cell dimensions were measured using software (ImageJ software version 1.42q; National Institutes of Health, Bethesda, Maryland, USA). Each measurement was replicated three times for every cross section on cells and tissues selected randomly in a systematic way. In the middle of the left, center and right thirds of the cross sections, tissue thicknesses were measured for upper and lower cuticle and epidermis and for spongy and palisade mesophyll. In the same three specific locations, cells from the upper and lower epidermis, and spongy and palisade mesophyll were measured for cell cross-sectional area and cell wall thickness (radial cell wall for epidermal cells), and the values for the three cells of each tissue per cross section were averaged. The xylem and bundle sheath cells were much more variable in lumen and cell wall diameters than mesophyll tissues, so a different approach was taken to determine mean values. From the bundle sheath surrounding each of two minor veins in each cross section, cells were measured for cross-sectional area and thickness; to determine a central value, the largest and smallest bundle sheath cells were measured, and the values were averaged. The xylem conduit lumen cross-sectional areas and wall thickness were determined for midrib and minor veins from each leaf. To determine xylem conduit lumen cross-sectional area, the conduits were considered as ellipses and their areas were calculated based on measurements of the major and minor axes; areas were averaged across all conduits. To determine xylem conduit wall thickness, we measured the two thickest and two thinnest conduit walls in the midrib and in each of two minor veins per cross section. These were averaged to give, respectively, the mean xylem conduit cell wall thickness for the midrib and minor vein. When cell walls were measured that separated two adjacent cells, the entire wall was measured and halved; this was typical when measuring xylem cell wall thicknesses and epidermal radial cell wall thickness, but for mesophyll cells and bundle sheath cells, cell walls could generally be measured adjacent to airspace.

Leaf areas were measured for leaves scanned (using a flatbed scanner; Epson Perfection 4490 Photo Scanner, Long Beach, California, USA; 1200 pixels per inch) and analyzed using ImageJ.

***Statistics*—**To test trait differences between moist and dry habitat species we performed ANOVAs with species nested within habitat type (Minitab release 15; Minitab, State College, Pennsylvania, USA). Prior to tests, data were log-transformed to improve normality and heteroscedasticity (Sokal and Rohlf, 1995).

A correlation matrix was determined to reveal the intercorrelative structure of leaf gross dimensions and anatomical traits. Spearman rank correlations are less sensitive to the effects of outliers, and thus, for a conservative estimation, correlations were considered significant only when $P < 0.05$ for both Spearman and Pearson coefficients (r_s and r_p, respectively).

To analyze allometric relationships, we used standard major axes (SMA; using SMATR; Warton et al., 2006). Standard major axes were used rather than ordinary least squares regression (OLS) to consider both x and y traits as independent variables with similar measurement error and to best estimate the functional relationship between the variables; OLS regression only considers the error in one trait (y) and the other variable (x) to be error-free and is most useful when the y variable is to be predicted from the x (Sokal and Rohlf, 1995; Sack et al., 2003a, b; Poorter and Sack, 2012). For each relationship, we fitted a standard major axis to the log-transformed data, determining a and b for $y = ax^b$, or, equivalently, using log-transformed data $\log y = \log a + b \log x$. We tested whether relationships differed in slopes and intercepts across tissues. We tested whether allometric slopes differed from those expected from geometric similarity (see *Introduction*).

For allometries relating to spongy mesophyll, we excluded *Romneya coulterii*, which lacked this tissue. All other allometric relationships were analyzed with and without including *R. coulterii,* as it was an outlier in other aspects of its leaf anatomy (see Results). We only tested allometric relationships among mesophyll anatomical traits, and not among xylem anatomical traits, which were hypothesized, and confirmed to be uncorrelated with mesophyll traits (see Introduction and Results).

Given our testing of a large number of hypothesized allometric relationships, we additionally tested for significance using two methods that accounted for multiple significance tests. We applied the sequential Bonferroni correction (Rice, 1989) and the false detection rate test (Benjamini and Hochberg, 1995; Harrington, 2002).

Our aim was to test hypothesized relationships that were likely to be very strong and thus to be resolved across a relatively small set of diverse species. Our general approach was consistent with that of previous studies often using 10–20, or even fewer species, to resolve important relationships among leaf anatomical and physiological traits that are later to be confirmed across yet larger, global data sets (e.g., 14 species in Garnier and Laurent, 1994; Garnier et al., 1999; 10 species in Sack and Frole, 2006; 4 species in Brodribb and Holbrook, 2003; 10 species in Zwieniecki et al., 2007; 7 species in Sperry et al., 2005; 7 species in Edwards, 2006; 7 species in Lens et al., 2011; 6 species in Nardini et al., 2012). These studies provide important and logistically challenging data especially as the field of plant anatomy is still in the discovery phase. However, a frequently voiced concern is that in such studies too few species were included; here we justify this general approach for investigation of novel structure–function trait correlations by addressing five potential objections: (1) A low species number might lead to detection of false trends (type I error), or (2) a failure to discern a true trend (type II error), and (3) trends might be too strongly affected by potential outlier species, and (4) when a significant trend is found, the parameters of the fitted line might be too imprecise, and (5) the results for few species might not be representative of larger species data sets, and especially global trends. Some of these objections are misplaced, and the others can be easily minimized through care in interpretation of the findings of these studies.

Concern (1) is misplaced, as a low $n > 2$ in fact reduces the power to detect a false positive trend, and is thus a conservative approach for testing correlations (Sokal and Rohlf, 1995). When trends *are* identified in studies with low n, they are likely to be robust. In particular, in our study, we explicitly hypothesized very strong scaling relationships that would be evident even for relatively few species. Concern (2) on the other hand is valid, and true trends might be missed when studying small species sets. Thus, the absence of a trend for a few species is not evidence that such trends would not be found in larger or more diverse datasets. To illustrate the value of testing trends among anatomical variables using 14 species, as in our study, we performed a randomization analysis of a published correlation data set, for the positive relationship of cell volume to leaf thickness for 91 alpine desert species ($R^2 = 0.59$; $P < 0.001$; Pyankov et al., 1999). We tested the ability to resolve this true correlation and avoid resolving a false trend by subsampling 14 species randomly with replacement from the 91 species and testing for a correlation, repeating 1000 times (using the program R; R Development Core Team, 2005). This analysis showed a very low risk of discerning a significant false trend, as only 3% of the 14-species data sets showed a significant negative correlation. However, there was a reasonable

power to discover the true trend, as 67% of the 14-species data sets showed a significant positive correlation. Presumably, the probability of discovering the true trend would be stronger if species were sampled explicitly for their diversity, as they were in our study. To test the danger of resolving correlations in a random data set, we repeated this subsampling analysis after randomizing the Pyankov et al. (1999) cell volume and leaf thickness data to remove the correlation ($R^2 = 0.042$, $P = 0.69$). Here, only 3% of 14-species data sets showed a significant positive correlation, and 2% showed a significant negative correlation. This simple case illustrates the potential to discover true trends and the low risk of determining false trends even when using small data sets.

Concern (3) can be minimized with analyses to specifically reduce the influence of outliers. In our study, we tested Spearman correlations, which are more robust to outliers, in addition to Pearson correlations. We also performed tests with and without including the outlier species, *Romneya coulterii*, which was easily identified (see previous section).

Concerns (4) and (5) are meaningful, but do not invalidate studies of relatively small species sets, as long as the interpretation takes these into account. Thus, the confidence intervals for parameters of the fitted lines for trends based on few species may be wide; however, these trends provide a baseline for future work with larger species sets, which will likely reduce the uncertainty around the mean parameter values. Further, the trends discerned for a few, diverse species may not necessarily be generalized to all plants, but these trends represent the "state-of-the-art", refined hypothesis for the relationship that can motivate further tests with larger species data sets. These tests would extend or constrain the generality of the relationship, or show variation in the relationship, e.g., across different lineages, communities or functional types.

For these reasons, we pursued our tests of hypothesized cell, tissue, and leaf anatomical allometries for 14 diverse species to accomplish our goal of discovering strong relationships which can be further tested and extended on larger and global data sets in future studies.

