Causes of ecological gradients in leaf margin entirety: Evaluating the roles of biomechanics, hydraulics, vein geometry, and bud packing[1]

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PREMISE OF THE STUDY: A recent commentary by Edwards et al. (*Am. J. Bot.* 103: 975–978) proposed that constraints imposed by the packing of young leaves in buds could explain the positive association between non-entire leaf margins and latitude but did not thoroughly consider alternative explanations.

METHODS: We review the logic and evidence underlying six major hypotheses for the functional significance of marginal teeth, involving putative effects on (1) leaf cooling, (2) optimal support and supply of the areas served by major veins, (3) enhanced leaf-margin photosynthesis, (4) hydathodal function, (5) defense against herbivores, and (6) bud packing.

KEY RESULTS: Theoretical and empirical problems undermine all hypotheses except the support–supply hypothesis, which implies that thinner leaves should have non-entire margins. Phylogenetically structured analyses across angiosperms, the El Yunque flora, and the genus *Viburnum* all demonstrate that non-entire margins are indeed more common in thinner leaves. Across angiosperms, the association of leaf thickness with non-entire leaf margins is stronger than that of latitude.

CONCLUSION: We outline a synthetic model showing how biomechanics, hydraulics, vein geometry, rates of leaf expansion, and length of development within resting buds, all tied to leaf thickness, drive patterns in the distribution of entire vs. non-entire leaf margins. Our model accounts for dominance of entire margins in the tropics, Mediterranean scrub, and tundra, non-entire margins in cold temperate deciduous forests and tropical vines and early-successional trees, and entire leaf margins in monocots. Spinose-toothed leaves should be favored in short-statured evergreen trees and shrubs, primarily in Mediterranean scrub and related semiarid habitats.

KEY WORDS adaptation; convergence; development; entire leaf margins; non-entire leaf margins; logistic regression

A century ago, Bailey and Sinnott (1916) discovered a remarkable pattern of evolutionary convergence: in dicotyledonous (term of the authors) flowering plants, most woody species in lowland tropical forests have entire leaf margins, while most in cold temperate forests have toothed or lobed leaves (Fig. 1). Bailey and Sinnott recognized the possibility that this pattern might have arisen through lineages fixed for entire vs. non-entire leaf margins having differential latitudinal distributions for reasons other than leaf form. However, they dismissed that possibility, because most of the families studied have both kinds of foliage and the association of marginal entirety with latitude repeatedly holds within families as well. Bailey and Sinnott (1916) also found that arctic and alpine shrubs often have entire leaves, reversing the latitudinal trend in marginal entirety; that certain warm temperate areas (e.g., southern California, Western Australia) have a high proportion of species with entire leaf margins; and that the proportion of species with non-entire margins is greater among herbs and vines than trees in tropical regions. These latter patterns, however, have been largely ignored by subsequent researchers. Paleobotanists have often used the fraction of leaves with entire margins in paleofloras to reconstruct mean annual temperature and/or paleolatitude, calibrated against the tropical to cold temperate pattern observed in modern floras (Wolfe, 1971; Peppe et al., 2011; Yang et al., 2015).

Despite the marked latitudinal gradient in leaf margin entirety, surprisingly little consensus has emerged over the past hundred years as to its adaptive significance. Edwards et al. (2016) briefly discussed some of the arguments about the functional significance

FIGURE 1 Leaves of *Tilia heterophylla* (left) and *Mollia lepidota* of Malvaceae subfamily Tilioideae tribe Tilleae. *Tilia* exemplifies the leaves of many cold temperate woody plants, with toothed margins and craspedodromous venation, with secondary veins penetrating to the margin; *Mollia* exemplifies the leaves of many tropical species, with entire margins and camptodromous (in this case, brochidodromous to reticulodromous) venation, with secondary veins fusing in loops that parallel the margin. Images derived with permission from the National Cleared Leaf Collection.

HYPOTHETICAL ADVANTAGES OF NON-ENTIRE LEAF MARGINS

(1) Leaf cooling—Gates (1968) calculated that sunlit leaves dissected into narrow lobes or leaflets should equilibrate at cooler temperatures and transpire less per unit area. Vogel (1970) suggested that marginal teeth might also increase convective heat loss, and Gottschlich and Smith (1982) confirmed that convective heat loss from model leaves of a given average width increased with the depth of marginal teeth—but this happened only as the teeth became deep enough to narrow the interior of the leaf and reduce its actual width. Given that entire, lobed, compound, and toothed leaves can all have the same effective width and impact on heat exchange, transpiration, and photosynthesis averaged over a leaf's surface, Givnish and Vermeij (1976) concluded that the significance of such variations in leaf shape cannot lie in their impact on heat balance and gas exchange. The query raised by Edwards et al. (2016)—Why aren't leaves more dissected in the lowland tropics to dissipate the greater heat load there?—in our view cannot be resolved using heat-balance models alone. The seemingly paradoxical occurrence of large entire leaves in the humid air of lowland rainforests was first explained by Givnish and Vermeij (1976), based on natural selection maximizing the difference between photosynthesis and the root costs associated with transpiration as leaf temperature and the rate of water loss per unit area rise with increasing leaf width, yielding the prediction that moister or more humid conditions that reduce root costs favor broader leaves.

(2) Optimal support and supply—Givnish (1978, 1979) calculated that the optimal lamina area for a major vein to support mechanically and supply with fluids should taper linearly toward the distal tip of the vein. This shape results in a distal decrease of the vein cross section at each point along the vein, with a resulting decline in mechanical strength that exactly matches the bending torques caused by the leaf masses and lever arms beyond each point. This shape also results in a distal tapering of vein cross-sectional area and supply capacity that exactly matches the areas of leaf tissue that must be supplied with water at each point along the vein. The shape of this area close to the base of a leaf or the areas served by adjacent secondary veins should be further selected, however, to avoid unproductive shading by other leaves or duplication of mechanical/hydraulic service with the areas around adjacent secondary veins. This model explains the nearly universal tapering of leaves around their midribs toward their tips. Givnish (1978, 1979) proposed that, in thin leaves, the areas supported by adjacent secondaries should be somewhat mechanically independent of each other, because of the weak mechanical strength of a thin lamina. Thin leaves should thus favor tapering, triangular teeth toward the distal end of each secondary vein—like

of marginal teeth in terms of how they might (1) cool leaves, (2) optimize the areas supported and supplied by major veins, (3) enhance photosynthesis along the leaf margin early in the growing season, (4) serve as hydathodes to prevent water supplied by root pressure from flooding the mesophyll, or (5) reduce herbivory. They concluded (p. 975) that "none of [these hypotheses] is terribly well supported", and instead explored the hypothesis that leaf shape and non-entire leaf margins may instead reflect constraints imposed by the (6) packing of leaf primordia within resting buds.

In our opinion, the evidence for each of the six potential explanations for the significance of leaf margin entirety merits further examination. We briefly address the empirical and theoretical issues, suggest that the preponderance of evidence strongly favors the support–supply hypothesis, and propose an alternative framework that takes into account how biomechanics, hydraulics, gas exchange, bud packing, and the geometry of leaf venation can explain *all* of the major patterns discovered by Bailey and Sinnott (1916), not merely the partial latitudinal gradient discussed by Edwards et al. (2016) and others.

the distal portion of a leaf around its midrib—whereas the mechanical support provided by a thick lamina should work against the formation of separate teeth around each secondary. We add that, in thinner leaves, the areas supplied by adjacent major veins should also be more hydraulically independent of each other, given the fewer parallel pathways by which water can move from the xylem through the mesophyll (Sack et al., 2013) and the large fraction of total leaf conductance found outside the xylem (Sack and Holbrook, 2006). Mechanical and hydraulic independence of the areas supported and supplied by individual secondary veins means that those areas should taper distally, like leaves supported by a midrib, thus favoring marginal teeth in thin leaves. Simulations suggest that hydraulic supply can be critically limiting at the leaf margin; hydraulically vulnerable areas—that is, those far from secondary veins near their tips, on the leaf margin—are absent in toothed leaves (Roth et al., 1995). A completely different argument, based on minimizing the costs of supportive tertiary veins, accounts for the adaptive significance of lobed leaves, predicting their occurrence in foliage that is thin in cross section *and* effectively broad (i.e., with substantial widths of leaves, lobes, or leaflets), which matches the distribution of lobed leaves primarily in temperate deciduous forests and in the short-lived leaves of rapidly growing vines and early-successional trees of the lowland tropics (Givnish, 1979).

