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Examining the support–supply and bud-packing hypotheses for the increase in toothed leaf margins in northern deciduous floras

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PREMISE: The proportion of woody dicots with toothed leaves increases toward colder regions, a relationship used to reconstruct past mean annual temperatures. Recent hypotheses explaining this relationship are that (1) leaves in colder regions are thinner, requiring thick veins for support and water supply, with the resulting craspedodromous venation leading to marginal teeth (support–supply hypothesis) or that (2) teeth are associated with the packing of leaf primordia in winter buds (bud-packing hypothesis).

METHODS: We addressed these hypotheses by examining leaf thickness, number of primordia in buds, growing season length (mean annual temperature, MAT), and other traits in 151 deciduous woody species using georeferenced occurrences and a Bayesian model controlling for phylogeny. We excluded evergreen species because longer leaf life spans correlate with higher leaf mass per area, precluding the detection of independent effects of leaf thickness on leaf-margin type.

RESULTS: The best model predicted toothed leaves with 94% accuracy, with growing season length the strongest predictor. Neither leaf thickness nor number of leaves preformed in buds significantly influenced margin type, rejecting the support–supply and bud-packing hypotheses.

CONCLUSIONS: A direct selective benefit of leaf teeth via a carbon gain early in the spring as proposed by Royer and Wilf (2006) would match the strong correlation between toothed species occurrence and short growing season found here using Bayesian hierarchical models. Efforts should be directed to physiological work quantifying seasonal photosynthate production in toothed and nontoothed leaves.

KEY WORDS Bayesian models; leaf teeth; leaf primordia; leaf thickness; leaf venation; preformed leaves in winter buds.

It is well documented that trees and shrubs from cold-temperate climates often have toothed leaves, while trees and shrubs from warm-tropical climates usually have entire-margined leaves (Bailey and Sinnott, 1915, 1916; Wolfe, 1971, 1993; Peppe et al., 2011; Royer, 2012a). What drives these correlations has remained enigmatic (Royer and Wilf, 2006; Royer, 2012a; Edwards et al., 2016, 2017; Givnish and Kriebel, 2017). One explanation for why low mean annual temperatures (MATs) favor species with toothed leaf margins is that cooler climates favor thinner leaves, which in turn require a strong vein system, with veins penetrating to the margin, resulting in craspedodromous venation (Fig. 1), to ensure sufficient mechanical strength and nutrient supply of the mesophyll. This explanation has been termed the support–supply hypothesis (Givnish, 1978, 1979; Givnish and Kriebel, 2017). The correlation between thinner leaves and cooler climates arises because a shorter growing season favors deciduousness and hence short-lived leaves. In craspedodromous venation, veins, ending in teeth, are formed *before* primordium growth ends, and the tissue between the vein never catches up in growth (Hickey, 1979; Roth et al., 1995; Edwards et al., 2017; Givnish and Kriebel, 2017).

An alternative explanation for the correlation between cold climates and high proportions of toothed leaves is that toothed leaves are a side effect of denser bud packing in climates with a pronounced cold season. This alternative is the so-called bud-packing
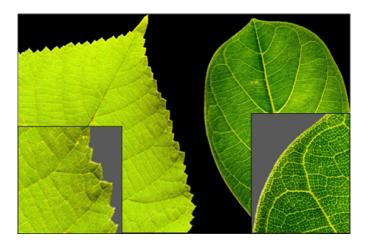
FIGURE 1. Examples of the leaf venation and margin types distinguished in this study. Left, toothed leaf margin and craspedodromous venation (secondary veins penetrating to the margin) in *Tilia platyphyllos*; right, entire-margin and noncraspedodromous venation (secondary veins fusing in loops parallel to the margin) in *Artocarpus heterophyllus*.

hypothesis (Edwards et al., 2016, 2017). Dense packing of young leaves in winter buds might cause prominent secondary veins because of how the developing leaves in the bud interdigitate. In some species, leaves that have been packed in overwintering buds possess teeth, while leaves formed later during the season are entire-margined, fitting with the bud-packing hypothesis (Edwards et al., 2016; Spriggs et al., 2018).