RESULTS

***Variation across species in cell and tissue anatomy*—**The species were diverse in their leaf size and in their leaf tissue thicknesses (Fig. 1; Table 1; Appendix S1a and S1b, see Supplemental Data with the online version of this article). For all traits, a substantial portion of variation was explained by species differences, a small amount by wet vs. dry habitat, and typically a minority of trait variation related to differences among leaves of given species (accounting for the residual error; Appendix S1b, last column). Species varied 20-fold in leaf area, 6-fold in thickness, 4-fold in the thickness of upper and lower epidermis, 8-fold in spongy and 11-fold in palisade mesophyll thickness, and 18-fold in the thicknesses of upper and lower cuticle. Species varied in having 1–2 cell layers in the epidermis (upper and lower) and 0–8 layers of spongy mesophyll (as *Romneya couterii* had only palisade mesophyll; Fig. 1) and 1–9 layers of palisade mesophyll.

Species also varied strongly in cell sizes and cell wall thicknesses (Fig. 1). Species varied 6-fold in cell cross-sectional areas for the upper epidermis, 13-fold for the lower epidermis, 12-fold for the spongy mesophyll, 8-fold for the palisade mesophyll, 9-fold for the bundle sheath, and 11-fold and 8-fold, respectively, for xylem conduits in the midrib and minor veins. Species varied 3-fold to 4-fold in cell wall thickness for each tissue.

We found very strong variation in cell sizes and cell wall thicknesses among the leaf tissues. Averaged across species, cell cross-sectional areas were smallest for minor vein xylem conduits, followed by, in order of increasing size, midrib vein xylem, bundle sheath, lower epidermis, spongy mesophyll, palisade mesophyll, and upper epidermis. Cell walls were thinnest for the minor vein xylem conduits, followed by, in order of increasing thickness, the bundle sheath, midrib vein xylem, palisade and spongy mesophyll, lower epidermis and upper epidermis. Thus, contrary to initial expectations, the xylem conduit cell walls were thin relative to mesophyll tissues, but they were very thick relative to their lumen areas compared with mesophyll cells.

***Differences in anatomy between species native to wet and dry habitat*—**We found significant differences in whole-leaf and anatomical traits between species native to wet and dry habitat (Appendix S1b). Species native to dry habitat had significantly smaller leaves, thinner epidermises and spongy mesophyll, but thicker palisade mesophyll, corresponding to a greater number of cell layers and larger cells. Indeed, species of dry habitat also had larger cells in the spongy mesophyll and lower epidermis.

***Hypothesis (1): Cell sizes in different tissues scale together isometrically*—**We found strong, novel relationships among the cell sizes of different tissues across leaves (Figs. 1, 2; Table 2; Appendix S1c, 1d; see online Supplemental Data). There were significant correlations among cell sizes in different tissues for 7 of 10 pairwise comparisons not including xylem ($r = 0.62$–0.90; $P < 0.001$–0.039).

The scaling slopes for the relationships between cell sizes in different tissues did not vary when considered across all species, but the slopes varied significantly after removing outlier *R. coulterii* ($P = 0.002$; Table 2). Cells of the upper and lower epidermis scaled isometrically in size (confidence intervals for b included 1), as did those of the spongy and palisade mesophyll, those of the palisade and bundle sheath, and those of the bundle sheath and lower epidermis. However, the cells of spongy mesophyll increased in size disproportionately to those of the upper and lower epidermis ($b > 1$; Table 2).

***Hypothesis (2): Cell wall thicknesses in different tissues scale together isometrically*—**We also found strong relationships between the thickness of cell walls across tissue types for each pair of tissues except for the xylem vs. other tissues, and the bundle sheath vs. upper epidermis (Figs. 1, 3; Table 2; Appendix S1c, 1d). Thus, correlations were statistically significant for 9 of 10 pairwise comparisons excluding the xylem ($r = 0.65$–0.91; $P < 0.001$–0.009).

The scaling slope for the relationships between cell wall thicknesses in different tissues was common across all the pairs of tissues with significant relationships (i.e., excluding that between bundle sheath and upper epidermis), and the scaling was isometric (confidence intervals for each species' b and for the common b included 1; Table 2).

***Hypothesis (3): Cell wall thickness scales geometrically with cell cross-sectional area within each tissue*—**We found strong, novel relationships between the thickness of cell walls and cell sizes (Fig. 4; Appendix S1c, 1d). Strong correlations were found of cell wall thickness with cell cross-sectional areas for palisade and spongy mesophyll, bundle sheath, and lower epidermis tissues ($r = 0.58$–0.84; $P < 0.001$–0.029). These relationships were not found for upper epidermis or xylem tissues ($r = 0.02$–0.48; $P = 0.084$–0.93).

For the tissues in which cell wall thickness related to cell size, the scaling was geometric. Thus, there were common allometric slopes for cell wall thickness vs. cell cross-sectional area across the palisade mesophyll, spongy mesophyll, bundle sheath, and lower epidermis (confidence intervals for each species' b and for the common b included 0.5; Table 2).

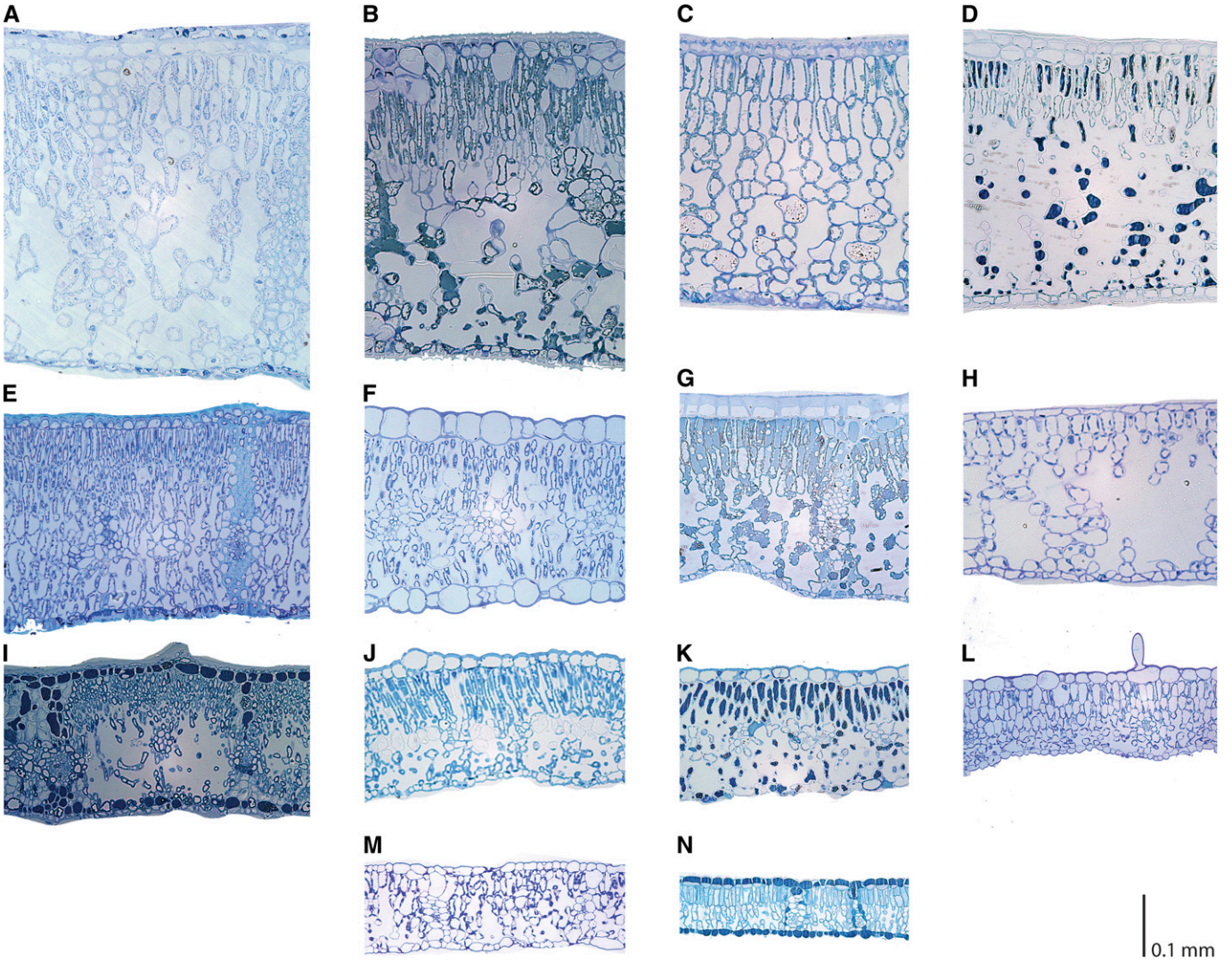

Fig. 1. Anatomical cross sections for 14 study species. (A) *Magnolia grandiflora*, (B) *Raphiolepis indica*, (C) *Camellia sasanqua*, (D) *Heteromeles arbutifolia*, (E) *Quercus agrifolia*, (F) *Romneya coulterii*, (G) *Comarostaphylis diversifolia*, (H) *Hedera canariensis*, (I) *Cercocarpus betuloides*, (J) *Lantana camara*, (K) *Platanus racemosa*, (L) *Salvia canariensis*, (M) *Helianthus annuus*, (N) *Bauhinia galpinii*.