Givnish (1978) provided evidence for this support–supply hypothesis by showing that the fraction of species with entire margins, based on binned data, increased with leaf thickness in the El Yunque dicot flora studied by Howard (1969). Even in a tropical flora including only evergreen species, the expected connection of entire margins to leaf thickness prevailed. Here we use StatPlus in Excel to execute a phylogenetically unstructured logistic regression on Howard's data to demonstrate a highly significant rise ($P <$ 0.0042 for 38 df) in the probability y of entire leaf margins as leaf thickness x increases, $y = 1/(1+\exp[-(\alpha + \beta x)]$ for $\alpha = 2.6611 \pm$ 1.1057 (SE) and $\beta = -0.0103 \pm 0.0036$ (SE), even when the data are unbinned (Fig. 2). Scripts and data for all statistical analyses are deposited in Dryad (available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.h1d1b; Givnish and Kriebel, 2017). The observed pattern is consistent with the high fraction of species with non-entire margins (90%) in tree species of the northern United States, most of which are deciduous and have thin leaves (mean thickness = 152 μm for 22 species from Iowa), and much lower fractions of native dicots in subtropical Florida (16%; mean thickness = 210 μm for 33 species) and New Zealand (47%; mean thickness = 406 μm for 38 species), both of which have large fractions of evergreen taxa with much thicker leaves (leaf margin data from Bailey and Sinnott, 1916; leaf thickness data from Wylie, 1946, 1954). Shrubs of Mediterranean climates are characterized by thick, evergreen, sclerophyllous leaves, most of which have entire margins (but see point 4 below). These comparisons suggest that the connection between entire leaf margins and thick leaves seen in the El Yunque flora might apply much more broadly and help explain the latitudinal pattern in marginal entirety.

On a global scale, Royer et al. (2012) used phylogenetically unstructured analyses of a pool of 3549 species to show that the fraction of woody species with toothed leaf margins decreases sharply with leaf thickness, and that—after controlling for mean annual temperature—species with toothed leaf margins are more likely to be deciduous and have thin leaves, high leaf N content, a low leaf mass per unit area, and ring-porous wood. The first finding provides strong evidence for the support–supply hypothesis (see
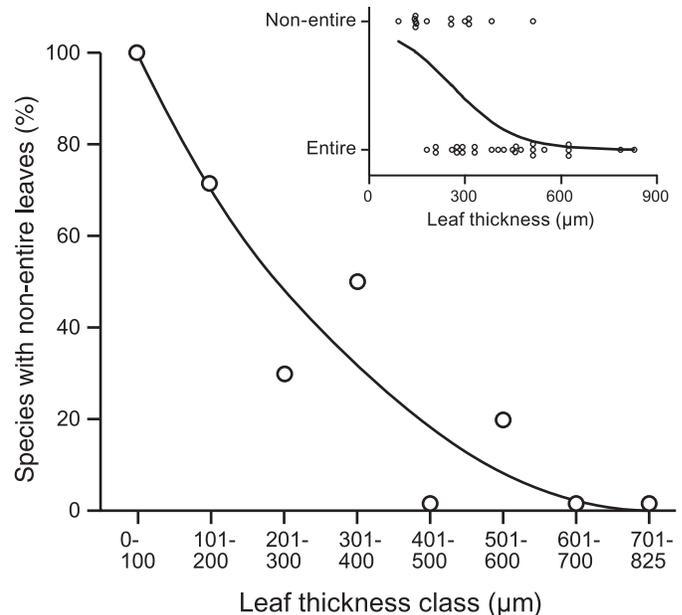
FIGURE 2 The fraction of species with non-entire leaves declines with leaf thickness in the tropical montane non-monocot flora of El Yunque, Puerto Rico (redrawn from Givnish, 1979). Inset: Distribution of leaf thicknesses and margin types by species, with a logistic regression showing the probability of a species having non-entire margins as a function of leaf thickness.

above). The latter findings are consistent with the leaf economics spectrum (Wright et al., 2004) and the functional integration of photosynthetic rate with stomatal conductance, transpiration rate, stem hydraulic conductance, xylem diameter, and wood density (Wong et al., 1979; Bucci et al., 2004; Santiago et al., 2004). In contrast to the data for woody species, Royer et al. (2012) found no significant latitudinal shift in the fraction of herbaceous species with toothed leaf margins. Royer et al. (2009) used phylogenetically structured analyses to show that the fraction and abundance of toothed woody species increase significantly in moving from ridges to riparian habitats and from shallow to deep soils across 100 sites in Australian subtropical notophyll vine rainforest (their table 4, fig. 6). Studies in North America (Kowalski and Dilcher, 2003) and South America (Burnham et al., 2001) have also shown a higher incidence of toothed species on moister sites at a given mean annual temperature. We suggest that all of these patterns are also consistent with the support–supply hypothesis, because thinner (and thus toothier) leaves with higher photosynthetic capacity are favored on moister and/or more fertile sites (Givnish, 1979, 1984).

Here we conduct the first phylogenetically structured tests of the correlations among leaf margin entirety (mostly due to the presence/absence of marginal teeth), leaf thickness, and latitude on a global basis, superimposing data for all traits gleaned from Royer et al. (2009) on a broad-scale angiosperm phylogeny provided by Zanne et al. (2014) and a more inclusive phylogeny to increase the match of species using S.PhyloMaker (Qian and Jin, 2015). Branch lengths are those given by the authors and based on time. Phylogenetic logistic regressions (Ives and Garland, 2010) using the phyloglm function in the R package phylolm (Ho and Ané, 2014) showed that the fraction of non-entire leaves decreases linearly with leaf

thickness (mm), significantly on the Zanne et al. tree ($\beta = -5.34 \pm 2.39$ [SE], $\alpha = 0.0068$, $P < 0.026$ for 181 non-entire species, 194 entire species), and in highly significant fashion on the larger Qian–Jin tree ($\beta = -3.02 \pm 0.55$, $\alpha = 0.0078$, $P < 4.7 \times 10^{-8}$ for 253 non-entire species and 362 entire species, including 97% of the 636 species for which Royer et al. tabulate leaf thickness and margin type) (Fig. 3, and Figs. S1 and S2 in Appendix S1 (see online Supplemental data with this article). Stochastic mapping of leaf entirety conducted with the R package phytools (Revell, 2012) under the best fitting all rates different (ARD) model of character evolution on the Qian–Jin tree identified 31.9 shifts from entire to non-entire leaves, and 95.1 from non-entire to entire margins. Marginal entirety is thus highly labile, and non-entire leaves were more frequently lost than gained. The lability of marginal entirety make its strong correlation with leaf thickness very likely to reflect replicated shifts in both variables, and thus strong evidence for the putative functional relationship of marginal entirety to leaf thickness (see Maddison and FitzJohn, 2015). We also found a significant increase in the incidence of entire leaves with leaf thickness after applying a phylogenetically structured logistic regression only to the 33 of the 40 El Yunque species that could be placed on the Qian–Jin tree ($\beta = -8.16 \pm 3.36$, $\alpha = 0.029$, $P < 0.016$). Together, based on modern analytical techniques, these results support the original findings of Givnish (1979) for the El Yunque flora, show that they apply at a global scale, and demonstrate that the highly significant pattern at the global scale does not depend on idiosyncrasies of species sampling in different data sets.

On the larger angiosperm tree, we also used phylogenetic generalized least squares regression (PGLS) to test whether leaf thickness declined with latitude, using the R package nlme (Pinheiro et al., 2016). As hypothesized, leaf thickness does decline with increasing latitude ($\beta = -2.18 \pm 0.81$, $P < 0.0074$, Ornstein–Uhlenbeck model for ln leaf thickness vs. latitude; decline not significant for untransformed data), while the fraction of non-entire leaves increased with latitude ($\beta = 0.032 \pm 0.010$, $\alpha = 0.0052$, $P < 1.2 \times 10^{-3}$) in a univariate PS logistic regression (see also Royer et al., 2009 and Little et al., 2010). In a PS logistic multiple regression, the incidence of non-entire margins increased with latitude L and decreased with leaf thickness θ ($\beta_L = 0.021 \pm 0.0095$, $P < 0.024$; $\beta_\theta = -5.73 \pm 1.24$; $P < 3.7 \times 10^{-6}$). We calculated the normalized effect of each trait x as $0.01 \cdot \beta_x \cdot SD(x)$, the impact of a change in a trait equal to 1% of its standard deviation across species. The normalized effect of leaf thickness on the incidence of entire leaves is 3.82 times that of latitude, and the significance of the partial effect of leaf thickness on leaf marginal entirety is nearly four orders of magnitude greater than that of latitude. The leaf margin–latitude nexus thus appears to be consistent with latitude (perhaps together with moisture seasonality in a more elaborate model) driving leaf thickness, and leaf thickness is driving leaf margin evolution, but with a small additional effect of latitude. On the basis of all the evidence, we find strong support for the support–supply hypothesis, the only previously proposed hypothesis providing a mechanism to predict that thinner leaves should be more likely to have non-entire margins.