Figure 2 illustrates why these two hypotheses for toothed leaf margins in northern woody species (Edwards et al., 2017; Givnish and Kriebel, 2017) are difficult to disentangle. The reason is that the correlation between thin leaves and leaf-margin type appears in both. Under the support–supply model (Fig. 2A), the correlation is seen as the causal mechanism, i.e., toothed margins simply result from a thin leaf needing dense and sturdy veins that run to the margin. Under the bud-packing model (Fig. 2B), the correlation arises because a third variable—winter dormancy in regions with long winters (low MATs)—requires the packing of young leaves in well-protected buds. As can be seen in Fig. 2C, the problem is that any internal or external variable showing a latitudinal gradient, be it leaf thickness, leaf size, presence of drip tips, the annual cycling of day length, or bud packing, can in principle "explain" leaf-margin type.
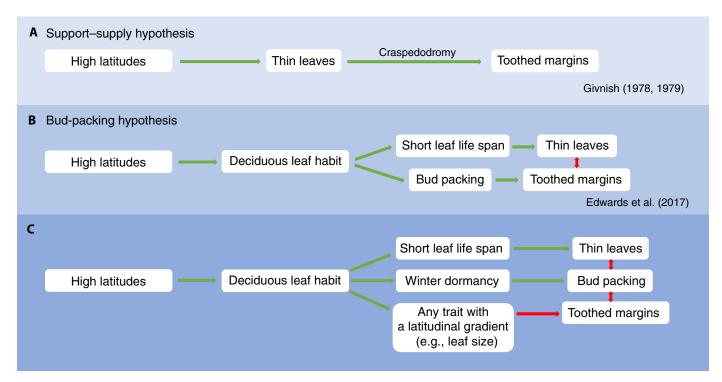

FIGURE 2. Alternative explanations for the latitudinal gradient in leaf margin type. Under all models, leaf thickness and margin type are assumed to be correlated. (A) The correlation may arise via a direct causal mechanism, such as envisioned by the support–supply hypothesis (Givnish, 1978, 1979; Givnish and Kriebel, 2017) where toothed margins emerge as a functional correlate of a thin lamina. (B, C) Alternatively, the correlation arises because both traits each are mechanistically connected to a third variable (deciduous leaf habit). (B) Under the bud-packing hypothesis (Edwards et al., 2017), toothed margins emerge as a functional correlate of winter buds. In this case, the evolution of a deciduous leaf habit in the temperate zone simultaneously selected for thinner leaves in response to a short growing season and leaf lifespan, and leaves with toothed margins then are the result of early leaf development inside of overwintering buds. In (C), we illustrate that any variable that features a latitudinal gradient can be used to explain leaf margin types, meaning that a strong trait correlation, by itself, cannot be used to support one model over the other. Causal mechanisms are indicated by green arrows; correlations that arise due to indirect connection are indicated by red arrows. In this study, we solely focused on species with a deciduous leaf habit, thereby excluding factors associated with this variable. If the support–supply model were true, leaf thickness should still be correlated with latitude and leaf-margin type even in a sample of only deciduous species. If, however, deciduous leaf habit were to drive the correlations, then our "deciduous-only" species sample should reveal no significant trait correlations.

Here we address the support–supply and bud-packing hypotheses by analyzing (1) the number of leaf primordia in winter buds, (2) leaf thickness, and (3) leaf-margin characteristics in deciduous species of trees and shrubs from throughout the temperate zone. We exclude evergreen species because evergreen plants usually have thicker leaves than deciduous species. Any analysis of the effect of leaf thickness on leaf-margin type (as postulated by the support–supply hypothesis) therefore needs to control for the functional differences between deciduous and evergreen species.

MATERIALS AND METHODS

Quantifying leaf and bud traits in 151 species

During 2017 and 2018, we documented and quantified bud packing, leaf thickness, and leaf venation type in 151 tree and shrub species (from 79 genera and 38 families) native to the northern hemisphere temperature zone and growing permanently outdoors in the Munich Botanical Garden (48°09′N, 11°30′E; 501 m a.s.l.; Appendix S1). Leaf buds were collected in winter 2017 and 2018 from five branches per species (one individual per species). The focus was on terminal buds, rather than on axillary buds at nodes, and we took great care not to include any reproductive buds. We counted the number of leaf primorida in five buds from one individual per species. A leaf blade was scored as present if venation was visible under the microscope after bud dissection. Buds in which only undifferentiated leaf primordia were visible were scored as zero. The values in Appendix S1 refer to the average number of preformed leaves within a bud. Leaf thickness (in millimeters) was measured in summer 2018 on five randomly chosen mature and healthy leaves per species (one individual per species) using a digital thickness gauge and taking care that the anvil of the thickness gauge fell only on the lamina between the veins, not the veins themselves.