Hypotheses (4–6): Leaf thickness scales geometrically with cell cross-sectional area in each tissue, cell wall thickness in each tissue, and the thicknesses of tissues— Across species, leaf thickness was positively related to cell size and to cell wall thickness for all tissues (Fig. 4; r = 0.56–0.98; P < 0.001–0.038; Appendix S1c, 1d) except for xylem ($|r|$ = 0.01–0.44; P = 0.099–0.98). The scaling of leaf thickness with cell cross-sectional area was geometric for cells in some tissues, i.e., palisade and spongy mesophyll (confidence intervals for b included 0.5), but not for others, i.e., the upper and lower epidermis and bundle sheath (b > 0.5), and thus across all species, leaf thickness increased disproportionately to cell cross-sectional area (common b > 0.5). Similarly, the scaling of leaf thickness with cell wall thickness was geometric for cells in some tissues, i.e., upper and lower epidermis and spongy mesophyll (confidence intervals for b included 1), but not for others, i.e., the palisade mesophyll and bundle sheath (b > 1), and thus across all species, leaf thickness increased disproportionately to cell wall thickness (common b > 0.5).

Leaf thickness correlated with tissue thickness for palisade mesophyll, spongy mesophyll, and upper and lower cuticle (Fig. 4; r = 0.56–0.98; P < 0.001–0.036; Appendix S1c, 1d), but not for upper and lower epidermis (r-values for Spearman correlations = 0.38–0.44; P > 0.05). The leaf thickness scaled geometrically with the thickness of palisade mesophyll (confidence intervals for b included 1), but the leaf thickness did not increase in proportion with tissue thickness for the spongy mesophyll and upper and lower cuticles (b < 1).

Hypotheses (7–9): Lack of relationship of leaf area with (7) cell cross-sectional area, (8) cell wall thickness, and (9) tissue thicknesses— We found no relationships across species of leaf size with cell size or cell wall thickness in any tissue, or with tissue thicknesses, or number of cell layers in given tissues (r = 0.03–0.48; P = 0.10–0.92; Appendix S1c and 1d).

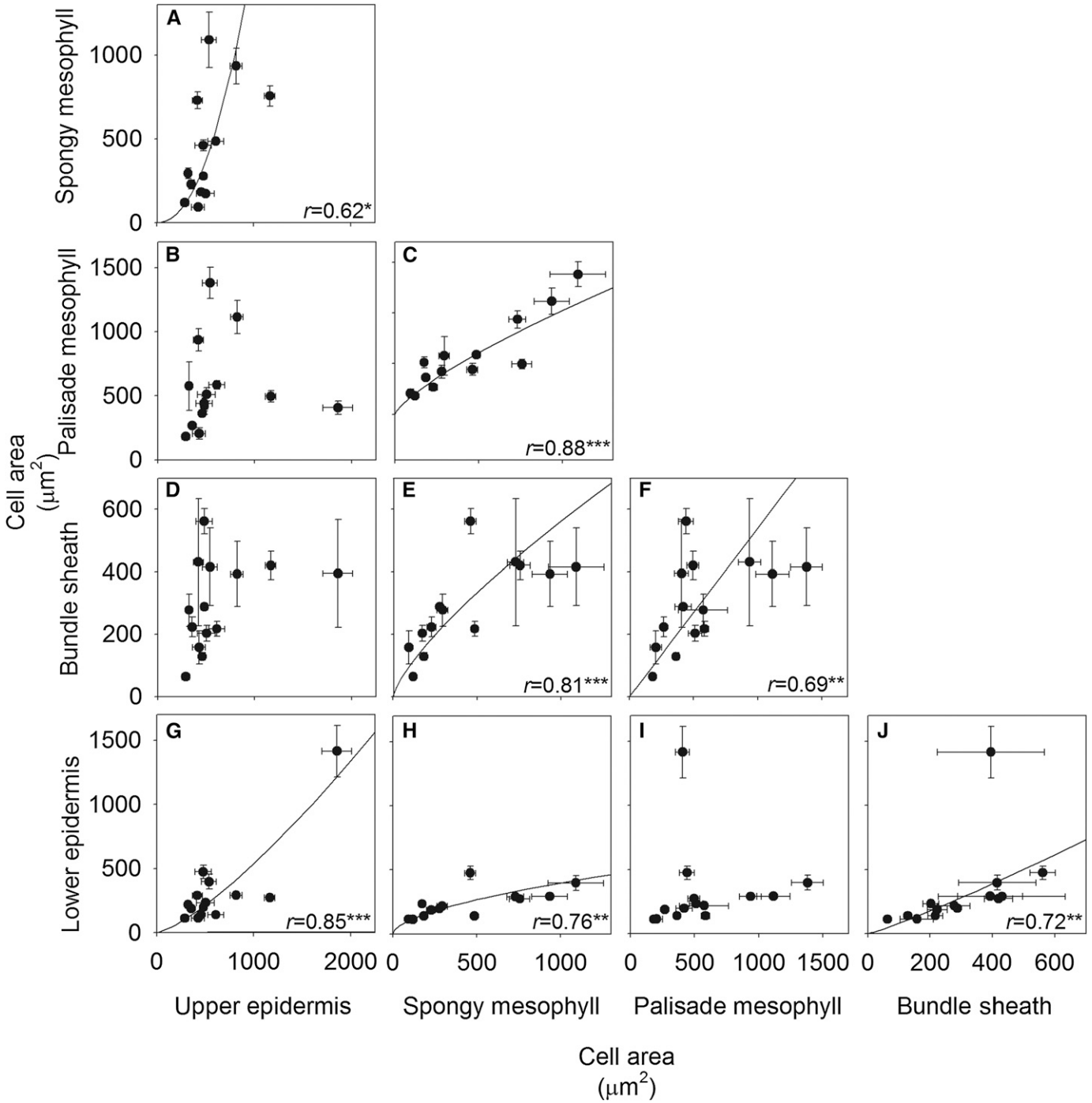

Fig. 2. Relationships between cell sizes (determined as cell cross-sectional areas) between leaf tissues for 14 diverse species. The plots are presented for all combinations of epidermal and mesophyll cell types; plots against leaf vein xylem cell sizes are not presented, as they were independent of those of other tissues (see text). Power laws were fitted for significant relationships using standardized major axes; parameters of allometric lines fitted by standard major axes are given in Table 2. The apparent outlier on the far right in panels B, D, and G, and at the top of panels I and J is the species *Romneya coulterii*. That species is not present in panels A, C, E, and H given its lack of spongy mesophyll. All statistical analyses were performed with and without this species, with minimal impact on overall trends and statistical significance (Table 2; Appendix S1c and S1d, respectively). Confirmation of the significance of correlations despite multiple testing using Bonferroni and false detection rate analyses is presented in Appendix S1e. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