(3) Enhanced leaf-margin photosynthesis—Early expansion and maturation of tissue at the tips of leaf teeth and lobes in deciduous species could yield high photosynthetic rates during leaf expansion early in the spring (Baker-Brosh and Peet, 1997). The abundance of veins and stomata near teeth and the thinner boundary layer at the

leaf margin might also enhance early photosynthesis there in expanding leaves, especially near teeth that might increase turbulence (Royer and Wilf, 2006). Baker-Brosh and Peet (1997) demonstrated higher photosynthetic rates per unit area near the leaf margins in eight of 15 woody deciduous species (all with toothed or lobed margins) from North Carolina and in none of four species with entire margins. Higher photosynthetic rates occurred within 2 mm of the leaf margin early in the growing season in 60 woody species from North Carolina and Pennsylvania and higher rates in toothed vs. untoothed margins (Royer and Wilf, 2006). Photosynthesis per unit area was up to 3–5 times higher in toothed margins than in the rest of the leaf early in the growing season. The area near the leaf margin is quite small in midsummer, but we estimate that it might account for 15–30% of total leaf area just after bud break. So, for a few days in early spring, toothed leaf margins might add a substantial amount to total photosynthesis, increasing leaf carbon gain by 30–120% above what it would have been if the margin had the same rate as the rest of the leaf. This contribution is probably caused mostly (and perhaps entirely) by the earlier maturation of the tips of secondary veins and adjacent lamina than tissue in the core of the leaf or at the margin of untoothed leaves, which often lack rapidly developing vein termini (see later Synthesis section). As development proceeds basipetally into the leaf interior, that area should gain photosynthetic competence and erase most or all of the photosynthetic "advantage" of the leaf margin.

Consequently, differences in marginal toothiness are unlikely to result in differences in leaf photosynthesis summed over the growing season. Plants might gain a substantial edge in carbon uptake via toothed leaf margins over an entire growing season if they (1) bear small leaves that mature more rapidly over their entire surface and have a longer active season as a result; or (2) achieve higher photosynthetic rates at the leaf margin via the reduced resistance to diffusion through the boundary layer there. The fact that most leaves, even in cold temperate areas, are far broader than their toothed margins suggests that marginal photosynthesis is simply not that important over the growing season. We know of no study showing enhanced photosynthetic rates at the leaf margin after full leaf expansion.

(4) Hydathodal teeth—Following earlier suggestions (Haberlandt, 1914; Rea, 1929), Feild et al. (2005) proposed that hydathodal teeth prevent water supplied by root pressure from flooding the mesophyll. This function would allow plants to refill xylem embolisms or drive leaf expansion without reducing photosynthesis via greatly increased resistance to CO_2 diffusion through water vs. air. Their experiments on *Chloranthus japonicus* support this conclusion; the mesophyll of leaves with intact hydathodes did not flood, while those with blocked hydathodes lost up to 40% of PSII photon yields, potentially for hours in highly humid habitats (Feild et al., 2005). Hydathodal teeth may thus be adaptive for cold temperate plants seeking to refill freeze–thaw embolisms (Edwards et al., 2016). Toothed leaves are significantly associated with ring-porous wood (Royer et al., 2012), whose wide vessels can easily embolize during frosts or drought; entire margins are associated with diffuse-porous wood with narrower vessels less susceptible to cavitation. However, large numbers of tropical plants possess hydathodes (Roth, 1990), many arctic and alpine shrubs exposed to frequent frost lack teeth of any kind (Bailey and Sinnott, 1916), species with toothed leaves often do not produce root pressure (Edwards et al., 2016), most temperate plants produce root pressure only briefly before the buds
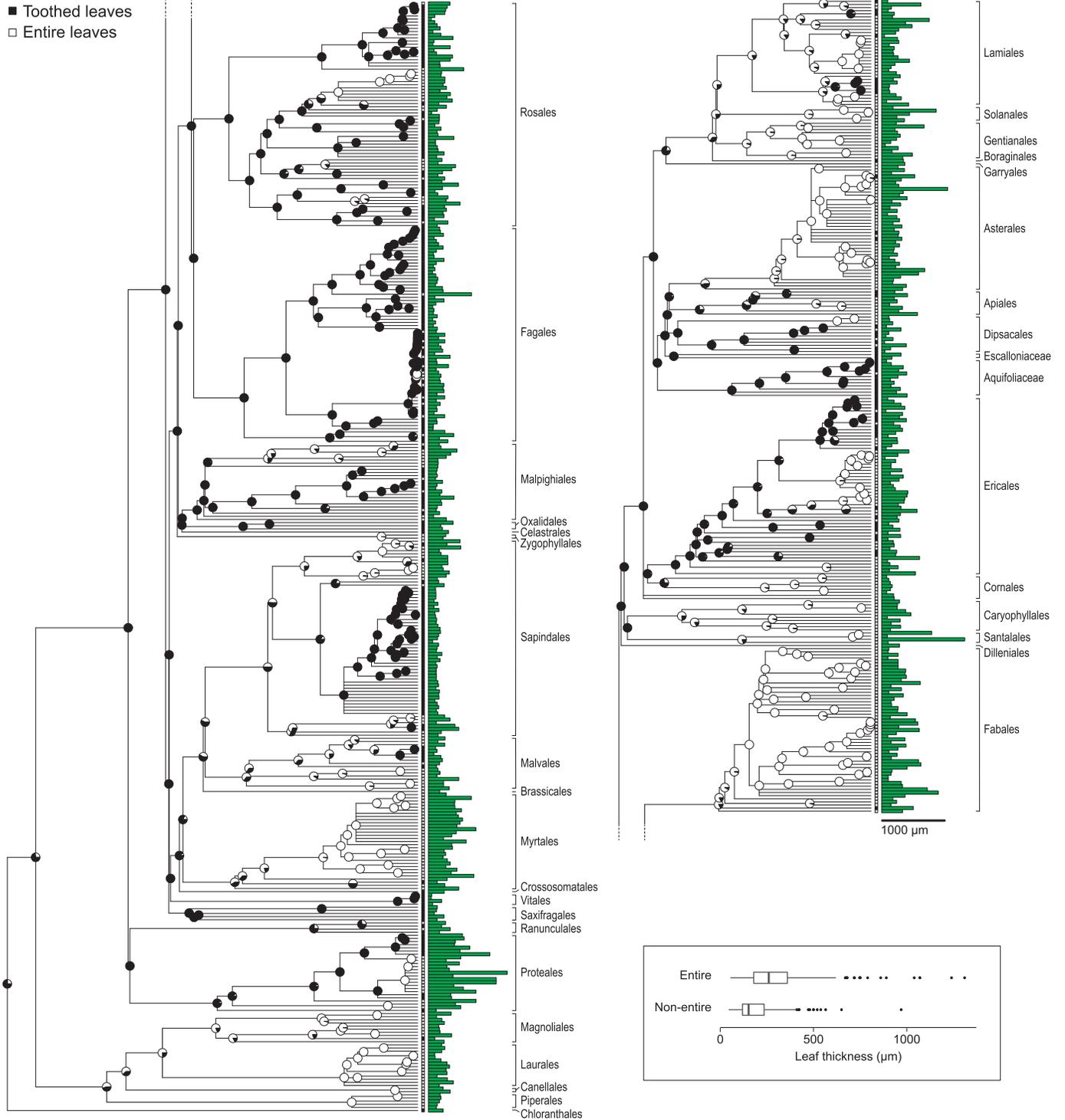
FIGURE 3 Leaf thickness and marginal type superimposed on the Qian–Jin molecular angiosperm phylogeny (Qian and Jin, 2015), showing repeated evolution of thin leaves with non-entire margins. Branch lengths related to extent of genetic divergence in ultrametric tree. Green bars indicate leaf thickness (black bar = 1000 μm). Black boxes indicate taxa with toothed leaf margins; white boxes, taxa with entire leaf margins. Pie charts indicate fraction of stochastic mapping runs in which the inferred ancestral taxa had toothed leaves, using the "all rates different" option selected by a ML test. Ordinal names are shown to the right.

open (Singh, 2016), monocots do produce root pressure (Singh, 2016) but almost none bear teeth, and several plants with entire leaf margins nevertheless have functional hydathodes (Fahn, 1979). Taken together, these facts strongly suggest that hydathodal activity cannot account for much of the ecological distribution of non-entire leaf margins.

The largest veins in leaves are the first to embolize (Brodribb et al., 2016), which may account for the position of hydathodes at the ends of primary, secondary, and often tertiary veins; root pressure could directly refill these veins and excess water be borne directly to the (potentially desiccated) leaf margin without flooding the epidermis. The frequent placement of hydathodes on marginal teeth may also allow the leaves to shed exudate rapidly and avoid damage by pathogens or epiphylls, with the teeth acting as mini-drip tips (Feild et al., 2005).

(5) Antiherbivore defense—Ehrlich and Raven (1964, 1967) first claimed that the spinose leaf teeth of European holly were adapted to deter herbivory by tent caterpillars, based on an experiment by Merz (1959) showing the edge-feeding caterpillar *Lasiocampa quercus* only fed on holly leaves after the spinose teeth were removed. Givnish (1979) reasoned, however, that those spinose teeth were set too far apart to act as an effective deterrent to feeding by insects. Subsequently, Potter and Kimmerer (1988) showed that Ehrlich and Raven had misinterpreted Merz's experiment, which entailed the removal of *both* spinose teeth and the fibrous leaf margin.

They demonstrated that it was the removal of that leaf margin—*not* the spinose teeth—that deterred feeding on American holly by edge-feeding caterpillars.