TABLE 2. Parameters of fitted allometries for statistically significant relationships of cell and tissue anatomy and leaf form for 14 diverse species. Allometries were fitted for six types of relationships to data for all species (one point per species) for the relationships that were significant (see full correlation matrices in Appendix S1c and S1d). For each relationship, linearized power laws ($y = \log a + b \log x$) were fitted by standard major axis (SMA) to log-transformed data with and without outlier *Romneya coulterii*. For each relationship, the expected b value from geometric scaling ($\hat{b}$) is presented (see introduction), and the R^2 value is presented with its significance, and slope b and intercept log a values, in bold, in between the lower and upper 95% confidence limits. For each allometry type, we also tested lines for differences in slopes and intercepts, and when a common slope was found across tissue allometries (i.e., $P > 0.05$ for a test for difference in b among slopes), the common slope is presented with confidence limits and the log a values are presented for each species given the common slope; in each case, the lines differed in intercepts ($P < 0.001$). A common slope was found for all relationships except where the outlier *Romneya coulterii* was removed in allometry types 1 and 6. ^{ns}: $P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Relationships were also subjected to significance testing corrected for multiple comparisons using the sequential Bonferroni test for tablewide significance, and the false detection rate test, and remained significant except for †failed sequential Bonferroni test for tablewide significance, and ‡failed false detection rate test (Appendix S1e). Findings for hypotheses (7), (8), (9), and (10) are not presented given that in each case the hypothesis for a lack of significant relationship (see introduction) was supported.

		All data				Outlier removed			
Allometry type/Tissues	$\hat{b}$	R^2	b	log a	Common b, and log a	R^2	b	log a	Common b and log a
(1) Cell cross-sectional areas in different tissues	1				b = 1.12, **1.29**, 1.48				b =N/A
Lower epidermis vs. upper epidermis		0.500**	0.860, **1.32**, 2.04	−2.84, **−1.23**, 0.385	−1.13	0.142 ns†‡	0.682, **1.22**, 2.18	−2.97, **-0.951**, 1.07	(P = 0.002)
Spongy mesophyll vs. upper epidermis		0.386*†‡	1.29, **2.14**, 3.52	−6.22, **−3.22**, −0.222	−0.936	0.386*†‡	1.30, **2.14**, 3.52	−6.22, **-3.22**, −0.222	
Spongy mesophyll vs. lower epidermis		0.577**	1.15, **1.75**, 2.67	−3.32, **−1.55**, 0.216	−0.471	0.577**	1.15, **1.75**, 2.67	−3.32, −1.55, 0.216	
Spongy mesophyll vs. palisade mesophyll		0.777***	0.959, **1.32**, 1.78	−2.08, **−0.975**, 0.131	−0.927	0.777***	0.959, **1.31**, 1.78	−2.08, **-0.975**, 0.131	
Spongy mesophyll vs. bundle sheath		0.656**	0.909, **1.33**, 1.94	−1.91, **−0.658**, 0.591	−0.561	0.656**	0.909, **1.33**, 1.94	−1.91, **-0.658**, 0.591	
Bundle sheath vs. palisade mesophyll		0.472**	0.643, **1.00**, 1.56	−1.50, **−0.267**, 0.965	−1.04	0.519**	0.630, **0.983**, 1.53	−1.46, **-0.239**, 0.983	
Lower epidermis vs. bundle sheath		0.521**	0.743, **1.13**, 1.73	−1.55, **−0.350**, 0.850	−0.719	0.785***	0.560, **0.758**, 1.03	−0.0516, **0.511**, 1.07	
Lower epidermis vs. palisade mesophyll		0.160 ns†‡	0.656, **1.14**, 1.96	−2.42, **−0.653**, 1.11	−1.106	0.499**	0.473, **0.745**, 1.17	−0.614, **0.330**, 1.27	
(2) Cell wall thickness in different tissues	1				b = 1.03, **1.15**, 1.27				b = 1.03, **1.15**, 1.28
Lower epidermis vs. upper epidermis		0.630**	0.753, **1.10**, 1.59	−0.231, **−0.108**, 0.0143	−0.121	0.626**	0.730, **1.08**, 1.61	−0.236, **-0.109**, 0.0192	−0.124
Spongy mesophyll vs. upper epidermis		0.747***	0.826, **1.15**, 1.59	−0.307, **−0.197**, −0.0860	−0.197	0.757***	0.826, **1.15**, 1.59	−0.307, **-0.197**, −0.0860	−0.197
Spongy mesophyll vs. lower epidermis		0.823***	0.803, **1.06**, 1.39	−0.149, **−0.0818**, −0.0147	−0.0960	0.823***	0.803, **1.06**, 1.39	−0.149, **-0.0818**, −0.0146	−0.0960
Spongy mesophyll vs. palisade mesophyll		0.812***	0.964, **1.28**, 1.70	−0.0131, **0.0389**, 0.0909	0.0440	0.812***	0.964, **1.28**, 1.70	−0.0131, **0.0389**, 0.0909	0.0440
Spongy mesophyll vs. bundle sheath		0.522**	0.874, **1.36**, 2.13	0.0602, **0.150**, 0.239	0.139	0.522**	0.874, **1.36**, 2.13	0.0602, **0.150**, 0.240	0.140
Bundle sheath vs. palisade mesophyll		0.418*	0.582, **0.926**, 1.47	−0.157, **−0.0901**, −0.0233	−0.0990	0.454*	0.586, **0.940**, 1.51	−0.151, **-0.0815**, −0.0125	−0.0890
Lower epidermis vs. bundle sheath		0.608**	0.890, **1.31**, 1.92	0.160, **0.232**, 0.303	0.223	0.653**	0.880, **1.29**, 1.89	0.149, **0.219**, 0.290	0.212
Lower epidermis vs. palisade mesophyll		0.798***	0.915, **1.21**, 1.60	0.0657, **0.114**, 0.162	0.117	0.792***	0.899, **1.21**, 1.63	0.0623, **0.114**, 0.166	0.116
Upper epidermis vs. palisade mesophyll		0.780***	0.826, **1.11**, 1.48	0.156, **0.203**, 0.249	0.201	0.780***	0.823, **1.12**, 1.52	0.156, **0.206**, 0.255	0.205

TABLE 2. Continued.