Givnish (1976, 1979) proposed that spinose teeth on hollies and similar plants were a defense against mammalian browsers (a view subsequently adopted by Brown and Lawton, 1991 and others) and that leaves would have to be very stiff for marginal spines to be an effective defense. Spinose leaves should be favored in short woody plants with tough, evergreen, sclerophyllous leaves accessible to browsers because placement on the leaf margin would directly enhance the defense of the leaves against browsers and because marginal spines mounted on a stiff leaf would not fold up when ingested and become ineffective. As a consequence, leaves with spinose teeth should be favored in semiarid to arid areas that select for thick, stiff evergreen foliage. Indeed, numerous shrubby species with holly-like leaves are native to areas of Mediterranean climate (Fig. 4), including several scrub oaks (e.g., *Quercus chrysolepis*, *Q. dumosa*, *Q. durata*), *Heteromeles arbutifolia*, *Prunus ilicifolia*, and *Rhamnus ilicifolia* in California chaparral (Givnish, 1979; Jepson Flora Project, 2016); numerous *Quercus* species (e.g., *Q. ilex*) in Mediterranean macchia; *Kageneckia oblonga*, *Colliguaya odorifera*, *Colletia spinosissima*, and *Retanilla ephedra* (leaves are photosynthetic spines in last two species) in Chilean matorral (see Schultz, 1995 for plant lists), and many saw-toothed leaves and leafy spines in *Banksia*, *Grevillea*, and *Hakea* in Australia, especially in the southwest (Carlquist, 1974; Givnish, 1979; Groom and Lamont, 2015). Relatively
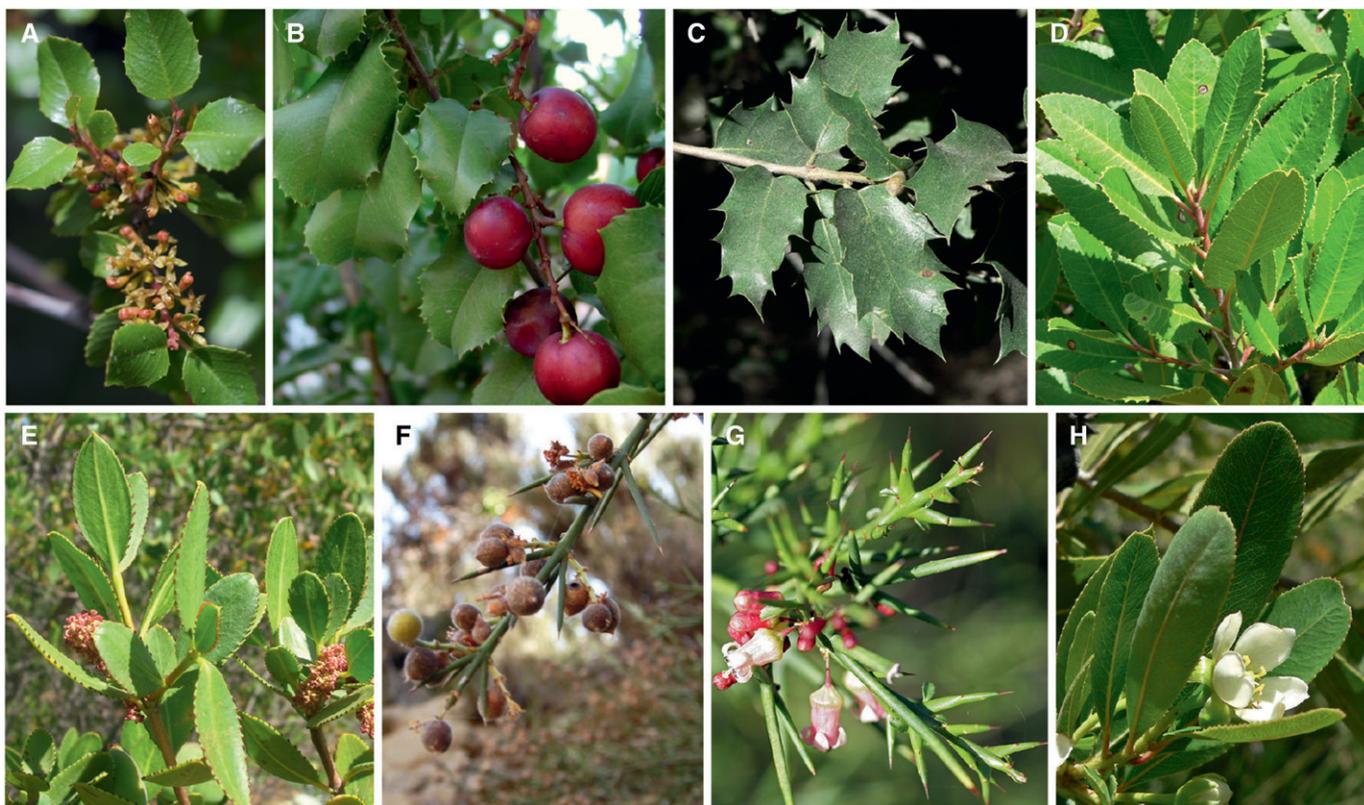
FIGURE 4 Holly-like leaves from (A–D) Californian chaparral and (E–H) Chilean mattoral, two forms of Mediterranean scrub from western North and South America. (A) *Rhamnus ilicifolia* (Rhamnaceae); (B) *Prunus ilicifolia* (Rosaceae); (C) *Quercus chrysolepis* (Fagaceae); (D) *Heteromeles arbutifolia* (Rosaceae); (E) *Colliguaya odorifera* (Euphorbiaceae); (F) *Retanilla ephedra* (Rhamnaceae); (G) *Colletia spinosissima* (Rhamnaceae); (H) *Kageneckia oblonga* (Rosaceae).

few spinescent plants are found in fynbos (Campbell, 1986), but half of them have holly-like leaves (Milton, 1991).

In general, evergreen oaks with holly-like leaves appear to be common only in semiarid areas of winter rainfall that favor evergreens, including southern California, the Mediterranean region, and the western Himalayan region (Brenner, 1902). Givnish (1976, 1979) reasoned that thorns on branches should replace spinose leaves in short plants with deciduous foliage; such thorns could defend several leaf cohorts, and soft deciduous leaves could too easily fold in a browser's mouth, rendering marginal leaf spines ineffective. The species of *Prunus* native to southern California nicely illustrate these principles (Givnish, 1976). Evergreen shrubs accessible to browsers on the mainland have spinose leaves (*P. ilicifolia* subsp. *ilicifolia*), while those occurring on the Channel Islands were unlikely to have been attacked by native mammalian herbivores and have entire leaves (*P. ilicifolia* subsp. *lyonii*). Short (<3 m) deciduous species (*P. andersonii*, *P. fasciculata*, *P. fremontii*, *P. subcordata*) have thorny branches and toothed leaves; tall deciduous species (*P. emarginata*, *P. virginiana*) also have toothed leaves but lack thorns (data from Jepson Flora Project, 2016). More broadly, Bowen and van Vuren (1997) found that sheep preferred feeding on the Channel Island form over the California mainland form of closely related species or subspecies in all six shrub genera examined (*Ceanothus*, *Cercocarpus*, *Dendromecon*, *Heteromeles*, *Prunus*, *Quercus*). Although island and mainland taxa differed from each other chemically in one or a few traits in one or two genera, the principal difference was that island forms had a sparser array of shorter leaf spines than their mainland relatives, clearing implicating the mechanism proposed by Givnish (1976).

So, contrary to Edwards et al. (2016), we believe there is strong comparative evidence for spinose teeth being effective as defenses against vertebrate herbivores in semiarid environments favoring evergreen foliage. However, there appears to be no evidence that more typical teeth in thinner leaves serve any defensive purpose. They might protect emerging leaves from tiny insects, but there are presently no data supporting such a role.

Taking into account the defensive spines of very thick leaves greatly clarifies the connection of leaf margins to leaf thickness and latitude. If we recode the spinose-leaved species on the larger angiosperm tree as having entire margins—distinguishing them from plants with teeth favored by support–supply considerations—the normalized effect of latitude on marginal entirety and its significance vs. that of leaf thickness both decline relative to our earlier analysis, while the relative significance of leaf thickness increases ($\beta_L = 0.030 \pm 0.0099$, $P < 0.0024$; $\beta_\theta = -8.24 \pm 1.42$; $P < 6.8 \times 10^{-9}$). Results are nearly identical if the spinose-leaved taxa are simply excluded ($\beta_L = 0.030 \pm 0.0099$, $P < 0.0028$; $\beta_\theta = -8.06 \pm 1.42$; $P < 1.2 \times 10^{-8}$). The normalized effect of leaf thickness on marginal entirety is now 3.7 times that of latitude and, as before, accounts for 79% of the explanatory power in the multiple regression. Excluding the unusual cases of spinose teeth increases the significance of the relationship of marginal teeth to thin leaves and latitude by roughly two orders of magnitude, while leaving the strength of the effect of each variable on leaf margins unchanged.

Spinose leaves do differ dramatically from most other leaves, with or without toothed margins. In the Royer et al. (2012) data set, spinose leaves average 487 ± 183 μm in thickness; leaves with non-entire margins, 175 ± 87 μm; and leaves with entire margins, 297 ± 181 μm. Including spinose leaves among those with non-entire margins raises the average for that category to 191 ± 117 μm,

increasing the SD by 33.4% and decreasing the difference in means between entire- and non-entire-margined leaves by 13%. Treating spinose leaves as having entire margins had little impact on the number of state transitions estimated using stochastic mapping, with 33.3 from entire to toothed margins, and 98.9 in the reverse direction.