Allometry type/Tissues	All data					Outlier removed				
	$\hat{b}$	R^2	b	log a	Common b, and log a	R^2	b	log a	Common b and log a
(3) Cell wall thickness vs. cell cross-sectional area	0.5				b = 0.463, **0.565,** 0.692				b = 0.516, **0.639,** 0.798
Upper epidermis		0.228 ns†‡	0.422, **0.716**, 1.21	−2.79, **−1.71**, −0.623	−1.30	0.320*	0.594, **1.00**, 1.69	−3.93, **-2.45**, −0.974	−1.48
Palisade mesophyll		0.356*†	0.342, **0.556**, 0.903	−2.20, **−1.45**, −0.692	−1.47	0.390*	0.333, **0.548**, 0.901	−2.20, **-1.44**, −0.670	−1.68
Spongy mesophyll		0.468*	0.339, **0.537**, 0.857	−1.94, **−1.28**, −0.613	−1.35	0.468*	0.337, **0.537**, 0.857	−1.94, **-1.28**, −0.613	−1.54
Bundle sheath		0.627**	0.353, **0.514**, 0.748	−1.77, **−1.29**, −0.814	−1.42	0.683***	0.363, **0.523**, 0.754	−1.78, **-1.31**, −0.834	−1.58
Lower epidermis		0.339*†	0.362, **0.592**, 0.968	−1.98, **−1.25**, −0.521	−1.19	0.479**	0.561, **0.890**, 1.41	−2.92, **-1.92**, −0.921	−1.33
(4) Leaf thickness vs. cell area	0.5				b = 0.639, **0.757**, 0.897				b = 0.725, **0.888**, 1.09
Upper epidermis		0.437*	0.609, **0.960**, 1.52	−1.42, **−0.179**, 1.06	0.377	0.596**	0.884, **1.33**, 2.01	−2.67, **-1.15**, 0.366	0.0450
Palisade mesophyll		0.360*	0.508, **0.824**, 1.34	−0.878, **0.239**, 1.36	0.418	0.391*	0.495, **0.814**, 1.34	−0.888, **0.250**, 1.39	0.0510
Spongy mesophyll		0.623**	0.419, **0.623**, 0.927	0.209, **0.857**, 1.51	0.520	0.623**	0.419, **0.623**, 0.927	0.209, **0.858**, 1.51	0.187
Bundle sheath		0.713***	0.591, **0.823**, 1.14	−0.215, **0.459**, 1.13	0.618	0.704***	0.582, **0.828**, 1.18	−0.274, **0.448**, 1.17	0.303
Lower epidermis		0.311*	0.440, **0.726**, 1.20	−0.201, **0.712**, 1.63	0.638	0.440*	0.677, **1.09**, 1.76	−1.38, **-0.111**, 1.16	0.366
(5) Leaf thickness vs. cell wall thickness	1				b = 1.17, **1.32**, 1.51				b = 1.14, **1.31**, 1.51
Upper epidermis		0.585**	0.904, **1.34**, 1.99	1.95, **2.11**, 2.27	2.12	0.580**	0.876, **1.33**, 2.02	1.95, **2.11**, 2.28	2.12
Palisade mesophyll		0.778***	1.11, **1.48**, 1.99	2.3, **2.39**, 2.45	2.39	0.771***	1.09, **1.49**, 2.03	2.32, **2.39**, 2.45	2.39
Spongy mesophyll		0.751***	0.838, **1.16**, 1.61	2.27, **2.34**, 2.42	2.33	0.751***	0.838, **1.16**, 1.61	2.27, **2.34**, 2.42	2.33
Bundle sheath		0.545**	1.06, **1.60**, 2.42	2.43, **2.53**, 2.63	2.52	0.584**	1.04, **1.58**, 2.40	2.42, **2.52**, 2.61	2.50
Lower epidermis		0.890***	0.997, **1.23**, 1.51	2.19, **2.25**, 2.30	2.23	0.887***	0.984, **1.23**, 1.53	2.18, **2.25**, 2.31	2.24
(6) Leaf thickness vs. tissue thickness	1				b = 0.705, **0.792**, 0.890				b = N/A (P = 0.05)
Upper cuticle		0.500**	0.404, **0.622**, 0.957	1.78, **2.00**, 2.22	1.87	0.532**	0.395, **0.613**, 0.952	1.76, **1.99**, 2.22	
Palisade mesophyll		0.600**	0.607, **0.895**, 1.32	–0.0375, **0.673**, 1.38	0.879	0.724***	0.781, **1.10**, 1.55	–0.450, **0.299**, 1.05	
Spongy mesophyll		0.952***	0.683, **0.790**, 0.913	0.509, **0.756,** 1.00	0.752	0.952***	0.683, **0.790**, 0.913	0.509, **0.756**, 1.00	
Lower cuticle		0.711***	0.444, **0.619**, 0.863	2.03, **2.15**, 2.27	2.07	0.707***	0.433, **0.615**, 0.874	2.02, **2.15**, 2.28	

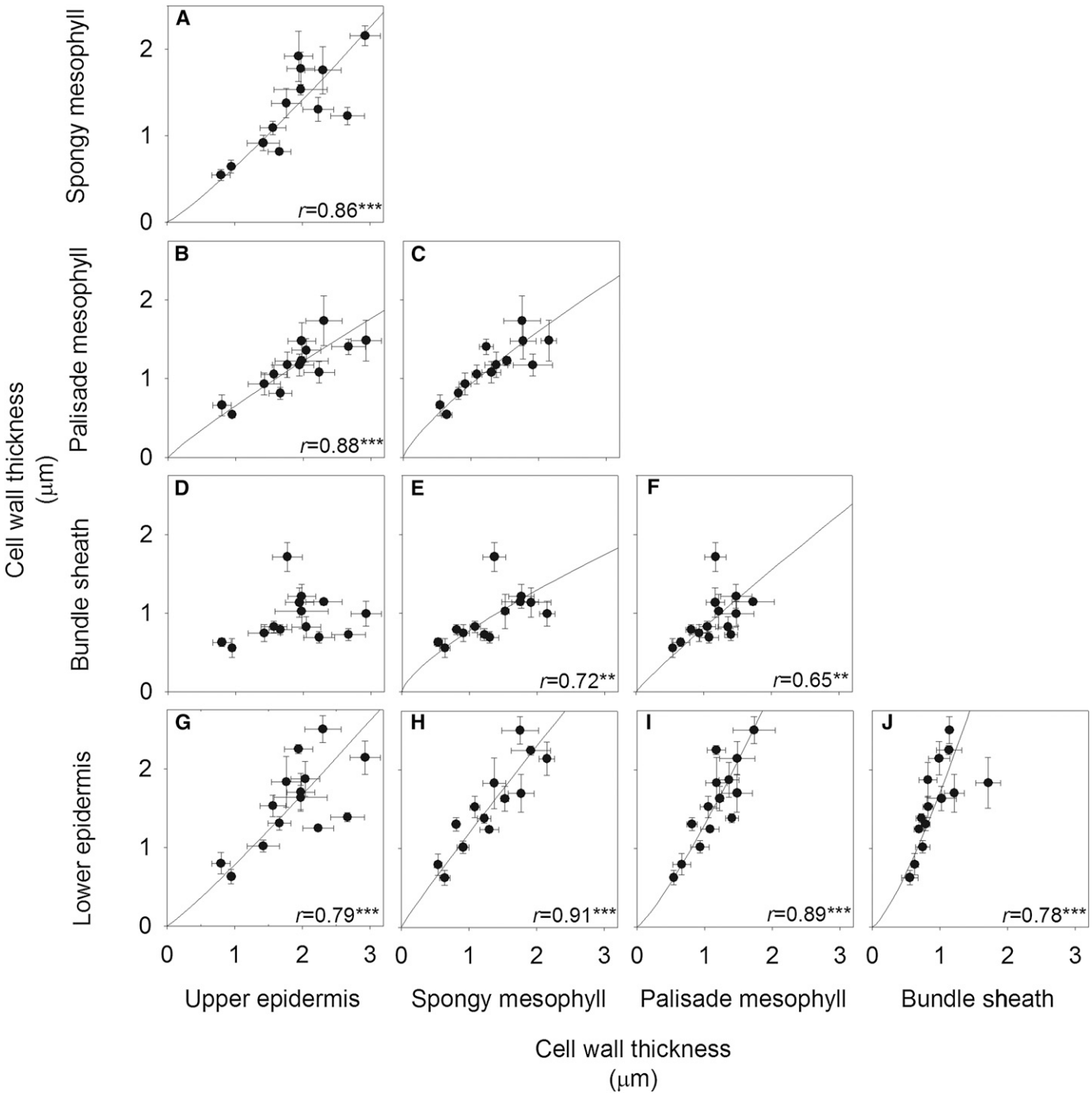

Fig. 3. Relationships between cell wall thicknesses between leaf tissues for 14 diverse species. Plots are presented for all combinations of epidermal and mesophyll cell types; plots against leaf vein xylem cell sizes are not presented, as they were independent of those of other tissues (see Results). Power laws were fitted for significant relationships using standardized major axes; parameters of allometric lines fitted by standard major axes are given in Table 2. The apparent outlier on the far right in panels B, D, and G, and at the top of panels I and J is the species *Romneya coulterii*. That species is not present in panels A, C, E, and H given its lack of spongy mesophyll. All statistical analyses were performed with and without this species, with minimal impact on overall trends and statistical significance (Table 2; Appendix S1c and S1d respectively). Confirmation of the significance of correlations despite multiple testing using Bonferroni and false detection rate analyses is presented in Appendix S1e. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Hypothesis (10): No relationships among xylem cells and mesophyll cell dimensions or leaf dimensions—We found no significant scaling relationships for xylem cell or cell wall thickness with those of other tissues (r = 0.01–0.43; P = 0.30–0.99; Appendix S1c, 1d). Further, we found no significant relationships between cell cross-sectional area and cell wall thickness within the xylem (r = −0.02–0.2; P = 0.48–0.95). Additionally, there were no significant relationships between xylem cell size or cell wall thickness and leaf thickness or area (r = 0.03–0.35; P = 0.20–0.91).

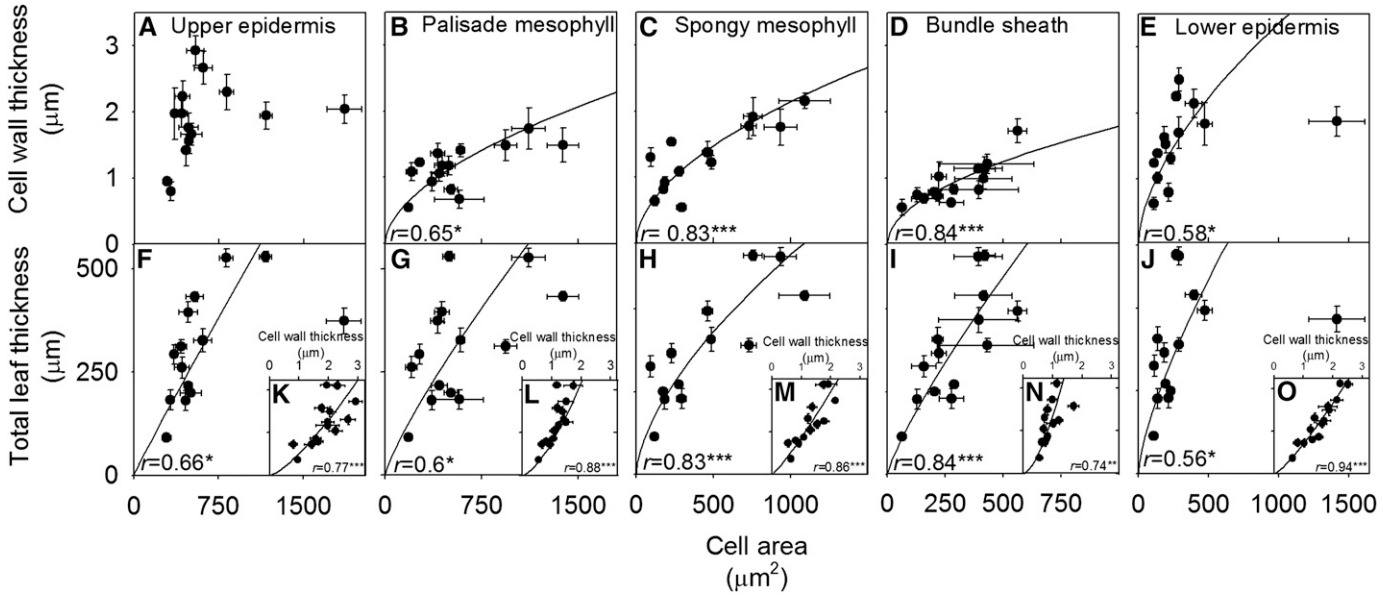

Fig. 4. Relationships between cell wall thickness and cell cross-sectional area (panels A–E), between total leaf thickness and cell cross-sectional area (panels F–J) and between total leaf thickness and cell wall thickness (insets in panels K–O) for given leaf tissues for 14 diverse species. The plots are presented for all combinations of epidermal and mesophyll cell types; plots against leaf vein xylem cell sizes are not presented, as they were independent of those of other tissues (see Results). Power laws were fitted for significant relationships using standardized major axes; parameters of allometric lines fitted by standard major axes are given in Table 2. The apparent outlier in panels A, E, F, and J is the species *Romneya coulterii*. That species is not present in panels C, H, and M given its lack of spongy mesophyll. All statistical analyses were performed with and without this species, with minimal impact on overall trends and statistical significance (Table 2; Appendix S1c and S1d, respectively). Confirmation of the significance of correlations despite multiple testing using Bonferroni and false detection rate analyses is presented in Appendix S1e. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

DISCUSSION

This study focused on 14 diverse angiosperm species, with strong differentiation in leaf anatomy, in part related to their adaptation to native wet or dry habitats. The principal novel findings of this study were fundamental and previously uncharacterized allometries for cells and tissues. We found strong relationships across diverse species relating the sizes of cells in the different tissues of the leaf and of cell wall thicknesses to the sizes of cells across tissues within the leaf. Additionally, we found that leaf thickness was strongly related to cell sizes across leaf tissues and to the thicknesses of component tissues, but that cell sizes and tissue thicknesses were independent of leaf area across species. In many cases, the allometries were consistent with the hypothesis of geometric scaling. These findings have important implications for understanding the development and design of whole leaves, with potential applications for modeling and estimating key functional traits from anatomical characteristics.

The first set of allometries tested was for the relationship among the sizes of cells in different tissues. As hypothesized, we found that in general, leaves with larger cells had proportionally larger cells in all their tissues, excluding the xylem. This pattern did not arise from uniform cell sizes across all leaf tissues for given species; cell sizes varied many-fold between tissues in given species. Rather, the scaling of cell sizes indicated a coordination of the relative cell sizes across all species, with isometric relationships among the cross-sectional areas. This finding extends to the internal anatomy the finding in a previous study of a correlation between epidermal pavement cell sizes and stomatal guard cell sizes (Beaulieu et al., 2008). In that study, the species with larger epidermal cells had larger genome sizes, likely relating to the well-known correlation of cell nucleus size with cell size (Bonner, 2006). Whether genome size is larger in species that have large cross-sectional internal cell sizes remains to be tested. Additionally, geometric scaling might be expected if there exists a developmental coordination of the rates and times of cell expansion rates and cell wall production in the different leaf tissues (Granier and Tardieu, 1998; Donnelly et al., 1999; Granier et al., 2000; Albersheim et al., 2011; Pantin et al., 2012) or if leaf function depends on a relatively conserved size proportionality of cells in different leaf tissues.

Notably, weak or absent relationships were found mostly for comparisons of internal and external tissues (i.e., mesophyll vs. epidermis). This was likely at least in part due to issues of measurement. While for the spongy and palisade mesophyll cells, the cross-sectional area can represent the cell volume, given their azimuthal symmetry (see Methods), for epidermal cells, volume may not be well represented by the cross-sectional area. Thus, the absence of a significant relationship among the size of epidermal cells and those in some other tissues needs to be confirmed using other estimates of cell size, especially as in some cases, nonsignificant empirical trends were observed (Fig. 2).

Additionally, as hypothesized, we found strong, novel geometric scaling between the thickness of cell walls and cell sizes in the mesophyll tissues. This finding was consistent with qualitative classical observations on the ecological anatomy of drought tolerance. F. W. Went is cited as having observed that "One of the most typical characters of xerophytes…is the thickness of the cell walls of *all* cells" (Metcalfe and Chalk, 1989, p. 142). Indeed, we found thicker cell walls coinciding with larger cells in the species of dry habitat. There are at least two explanations for the correlation of cell wall thickness with cell

size, a mechanical and a developmental explanation. As leaves transpire or dehydrate, cells undergo shrinkage, and the pressure required to deform a cell is a function of the cell wall thickness relative to the lumen diameter of the cell, a relationship well studied in wood xylem (Hacke et al., 2001), but not previously examined in leaf mesophyll cells, though the same principle should apply. Indeed, the geometric scaling of cell wall thickness with cell cross-sectional area would indicate a mechanism for the ability of the cell wall to withstand collapse to be independent of cell size across species. Notably, the xylem cells did not show this trend, but rather, cell walls were disproportionally thick across all xylem conduit sizes, and lumen diameter was not related to wall thickness, as previously observed in wood xylem studied across species of angiosperms and conifers (Pittermann et al., 2006; Cochard et al., 2008), and as confirmed in our analysis of the data for leaf minor veins for 20 diverse Australian angiosperms ($P > 0.05$ in Spearman rank correlation; Blackman et al., 2010). Such disproportional thickening would contribute to the ability to withstand strong tensions. Notably in the upper epidermis, there also was no correlation of cell cross-sectional area and cell wall thickness. This lack of a relationship might have been due to azimuthal assymetry of epidermal cells (see *Methods*) and a relationship might occur between cell volume and cell wall thickness. Alternatively, there may be no relationship due to disproportionate thickening of these cell walls, which would provide mechanical protection to the leaf (Haberlandt, 1914; Esau, 1965).