(6) Bud packing—Following Lubbock (1899) and Couturier et al. (2011, 2012), Edwards et al. (2016) suggest that the pattern and duration of packing of leaf primordia in resting buds plays a major role in determining the presence and latitudinal distribution of non-entire leaf margins. We question the role of leaf packing in explaining this pattern. First, the proposals made by Lubbock (1899) and Couturier et al. (2011, 2012) bear on leaf lobes, *not* teeth; so far as we can tell, nothing they stated explains how bud packing could account for toothed leaf margins. This point regarding toothed margins is crucial, because the great majority of non-entire leaf margins involve leaf teeth, not lobes (see Table 1). Of the species with non-entire margins in the Royer et al. (2012) global data set that we overlaid on the Qian–Jin tree, 78% are toothed only, 18.5% are lobed and toothed (to which our hypotheses regarding marginal teeth would also apply), and only 3.5% are lobed but not toothed.

Second, many plants produce toothed leaf margins but no resting buds whatsoever. For example, most late-summer herbs of indeterminate growth in deciduous forests of the eastern United States (e.g., *Aster*, *Circaea*, *Eupatorium*, *Impatiens*, *Pilea*, *Solidago*, *Urtica*) bear toothed leaves (Givnish, 1987) that develop directly from leaf primordia with no period of dormancy. Admittedly, some of these involve hydathodal teeth, but the presence of teeth without long periods of development in buds runs counter to the hypothesis advanced by Edwards et al. (2016). Third, Edwards et al. failed to provide any rationale as to how development within buds, prolonged or short, could favor the development of toothed margins or favor such margins to a greater extent at higher latitudes. Clearly, neither teeth nor lobes are required for rapid leaf deployment at high latitudes, as evidenced by the success of *Lindera*, *Pyrularia*, *Vaccinium*, and numerous other entire-margined, early-leafing shrubs at temperate and boreal latitudes. There has been no demonstration, moreover, that packing primordia in buds must result in either lobes or teeth, or that such leaves do in fact unfurl any faster than those of species with leaves of comparable area. The fact that arctic and alpine woody plants generally have entire leaves (Bailey and Sinnott, 1916) even though they have longer apparent periods of leaf development and packing inside buds than cold temperate species argues against this claim. Such tundra species, however, may accord with the support–supply hypothesis

TABLE 1. Numbers and percentages of species with different kinds of leaf (or leaflet) margins from Royer et al. (2012) that were overlaid on the Qian and Jin (2015) tree. Third column indicates the percentages of species with non-entire leaf margins that are toothed only, lobed and toothed, or lobed only.

Leaf margin	No. of species	% Total species	% Non-entire species
Entire	362	41.1	—
Non-entire	253	58.9	—
Toothed but not lobed	198	32.2	78.3
Lobed and toothed	46	7.4	18.2
Lobed but not toothed	9	1.5	3.6

because they often have thick leaves (e.g., *Diapensia*, *Empetrum*, *Loiseluria*, *Rhododendron lapponicum*, *Vaccinium vitis-idaea*), perhaps reflecting the cold soil from which roots must inefficiently harvest water, elevating the root costs of transpiration and favoring narrow, thick leaves (Givnish, 1979). Importantly, however, Edwards et al. (2016) observed that in *Viburnum*, the earliest-emerging leaves are sometimes more likely to be toothed than those that emerge later on the same shoots, suggesting that leaves that spend a long time developing in resting buds are more likely to have non-entire margins than leaves that spend little or no time in resting buds, though the causal pathway for this pattern remains unclear, and whether it involves time in bud or correlated factors.

Fourth, causes for variation in the form or physiology of any organ can always be found in terms of *how* (in developmental or genetic terms) that organ forms and *why* (in adaptive terms) it forms in a particular way. Addressing the first question—finding the proximal reasons for possession of a trait—does not in any way address the second issue—finding the ultimate drivers of that trait, if they exist. Or vice versa. We may one day discover the characteristic developmental pathway leading to toothed leaf margins. But it is not clear that such a discovery would explain the context-specific adaptive value of those margins. There are likely many different outcomes to the coadaptation of the shape of developing leaves and bud packing geometry, unlike the evolution of leaf size or growth form to environmental conditions. It is not at all clear why bud packing should shape leaves rather than leaf shape sculpt buds and why environmental conditions would drive either (i.e., if particular packings were to result in non-entire leaf margins resulting in faster leaf expansion).

Finally, the proposal that bud packing somehow explains the distribution of entire and non-entire leaf margins in *Viburnum* may be unnecessary: The support–supply hypothesis appears to account for this pattern. On the basis of the phylogeny and leaf thickness data for 81 *Viburnum* taxa presented by Chatelet et al. (2013) and the leaf margin types scored by Schmerler et al. (2012) for several dozen of these species, and by us for the remaining taxa (data sets available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.h1d1b; Givnish and Kriebel, 2017), we found a significant phylogenetically structured logistic regression relating leaf margin to leaf thickness, regardless of whether we pooled minutely toothed species with those having non-entire margins ($\beta = -10.14 \pm 3.71$ SE, $\alpha = 3.51$, $P < 0.007$) or entire margins ($\beta = -7.81 \pm 3.74$, $\alpha = 6.62$, $P < 0.037$) (Fig. 5 and Fig. S3 in Appendix S1). Leaf margins are highly labile. In the first of these analyses, stochastic mapping identified 22.7 transitions from entire to non-entire leaves and 23.3 in the opposite direction. The second analysis identified 19.8 transitions from entire to non-entire leaves and 23.9 in the opposite direction.

These reconstructions accord with the demonstration by Schmerler et al. (2012) of repeated transitions from entire to non-entire leaves, and vice versa, associated with latitude in a much smaller set of *Viburnum* species. Our findings extend those results by showing how they are coupled to shifts in leaf thickness. The high value of α in the *Viburnum* analyses vs. the across-angiosperm and El Yunque analyses (see above) point to a strong effect of phylogeny on the distribution of entire vs. non-entire leaves within *Viburnum*, probably due to the relatively small number of transitions inferred within a genus rather than across a flora or all angiosperms. In the pool of 81 *Viburnum* taxa surveyed, species with entire leaf margins have an average leaf thickness of 229 ± 65 μm ($N = 20$), significantly greater ($t = -3.03$, $P < 0.0035$, 2-tailed t test with unequal sample

variances for 69 df) than the thickness of 175 ± 68 μm ($N = 51$) for species with toothed leaf margins. Those with minutely toothed margins had an average thickness of 242 ± 54 μm ($N = 10$) and did not differ significantly in that measure from those with entire margins.

SYNTHESIS

Our analyses strongly point toward the support–supply hypothesis and the importance of leaf thickness in determining broad-scale ecological patterns in the distribution of entire vs. non-entire leaf margins. Bailey and Sinnott (1916, p. 35) themselves stated that "Among woody plants, well-developed non-entire margins occur commonly on comparatively thin, soft leaves with prominent veins. Entire margins, on the other hand, usually occur on thicker, stiffer, more leathery leaves which are provided with structures that seem to retard evaporation." We believe, however, that a comprehensive model for the evolution of entire vs. non-entire leaf margins must integrate the support–supply hypotheses with a few additional considerations, including the geometry of leaf venation, the relationship of that geometry to leaf thickness, rapid leaf expansion, and healing embolisms, and the relationship of leaf thickness to packing within resting buds (Fig. 6), as we now explain.

The most important factor ignored in all the studies reviewed here is the direct relationship of leaf margin geometry to venation pattern. Across angiosperms, we believe there is a strong association of toothed margins with craspedodromous or semicraspedodromous venation and of untoothed margins with brachidodromous, eucamptodromous, or reticulodromous venation (Fig. 7; see also Fig. 1). In craspedodromy, the secondary veins terminate in teeth; in semicraspedodromy, adjacent secondaries join near the margin, but then give to rise to another secondary vein at the top of their arch that penetrates to the margin. In brachidodromy, adjacent secondaries form loops and arches that parallel the margin; eucamptodromy involves arcuate venation, in which the secondaries come to parallel the leaf margin near their terminus; and reticulodromy includes secondary veins that vanish into a network of increasingly fine elements near the leaf margin (Fig. 7).

The association of toothed margins with craspedodromous or semicraspedodromous venation is evident in the illustrations of various tooth types defined by Hickey and Wolfe (1975), in which a strong vein lies at the center of individual teeth and penetrates completely or nearly completely to the leaf margin. We were able to document, for the first time, a strong quantitative association of venation pattern with leaf margin types, using the tree flora of the Great Smoky Mountains sampled by Whittaker (1956: table 2). Inspection of specimens in the National Cleared Leaf Collection (http://peabody.research.yale.edu/nclc/) revealed that essentially all Great Smoky Mountains tree genera with toothed margins (*Acer*, *Aesculus*, *Aralia*, *Carya*, *Clethra*, *Fagus*, *Fraxinus*, *Hamamelis*, *Ilex*, *Ostrya*, *Prunus*, *Quercus*, *Tilia*) have craspedodromous or semicraspedodromous venation (cleared leaves of *Amelanchier* and arborescent *Halesia* were unavailable for inspection). Great Smoky Mountains tree genera with untoothed margins (*Cornus*, *Liriodendron*, *Magnolia*, *Nyssa*, *Oxydendrum*, *Pyrularia*, *Rhododendron*, *Robinia*, *Sassafras*) all had brachidodromous, eucamptodromous, or reticulodromous venation. A casual examination of the National Cleared Leaf Collection suggests that this pattern may prove general. We observed similar patterns in cleared leaves and photographs of

Viburnum leaf-margin evolution

■ Toothed leaves
□ Entire* leaves

Leaf thickness (µm)

setigerum
luzonicum
erosum
betulifolium
wrightii
tashiroi
parvifolium
japonicum
integrifolium
annamensis
formosanum
corylifolium
dilatatum
hupehense
foetidum
mullaha
lobophyllum
ichangense
acerifolium
cylindricum
coriaceum
vernicosum
hispidulum
beccarii
sambucinum
inopinatum
sargentii
opulus
trilobum
lautum
stellatotomentosum
hartwegii
disjunctum
costaricanum
stenocalyx
loeseneri
discolor
acutifolium
dentatum
rafinesquianum
rafinesquianum affine
molle
bracteatum
davidii
cinnamomifolium
propinquum
tinus
taitoense
suspensum
henryi
erubescens
farreri
odoratissimum
awabuki
sieboldii
foetens
grandiflorum
plicatum
lutescens
amplificatum
taiwanianum
lantana discolor
lantana
burejaeticum
veitchii
rhytidophyllum
cotinifolium
carlesii
bitchiuense
utile
macrocephalum
punctatum
prunifolium
lentago
rufidulum
elatum
cassinoides
lantanoides
furcatum
sympodiale
clemensiae

400 µm

FIGURE 5 Leaf thickness and marginal type in *Viburnum* superimposed on the molecular phylogeny of Chatelet et al. (2013), showing repeated evolution of thin leaves with non-entire margins. Symbols as in Fig. 3; here, however, species with minutely dentate margins have been grouped with those having non-entire margins. Pie charts reflect the "all rates different" option selected by a ML test. Species names are shown to the right.

A

Higher latitudes, short growing season, short leaf lifespan → Rapid leaf expansion → Plication, rapid leaf expansion

Prolonged bud packing

Low extraxylary hydraulic conductance, high water stress near leaf margins → Craspedodromous or semicraspedodromous venation penetrates to leaf margins

Thin leaves → Mechanical and hydraulic independence of areas served by secondary veins → **Marginal teeth**

High photosynthetic capacity → High hydraulic conductance of major veins, embolisms → Hydathodes to heal embolisms

B

Lower latitudes, long growing season, long leaf lifespan → Slow leaf expansion → No plication

Shortened or absent bud packing

High extraxylary hydraulic conductance, low water stress near leaf margins → Brachidodromous, camptodromous, or reticulodromous venation parallels leaf margins

Thick leaves → Mechanical and hydraulic dependence of areas served by adjacent secondary veins → **Entire margins**

Low photosynthetic capacity → Low hydraulic conductance of major veins, few embolisms → No hydathodes

Short stature, evergreen habit → **Spinose margins**

FIGURE 6 Integrated model for the evolution of (A) non-entire leaf margins and (B) entire and spinose leaf margins. The support–supply hypothesis is the direct path leading from leaf thickness to marginal entirety. Leaf thickness should also affect leaf margins by favoring different kinds of venation, and being associated with different levels of photosynthetic capacity, tendency to embolisms, and thus presence/absence of hydathodes to heal embolism. Higher latitudes should favor deciduous leaves except in areas of winter rainfall at mid latitudes or in areas of heavy leaching in boreal and low polar latitudes (Givnish, 2002); short-lived deciduous leaves, adapted to favorable conditions during the growing season, are usually thin. Moist fertile sites and short leaf lifetimes should favor thin leaves at any latitude (Givnish, 1979; Reich et al., 1997). The indirect and somewhat noisy relationship of leaf thickness to latitude may help account for the stronger effect of leaf thickness on marginal entirety (see text). Short growing seasons favor rapid leaf expansion, which in turn should favor plication of craspedodromous or semicraspedodromous venation to ensure rapid leaf expansion, as well as thin leaf primordia to allow extensive preformation in buds. Short stature plus thick, evergreen leaves should favor spinose leaf margins.

uncleared leaves of *Viburnum*. The patterns observed are consistent with the support–supply hypothesis, in that no teeth would be favored if secondary veins run parallel to the leaf margin: such veins never penetrate to the leaf margin, and hence teeth based on areas supported and supplied independently by individual secondaries cannot arise (Fig. 6). We infer that this point regarding venation may also be the reason why almost all monocots have entire margins, given that most have parallel veins that run along the leaf margin, and even those lineages with strongly branching venation (Givnish et al., 2005) have veins that parallel the margin. The few cases of toothed leaves in monocots appear to involve defensive leaf spines, as in several bromeliads (e.g., *Bromelia*, *Dyckia*, *Encholirium*, *Hechtia*, *Puya*) from dry habitats in the neotropics, and several species of *Agave* from New World deserts.

What factors might favor craspedodromous or semicraspedodromous venation in non-monocot angiosperms and thus trigger the development of marginal teeth as the optimal areas to be supported and supplied by major veins reaching the margin? Moist, fertile conditions and short growing seasons favor the evolution of thin leaves with short lifetimes, high photosynthetic rates, high stomatal conductances adapted to those rates (Wong et al., 1979; Reich et al., 1997), and thus, high rates of transpirational water loss. Such conditions should apply to deciduous forest trees; rapid leaf overtopping in fast-growing vines and early successional trees of the humid lowland tropics (e.g., *Cecropia*, *Macaranga*) should also result in thin leaves with short lifetimes and high photosynthetic and transpiration rates (Fig. 6). Thin leaves should also have, other things being equal, low rates of extraxylary conductance and thus require large veins to provide high
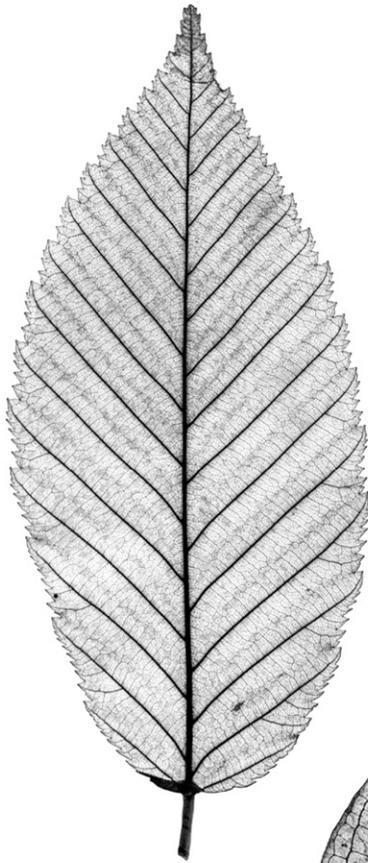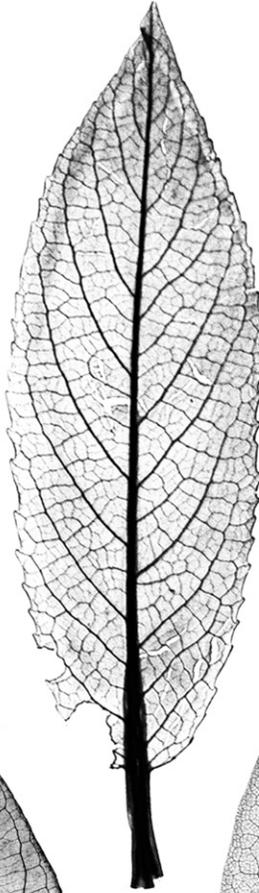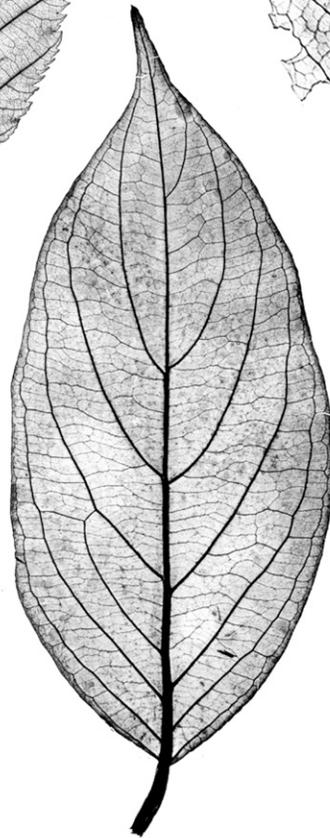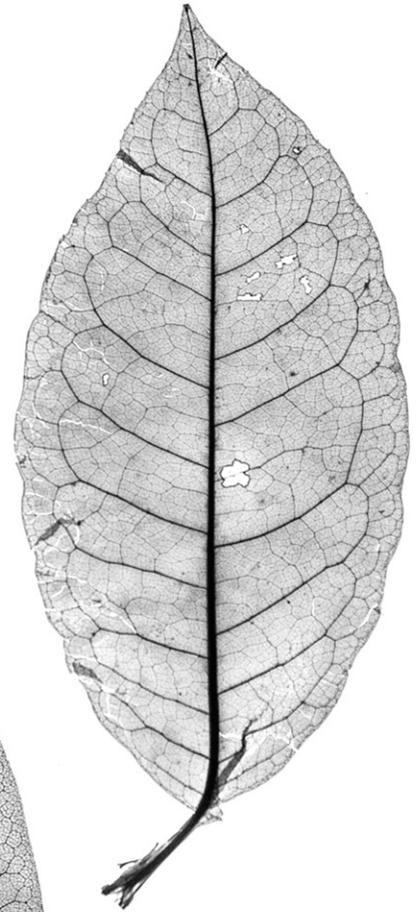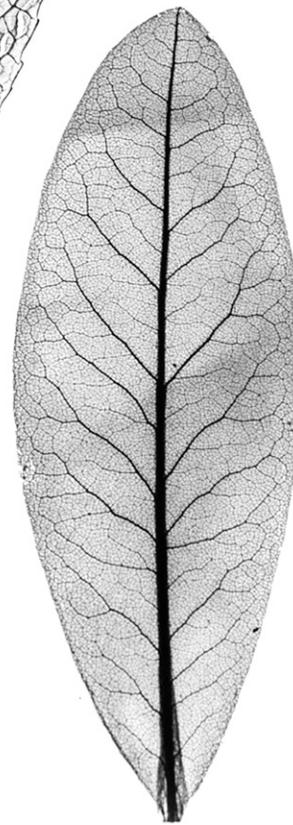
FIGURE 7 Examples of craspedodromous and semicraspedodromous venation, associated with non-entire leaf margins, and of brochidodromous, eucamptodromous, and reticulodromous venation, associated with entire leaf margins. Leaves not to scale. *Salix montana* from the National Cleared Leaf Collection; the others are from the ClearedLeavesDB (Das et al., 2014).