A second explanation for the geometric scaling of mesophyll cell wall thickness with cell size is developmental. Cell wall thickening may match cell expansion because these processes continue simultaneously from when leaf primordium cells begin to divide until leaf maturation (Esau, 1965; Albersheim et al., 2011). The lack of this trend in xylem and upper epidermal cells may correspond to their developing thick, lignified secondary cell walls. Thus, species with cells that expand to larger sizes, either via more rapid expansion in a given time or via a longer time for expansion would also develop thicker cell walls (cf. Donnelly et al., 1999). Such a developmental explanation for scaling trends across tissues has been recently proposed for the relationship of leaf major vein traits to leaf size across diverse species (Sack et al., 2012).

The relationships of whole-leaf thickness to mesophyll tissue thicknesses, to cell sizes, and cell wall thickness throughout the leaf may be a simple result of these components contributing to the whole-leaf thickness and/or it may involve a developmental mechanism. A recent review has shown that in typical leaves, leaf thickening is coordinated with the expansion of cells in the mesophyll, which continues after cell divisions slow (Pantin et al., 2012). Thus, thicker leaves would likely develop with larger cells and thicker mesophyll tissues, even across diverse species. Indeed, correlations of whole-leaf thickness with thickness of leaf tissues have been previously reported to hold across deciduous species and evergreen species (Garnier and Laurent, 1994; Roderick et al., 1999; Sack and Frole, 2006). Our allometric analyses indicated that whole-leaf thickness typically increased less than geometrically with component tissue thicknesses or cell sizes or cell wall thickness, as expected given that epidermal tissue thicknesses, also a component of whole-leaf thickness, were unrelated to leaf thickness in our data set, and also likely due to the variability in the contribution of different tissues to the whole-leaf thickness across species.

The finding of no relationship of leaf lamina area to tissue thicknesses and cell sizes is consistent with findings for across *A. thaliana* genotypes (Perez-Perez et al., 2011), across species within Proteaceae (Brodribb et al., 2013), across 94 alpine desert species (Pyankov et al., 1999), and also in comparisons of bonsai and natural tree leaves (Korner et al., 1989). This finding is analogous to the lack of correlation between cell size and body size in animals. The final size of the leaf (or organism) depends equally on the number of cell divisions as final cell size and thus can be independent of cell size and vary across species by many more orders of magnitude (Schmidt-Nielsen, 1984).

These strong relationships provide new insight into the general rules underlying the construction of leaves. The overall trend is that thicker leaves have thicker tissues, with larger cells and thicker cell walls, except in the xylem, and that leaf area is independent of these trends. The scaling of cells and cell walls tends to be geometric. These allometries, here shown for 14 diverse species, should be further tested, and the relationships extended, to include a yet wider diversity of plant species. The relationships should additionally be tested within lineages of closely related species, in a phylogenetic context, to determine their robustness in evolutionary trajectories. Within lineages, developmental trait coordination may be expected to be even stronger than when considering diverse species, as in this study; thus, in a study of species within Proteaceae, correlations among cell sizes were very strong, including among mesophyll and epidermal cell sizes and minor vein xylem conduit diameters (Brodribb et al., 2013). Indeed, the strength of these novel allometries across diverse species indicates that they are likely to be found more generally, and point to the need for future work to elucidate the underlying developmental and evolutionary mechanisms for their origin and ontogenetic basis, and their functional implications.

Future work is also needed to consider the possible influence of the environment on the allometries of cells and tissues within leaves. Many trait allometries (e.g., those among the mass of organs) shift substantially due to plasticity across different growing conditions, i.e., different supplies of light, nutrients, and/or water (e.g., Poorter et al., 2012; Poorter and Sack, 2012), and previous studies have shown that the relationships among tissue thicknesses and whole-leaf thickness across 22 herbaceous species shifted with irradiance and nutrient availability (Meziane and Shipley, 1999). The leaves in this study were for plants all grown under well-exposed, irrigated conditions. Additional experimental work will show how much the allometries among cell, tissue, and whole leaf dimensions can shift for given species in different conditions.

These fundamental relationships among the dimensions of cell walls, cells, tissues, and whole leaves will themselves scale up to influencing other leaf and plant traits. Indeed, an increasing focus of research is to clarify the anatomical basis of leaf mass per area (LMA), and other relationships pertaining to "leaf economics" traits, which tend to correlate with LMA, e.g., rates of photosynthesis and respiration, nutrient concentrations per leaf area and per leaf mass, and leaf longevity (cf. Garnier and Laurent, 1994; Shipley et al., 2006; Poorter et al., 2009; Sack et al., 2013). The allometries of cells and tissues will figure fundamentally in future attempts to determine the quantitative anatomical basis for these traits, their genetic and developmental basis, and their evolutionary and ecological differentiation, increasing the ability to select or engineer those traits in crop plants (Gowik and Westhoff, 2011; Kattge et al., 2011).

LITERATURE CITED

Albersheim, P., A. Darvill, K. Roberts, R. Sederoff, and A. Staehelin. 2011. Plant cell walls. Taylor & Francis, New York, New York, USA.

Beaulieu, J. M., I. J. Leitch, S. Patel, A. Pendharkar, and C. A. Knight. 2008. Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytologist* 179: 975–986.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society, B, Methodological* 57: 289–300.

Blackman, C. J., T. J. Brodribb, and G. J. Jordan. 2010. Leaf hydraulic vulnerability is related to conduit dimensions and drought resistance across a diverse range of woody angiosperms. *New Phytologist* 188: 1113–1123.

Bonner, J. T. 2006. Why size matters. Princeton University Press, Princeton, New Jersey, USA.

Brodribb, T. J., and N. M. Holbrook. 2003. Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiology* 132: 2166–2173.

Brodribb, T. J., G. J. Jordan, and R. J. Carpenter. 2013. Unified changes in cell size permit coordinated leaf evolution. *New Phytologist* 199: 559–570.

Brown, J. H., and G. B. West. 2000. Scaling in biology. Oxford University Press, New York, New York, USA.

Cochard, H., S. T. Barigah, M. Kleinhentz, and A. Eshel. 2008. Is xylem cavitation resistance a relevant criterion for screening drought resistance among *Prunus* species? *Journal of Plant Physiology* 165: 976–982.

Donnelly, P. M., D. Bonetta, H. Tsukaya, R. E. Dengler, and N. G. Dengler. 1999. Cell cycling and cell enlargement in developing leaves of *Arabidopsis*. *Developmental Biology* 215: 407–419.

Edwards, E. J. 2006. Correlated evolution of stem and leaf hydraulic traits in *Pereskia* (Cactaceae). *New Phytologist* 172: 479–489.

Esau, K. 1965. Plant anatomy. John Wiley, New York, New York, USA.

Garnier, E., and G. Laurent. 1994. Leaf anatomy, specific mass and water content in congeneric annual and perennial grass species. *New Phytologist* 128: 725–736.

Garnier, E., J. L. Salager, G. Laurent, and L. Sonie. 1999. Relationships between photosynthesis, nitrogen and leaf structure in 14 grass species and their dependence on the basis of expression. *New Phytologist* 143: 119–129.

Gowik, U., and P. Westhoff. 2011. The path from C_3 to C_4 photosynthesis. *Plant Physiology* 155: 56–63.

Granier, C., and F. Tardieu. 1998. Spatial and temporal analyses of expansion and cell cycle in sunflower leaves: A common pattern of development for all zones of a leaf and different leaves of a plant. *Plant Physiology* 116: 991–1001.

Granier, C., O. Turc, and F. Tardieu. 2000. Co-ordination of cell division and tissue expansion in sunflower, tobacco, and pea leaves: Dependence or independence of both processes? *Journal of Plant Growth Regulation* 19: 45–54.

Haberlandt, G. 1914. Physiological plant anatomy, 4th ed. MacMillan, London, UK.

Hacke, U. G., J. S. Sperry, W. T. Pockman, S. D. Davis, and K. A. McCulloch. 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* 126: 457–461.

Harrington, R. 2002. Controlling the false discovery rate in multiple hypothesis testing [online]. Benchmarks Online, vol. 5(4), available at http://www.unt.edu/benchmarks/archives/2002/april02/rss.htm. Research and Statistical Support, University of North Texas, Denton, Texas, USA.