rates of flow all the way to the hydraulically vulnerable areas nearest the leaf margin (Roth et al., 1995). Thicker leaves should provide greater extraxylary conductance and be associated with lower photosynthetic rates, longer lifetimes, and lower transpiration rates, and thus permit looping connections between the secondary veins in various forms of camptodromous venation; such connections could promote greater long-term safety of the leaf hydraulic network, and the major veins paralleling the leaf margin could be permitted when extraxylary conductance is high (Fig. 6). Thin leaves should also select for thicker veins with more mechanical tissue to support the otherwise floppy lamina. In many species, high flow rates in thin leaves may also lead to greater risks of embolism within the leaf, selecting for root pressure to heal embolisms, especially in larger veins (Brodribb et al., 2016), selecting in another way for craspedodromous or semicraspedodromous venation leading to the leaf margin and hydathodal teeth to prevent flooding of the mesophyll and possibly loss of photosynthetic capacity (Feild et al., 2005) (Fig. 6). Craspedodromous or semicraspedromous venation may also allow rapid expansion of preformed leaves in the bud; plication of areas between parallel secondary veins could allow them to unfold rapidly as the buds open, providing a potential advantage in areas with a short growing season. *Fagus grandifolia* exemplifies this strategy; it has craspedodromous venation, plication, and the highest rate of leaf expansion among the tree species of the Great Smoky Mountains studied by Lopez et al. (2008).

Finally, Edwards et al. (2016) could be correct that packing of developing leaves in resting buds may affect leaf margins, but for reasons other than those they envisioned. Long periods of active development of young leaves within buds might only be possible for thin leaves that occupy relatively little volume (Fig. 6); this might account for the shift from non-entire to entire leaf margins in preformed vs. neoformed leaves on the same shoots of *Viburnum* plants (Edwards et al., 2016). Given that repeated shifts from non-entire to entire leaf margins across species in *Viburnum* are associated with increases in leaf thickness, it would be surprising if developmental shifts within species involving the same leaf forms were not also associated with increases in leaf thickness.

The selection pressures we have outlined involve a combination of biomechanics, hydraulics, vein geometry, rates of leaf expansion, and length of development within resting buds—all tied primarily to differences among species in leaf thickness and leaf longevity—as driving ecological patterns in the distribution of angiosperms with entire vs. non-entire leaf margins. This synthetic model (Fig. 6) would account for a preponderance of entire leaves in the lowland tropics, Mediterranean scrub, and alpine/arctic tundra, of non-entire leaves in cold temperate deciduous forests and fast-growing vines and early-successional trees of the tropics, and of the virtual absence of non-entire leaves in monocots. Spinose-toothed leaves should be favored in short-statured evergreen trees and shrubs, primarily in Mediterranean scrub and related arid and semiarid areas. Future progress in our understanding of the evolution of leaf margins will likely depend on (1) a more thorough survey of leaf thickness and leaf margins across all major angiosperm lineages and a wide range of sites and latitudes; (2) tests of the assumptions of the leaf-thickness model via vein cutting experiments to assess the degree of mechanical and hydraulic independence of areas served by individual secondary veins; (3) determining whether the developmental fields shaped by expanding secondary veins are narrower in thinner leaves (and consequently might favor teeth, vs. a continuous margin favored by overlapping fields); and (4) quantifying the extent to which marginal teeth in different lineages function as hydathodes.

DATA ACCESSIBILITY STATEMENT

All data files, trees, and scripts for the analyses presented in this paper have been deposited at Dryad (http://dx.doi.org/10.5061/dryad.h1d1b).

LITERATURE CITED

Bailey, I. W., and E. W. Sinnott. 1916. The climatic distribution of certain types of angiosperm leaves. *American Journal of Botany* 3: 24–39.

Baker-Brosh, K. F., and R. K. Peet. 1997. The ecological significance of lobed and toothed leaves in temperate forest trees. *Ecology* 78: 1250–1255.

Bowen, E., and D. van Vuren. 1997. Insular endemic plants lack defenses against herbivores. *Conservation Biology* 11: 1249–1254.

Brenner, W. 1902. Klima und Blatt bei der Gattung *Quercus. Flora* 90: 114–160.

Brodribb, T. J., D. Bienaimé, and P. Marmottant. 2016. Revealing catastrophic failure of leaf networks under stress. *Proceedings of the National Academy of Sciences, USA* 113: 4865–4869.

Brown, V., and J. Lawton. 1991. Herbivory and the evolution of leaf size and shape. *Philosophical Transactions of the Royal Society of London, B, Biological Sciences* 333: 265–272.

Bucci, S. J., G. Goldstein, F. C. Meinzer, F. G. Scholz, A. C. Franco, and M. Bustamante. 2004. Functional convergence in hydraulic architecture of tropical savanna trees: From leaf to whole plant. *Tree Physiology* 24: 891–899.

Burnham, R. J., N. C. A. Pitman, K. R. Johnson, and P. Wilf. 2001. Habitat-related error in estimating temperatures from leaf margins in a humid tropical forest. *American Journal of Botany* 88: 1096–1102.

Campbell, B. M. 1986. Plant spinescence and herbivory in a nutrient poor ecosystem. *Oikos* 47: 168–172.

Carlquist, S. 1974. Island biology. Columbia University Press, New York, New York, USA.

Chatelet, D. S., W. L. Clement, L. Sack, M. J. Donoghue, and E. J. Edwards. 2013. The evolution of photosynthetic anatomy in *Viburnum* (Adoxaceae). *International Journal of Plant Science* 174: 1277–1291.

Couturier, E., N. Brunel, S. Douady, and N. Nakayama. 2012. Abaxial growth and steric constraints guide leaf folding and shape in *Acer pseudoplatanus. American Journal of Botany* 99: 1289–1299.

Couturier, E., S. Courrech du Pont, and S. Douady. 2011. The filling law: A general framework for leaf folding and its consequences on leaf shape diversity. *Journal of Theoretical Biology* 289: 47–64.

Das, A., A. Bucksch, C. A. Price, and J. S. Weitz. 2014. ClearedLeavesDB: An online database of cleared plant leaf images. *Plant Methods* 10: 8.

Edwards, E. J., E. L. Spriggs, D. S. Chatelet, and M. J. Donoghue. 2016. Unpacking a century-old mystery: Winter buds and the latitudinal gradient in leaf form. *American Journal of Botany* 103: 975–978.

Ehrlich, P. R., and P. H. Raven. 1964. Butterflies and plants: A study in coevolution. *Evolution* 18: 586–608.

Ehrlich, P. R., and P. H. Raven. 1967. Butterflies and plants. *Scientific American* 216: 104–113.

Fahn, A. 1979. Secretory tissues of plants. Academic Press, London, UK.

Feild, T., T. Sage, and C. Czerniak. 2005. Hydathodal leaf teeth of *Chloranthus japonicus* (Chloranthaceae) prevent guttation-induced flooding of the mesophyll. *Plant, Cell & Environment* 28: 1179–1190.

Gates, D. M. 1968. Energy exchange and ecology. *BioScience* 18: 90–95.