Huxley, J. S. 1932 [reprint 1993]. Problems of relative growth. Johns Hopkins University Press, Baltimore, Maryland, USA.

Kattge, J., S. Díaz, S. Lavorel, I. C. Prentice, P. Leadley, G. Bönisch, E. Garnier, et al. 2011. TRY—A global database of plant traits. *Global Change Biology* 17: 2905–2935.

Korner, C., S. P. Menendezriedl, and P. C. L. John. 1989. Why are bonsai plants small: A consideration of cell size. *Australian Journal of Plant Physiology* 16: 443–448.

Lens, F., J. S. Sperry, M. A. Christman, B. Choat, D. Rabaey, and S. Jansen. 2011. Testing hypotheses that link wood anatomy to cavitation resistance and hydraulic conductivity in the genus *Acer*. *New Phytologist* 190: 709–723.

Metcalfe, C. R., and L. Chalk. 1989. Anatomy of the dicotyledons, 2nd ed., vol. II, Wood structure and conclusion of the general introduction. Oxford University Press, New York, New York, USA.

Meziane, D., and B. Shipley. 1999. Interacting determinants of specific leaf area in 22 herbaceous species: Effects of irradiance and nutrient availability. *Plant, Cell & Environment* 22: 447–459.

Moghaddam, P. R., and D. Wilman. 1998. Cell wall thickness and cell dimensions in plant parts of eight forage species. *Journal of Agricultural Science* 131: 59–67.

Nardini, A., G. Peda, and N. La Rocca. 2012. Trade-offs between leaf hydraulic capacity and drought vulnerability: Morpho-anatomical bases, carbon costs and ecological consequences. *New Phytologist* 196: 788–798.

Niklas, K. J. 1992. Plant biomechanics: An engineering approach to plant form and function. University of Chicago Press, Chicago, Illinois, USA.

Niklas, K. J. 1994. Plant allometry. University of Chicago Press, Chicago, Illinois, USA.

Nobel, P. S. 1976. Photosynthetic rates of sun versus shade leaves of *Hyptis emoryi* Torr. *Plant Physiology* 58: 218–223.

Nobel, P. S. 2009. Physicochemical and environmental plant physiology, 5th ed. Academic Press, San Diego, California, USA.

Nobel, P. S., L. J. Zaragoza, and W. K. Smith. 1975. Relation between mesophyll surface area, photosynthetic rate, and illumination level during development for leaves of *Plectranthus parviflorus* Henckel. *Plant Physiology* 55: 1067–1070.

Oguchi, R., K. Hikosaka, and T. Hirose. 2005. Leaf anatomy as a constraint for photosynthetic acclimation: Differential responses in leaf anatomy to increasing growth irradiance among three deciduous trees. *Plant, Cell & Environment* 28: 916–927.

Pantin, F., T. Simonneau, and B. Muller. 2012. Coming of leaf age: Control of growth by hydraulics and metabolics during leaf ontogeny. *New Phytologist* 196: 349–366.

Pasquet-Kok, J., C. Creese, and L. Sack. 2010. Turning over a new "leaf": Multiple functional significances of leaves versus phyllodes in Hawaiian *Acacia koa*. *Plant, Cell & Environment* 33: 2084–2100.

Pearsall, W. H. 1927. Growth studies. VI. On the relative sizes of growing plant organs. *Annals of Botany* 41: 549–559.

Pérez-Pérez, J. M., S. Rubio-Díaz, S. Dhondt, D. Hernández-Romero, J. Sánchez-Soriano, G. T. S. Beemster, M. Rosa Ponce, et al. 2011. Whole organ, venation and epidermal cell morphological variations are correlated in the leaves of Arabidopsis mutants. *Plant, Cell & Environment* 34: 2200–2211.

Pittermann, J., J. S. Sperry, J. K. Wheeler, U. G. Hacke, and E. H. Sikkema. 2006. Mechanical reinforcement of tracheids compromises the hydraulic efficiency of conifer xylem. *Plant, Cell & Environment* 29: 1618–1628.

Poorter, H., U. Niinemets, L. Poorter, I. J. Wright, and R. Villar. 2009. Causes and consequences of variation in leaf mass per area (LMA): A meta-analysis. *New Phytologist* 182: 565–588.

Poorter, H., K. J. Niklas, P. B. Reich, J. Oleksyn, P. Poot, and L. Mommer. 2012. Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. *New Phytologist* 193: 30–50.

Poorter, H., and L. Sack. 2012. Pitfalls and possibilities in the analysis of biomass allocation patterns in plants. *Frontiers in Plant Science* 3: 259.

Pyankov, V. I., and A. V. Kondrachuk. 2003. Basic types of structural changes in the leaf mesophyll during adaptation of Eastern Pamir plants to mountain conditions. *Russian Journal of Plant Physiology: A Comprehensive Russian Journal on Modern Phytophysiology* 50: 34–42.

PYANKOV, V. I., A. V. KONDRATCHUK, AND B. SHIPLEY. 1999. Leaf structure and specific leaf mass: the alpine desert plants of the Eastern Pamirs, Tadjikistan. *New Phytologist* 143: 131–142.

R DEVELOPMENT CORE TEAM. 2005. R: A language and environment for statistical computing, version 2.2.0. R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org/.

RICE, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43: 223–225.

RODERICK, M. L., S. L. BERRY, I. R. NOBLE, AND G. D. FARQUHAR. 1999. A theoretical approach to linking the composition and morphology with the function of leaves. *Functional Ecology* 13: 683–695.

SACK, L., P. D. COWAN, N. JAIKUMAR, AND N. M. HOLBROOK. 2003a. The 'hydrology' of leaves: Co-ordination of structure and function in temperate woody species. *Plant, Cell and Environment* 26: 1343–1356.

SACK, L., P. J. GRUBB, AND T. MARAÑÓN. 2003b. The functional morphology of juvenile plants tolerant of strong summer drought in shaded forest understories in southern Spain. *Plant Ecology* 168: 139–163.

SACK, L., AND K. FROLE. 2006. Leaf structural diversity is related to hydraulic capacity in tropical rain forest trees. *Ecology* 87: 483–491.

SACK, L., C. SCOFFONI, G. P. JOHN, H. POORTER, C. M. MASON, R. MENDEZ-ALONZO, AND L. A. DONOVAN. 2013. How do leaf veins influence the worldwide leaf economic spectrum? Review and synthesis. *Journal of Experimental Botany*: in press.

SACK, L., C. SCOFFONI, A. D. MCKOWN, K. FROLE, M. RAWLS, J. C. HAVRAN, H. TRAN, ET AL. 2012. Developmentally based scaling of leaf venation architecture explains global ecological patterns. *Nature Communications* 3: 837.

SCHMIDT-NIELSEN, K. 1984. Scaling. Cambridge University Press, Cambridge, UK.

SHIPLEY, B., M. J. LECHOWICZ, I. WRIGHT, AND P. B. REICH. 2006. Fundamental trade-offs generating the worldwide leaf economics spectrum. *Ecology* 87: 535–541.

SOKAL, R. R., AND F. J. ROHLF. 1995. Biometry, 3rd ed. Freeman, New York, New York, USA.

SPERRY, J. S., U. G. HACKE, AND J. K. WHEELER. 2005. Comparative analysis of end wall resistivity in xylem conduits. *Plant, Cell & Environment* 28: 456–465.

TOSENS, T., U. NIINEMETS, M. WESTOBY, AND I. J. WRIGHT. 2012. Anatomical basis of variation in mesophyll resistance in eastern Australian sclerophylls: News of a long and winding path. *Journal of Experimental Botany* 63: 5105–5119.

TURRELL, F. M. 1936. The area of the internal exposed surface of dicotyledon leaves. *American Journal of Botany* 23: 255–264.

WARTON, D. I., I. J. WRIGHT, D. S. FALSTER, AND M. WESTOBY. 2006. Bivariate line-fitting methods for allometry. *Biological Reviews of the Cambridge Philosophical Society* 81: 259–291.

ZWIENIECKI, M. A., T. J. BRODRIBB, AND N. M. HOLBROOK. 2007. Hydraulic design of leaves: Insights from rehydration kinetics. *Plant, Cell & Environment* 30: 910–921.