Givnish, T. J. 1976. Leaf form in relation to environment. Ph.D. dissertation, Princeton University, Princeton, New Jersey, USA.

Givnish, T. J. 1978. Ecological aspects of plant morphology: Leaf form in relation to environment. *Acta Biotheoretica (Supplement: Folia Biotheoretica no. 7)* 27: 83–142.

Givnish, T. 1979. On the adaptive significance of leaf form. *In* O. T. Solbrig, P. H. Raven, S. Jain, and G. Johnson [eds.], Topics in plant population biology, 375–407. Columbia University Press, New York, New York, USA.

Givnish, T. J. 1984. Leaf and canopy adaptations in tropical forests. *In* E. Medina, H. A. Mooney, and C. Vásquez-Yánes [eds.], Physiological ecology of plants of the wet tropics, 51–84. Dr. Junk, The Hague, Netherlands.

Givnish, T. J. 1987. Comparative studies of leaf form: Assessing the relative roles of selective pressures and phylogenetic constraints. *New Phytologist* 106 (supplement): 131–160.

Givnish, T. J. 2002. On the adaptive significance of evergreen vs. deciduous leaves: Solving the triple paradox. *Silva Fennica* 36: 703–743.

Givnish, T. J., and Kriebel, R. Data from: Causes of ecological gradients in leaf margin entirety: evaluating the roles of biomechanics, hydraulics, vein geometry, and bud packing. Dryad Digital Repository. doi:10.5061/dryad.h1d1b

Givnish, T. J., J. C. Pires, S. W. Graham, M. A. McPherson, L. M. Prince, et al. 2005. Repeated evolution of net venation and fleshy fruits among monocots in shaded habitats confirms *a priori* predictions: Evidence from an *ndhF* phylogeny. *Proceedings of the Royal Society, B, Biological Sciences* 272: 1481–1490.

Givnish, T. J., and G. J. Vermeij. 1976. Sizes and shapes of liane leaves. *American Naturalist* 110: 743–778.

Gottschlich, D. E., and A. P. Smith. 1982. Convective heat transfer characteristics of toothed leaves. *Oecologia* 53: 418–420.

Groom, P. K., and B. Lamont. 2015. Plant life in southwestern Australia: Adaptations for survival. De Gruyter Open Ltd., Warsaw, Poland.

Haberlandt, G. 1914. Physiological plant anatomy. Macmillan, London, UK.

Hickey, L. J., and J. A. Wolfe. 1975. The bases of angiosperm phylogeny: Vegetative morphology. *Annals of the Missouri Botanical Garden* 62: 538–589.

Ho, L. S. T., and C. Ané. 2014. A linear-time algorithm for Gaussian and non-Gaussian trait evolution models. *Systematic Biology* 63: 397–408.

Howard, R. A. 1969. The ecology of an elfin forest in Puerto Rico, 8. Studies of stem growth and form and of leaf structure. *Journal of the Arnold Arboretum* 50: 225–267.

Ives, A. R., and T. Garland Jr. 2010. Phylogenetic logistic regression for binary dependent variables. *Systematic Biology* 59: 9–26.

Jepson Flora Project [eds]. 2016. Jepson eFlora. Available at http://ucjeps.berkeley.edu/eflora/ [accessed on 13 July 2016].

Kowalski, E. A., and D. L. Dilcher. 2003. Warmer paleotemperatures for terrestrial ecosystems. *Proceedings of the National Academy of Sciences, USA* 100: 167–170.

Little, S. A., S. W. Kembel, and P. Wilf. 2010. Paleotemperature proxies from leaf fossils reinterpreted in light of evolutionary history. *PLoS One* 5: e15161.

Lopez, O. R., K. Farris-Lopez, R. A. Montgomery, and T. J. Givnish. 2008. Leaf phenology in relation to canopy closure in southern Appalachian trees. *American Journal of Botany* 95: 1395–1407.

Lubbock, J. 1899. On buds and stipules. K. Paul, Trench, Tribner & Co., London, UK.

Maddison, W. P., and R. G. FitzJohn. 2015. The unsolved challenge to phylogenetic correlation tests for categorical characters. *Systematic Biology* 64: 227–136.

Merz, E. 1959. Pflanzen und Raupen. Über einige Prinzipien der Futterwahl bei Gross- schmetterlingsraupen. *Biologisches Zentralblatt* 78: 152–188.

Milton, S. J. 1991. Plant spinescence in arid southern Africa: Does moisture mediate selection by mammals? *Oecologia* 87: 279–287.

Peppe, D., D. Royer, B. Cariglino, S. Oliver, S. Newman, E. Leight, R. Enikolopov, et al. 2011. Sensitivity of leaf size and shape to climate: Global patterns and paleoclimatic applications. *New Phytologist* 190: 724–739.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2016. nlme: Linear and nonlinear mixed effects models. R package version 3.1-128, available at http://CRAN.R-project.org/package=nlme.

Potter, D. A., and T. W. Kimmerer. 1988. Do holly leaf spines really deter herbivory? *Oecologia* 75: 216–221.

Qian, H., and Y. Jin. 2015. An updated megaphylogeny of plants, a tool for generating plant phylogenies and an analysis of phylogenetic community structure. *Journal of Plant Ecology* 9: 233–239.

Rea, M. W. 1929. Stomata and hydathodes of *Campanula rotundifolia* L. and their relation to environment. *New Phytologist* 22: 56–72.

Reich, P. B., M. B. Walters, and D. S. Ellsworth. 1997. From tropics to tundra: Global convergence in plant functioning. *Proceedings of the National Academy of Sciences, USA* 94: 13730–13734.

Revell, L. J. 2012. phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3: 217–223.

Roth, A., V. Mosbrugger, G. Belz, and H. J. Neugebauer. 1995. Hydrodynamic modeling study of angiosperm leaf venation types. *Botanica Acta* 108: 121–126.

Roth, I. 1990. Peculiar surface structures of tropical leaves for gas exchange, guttation, and light capture. *In* B. Rollet, C. Högermann, and I. Roth [eds.], Stratification of tropical forests as seen in leaf structure, part 2, 185–238. Kluwer, Dordrecht, Netherlands.

Royer, D. L., R. M. Kooyman, and P. Wilf. 2009. Ecology of leaf teeth: A multisite analysis from an Australian subtropical rainforest. *American Journal of Botany* 96: 738–750.

Royer, D. L., D. J. Peppe, E. A. Wheeler, and Ü. Niinemets. 2012. Roles of climate and functional traits in controlling toothed vs. untoothed leaf margins. *American Journal of Botany* 99: 915–922.

Royer, D. L., and P. Wilf. 2006. Why do toothed leaves correlate with cold climates? Gas exchange at leaf margins provides new insights into a classic paleotemperature proxy. *International Journal of Plant Sciences* 167: 11–18.

Sack, L., and N. M. Holbrook. 2006. Leaf hydraulics. *Annual Review of Plant Biology* 57: 361–381.

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* 64: 4053–4080.

Santiago, L. S., G. Goldstein, F. C. Meinzer, J. B. Fisher, K. Machado, D. Woodruff, and T. Jones. 2004. Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. *Oecologia* 140: 543–550.

Schmerler, S. B., W. L. Clement, J. M. Beaulieu, D. S. Chatelet, L. Sack, M. J. Donoghue, and E. J. Edwards. 2012. Evolution of leaf form correlates with tropical–temperate transitions in *Viburnum* (Adoxaceae). *Proceedings of the Royal Society, Series B* 279: 3905–3913.

Schultz, J. 1995. The ecozones of the world: The ecological divisions of the geosphere. Springer Verlag, Heidelberg, Germany.

Singh, S. 2016. Root pressure: Getting to the root of pressure. *Progress in Botany* 77: 105–150.

Vogel, S. 1970. Convective cooling at low air speeds and shapes of broad leaves. *Journal of Experimental Botany* 21: 91–101.

Whittaker, R. H. 1956. Vegetation of the Great Smoky Mountains. *Ecological Monographs* 26: 1–80.

Wolfe, J. A. 1971. Tertiary climatic fluctuations and methods of analysis of Tertiary floras. *Palaeogoegraphy, Palaeoclimatology, Palaecology* 9: 27–57.

Wong, S. C., I. R. Cowan, and G. D. Farquhar. 1979. Stomatal conductance correlates with photosynthetic capacity. *Nature* 282: 424–426.

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, et al. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821–827.

Wylie, R. B. 1946. Relations between tissue organization and vascularization in leaves of certain tropical and subtropical dicotyledons. *American Journal of Botany* 33: 721–726.

Wylie, R. B. 1954. Leaf organization of some woody dicotyledons from New Zealand. *American Journal of Botany* 41: 186–191.

Yang, J., R. A. Spicer, T. E. V. Spicer, N. C. Arens, F. M. B. Jacques, T. Su, et al. 2015. Leaf form-climate relationships on the global stage: An ensemble of characters. *Global Ecology and Biogeography* 24: 1113–1125.

Zanne, A. E., D. C. Tank, W. K. Cornwell, J. M. Eastman, S. A. Smith, et al. 2014. Three keys to the radiation of angiosperms into freezing environments. *Nature* 506: 89–92.