Leaf venation was scored as craspedodromous or noncraspedodromous (Fig. 1). The secondary veins of craspedodromous leaves penetrate to the leaf margin; for all other venation types, veins do not penetrate to the margin and instead parallel it. To confirm that species have toothed leaves throughout their range, we studied herbarium material and photographed leaf margins in randomly selected specimens from the natural range of each species. (Images are accessible at https://www.gfbio.org, accession 37fa1c25-5751-4894-941d-b87fc2435b9f.)

Species ranges and temperature characteristics

Georeferenced occurrences of the 151 species were obtained from the Global Biodiversity Information Facility (GBIF, http://www.gbif.org/) using the gbif function of the R-package dismo (Hijmans et al., 2011). Filtering scripts were applied to exclude unreliable records and reduce spatial clustering using the following three criteria (see Zohner et al., 2016, 2017): For each species, we removed (1) records from continents where the species is not native, (2) coordinate duplicates at a resolution of 2.5 arc min, and (3) records based on fossil material, germplasm (cultivated plants), or literature. After applying the filtering scripts, we were left with a total of 479,488 occurrence records (on average 3175 occurrence records per species, minimally 30 occurrence records per species [threshold used by Zohner et al., 2016, 2017]). Occurrences were then queried against a grid file (2.5 arc

min spatial resolution) for MAT from the Worldclim version 2.0 data set (Hijmans et al., 2011; Fick and Hijmans, 2017). For each species, we determined the optimum MAT by calculating the 0.5 quantile (median) of all values.

Data analyses

We used a hierarchical Bayesian (HB) approach for analyzing the trait–trait and trait–climate correlations proposed in Fig. 3 in a single modelling framework. This approach allowed us (1) to control for phylogenetic structure and different functional types (shrub or tree growth habit) in the data and (2) to simultaneously fit slope parameters of functional and climatic traits relevant to our questions without concerns of P-value correction or multiple testing. A phylogenetically informed approach was necessary to account for the lack of statistical independence among species owing to the strong evolutionary signal in leaf-margin types and related traits (Little et al., 2010). Our model includes four dependent variables of which two are continuous variables (leaf thickness and number of leaves in winter buds [called "budpacking" in the model]), and two are binary variables (whether the species has craspedodromous venation or not and leaf teeth or not). Regression components of the models are of the form:

$$\text{Leafteeth} = \alpha_1 + \beta_1 \text{venation}_i + \beta_6 \text{budpacking}_i + \text{habit}_i + \text{family}_i \,(1)$$

$$\text{Venation} = \alpha_2 + \beta_2 \times \text{MAT}_i + \beta_3 \times \text{thickness}_i + \\ \beta_7 \times \text{budpacking}_i + \text{habit}_i + \text{family}_i \qquad (2)$$

$$\text{Thickness} = \alpha_3 + \beta_4 \times \text{MAT}_i + \beta_5 \times \text{budpacking}_i + \text{habit}_i \quad (3)$$

$$\text{Budpacking} = \alpha_4 + \beta_8 \times \text{MAT}_i + \text{habit}_i \qquad (4)$$

Number of leaf primordia in buds, leaf teeth, thickness, MAT, and venation refer to species values (i) in Appendix S1, α refers to the intercept, β refers to the estimated slopes of the respective variable, habit refers to the random intercept effect of whether a species has a shrub- or tree-like growth, and family refers to the family-level Non craspedodromous random intercept effect inserted in the binary leaf venation and leaf teeth submodels (Eqs. 1 and 2). In contrast to previous studies (Zohner et al., 2016, 2017), we did not insert a genus-level random effect because this prevented model convergence; the subpopulations of the model became too small (each genus, on average, included only two species). The variables leaf primordium number, leaf teeth, and leaf thickness were log-transformed to obtain normally distributed vectors. All variables were standardized before analysis (subtracting their mean and dividing by 2 standard deviations) to obtain relative, comparable effect sizes (Gelman and Hill, 2007). The resulting posterior distributions are a direct probability statement of the hypotheses.

We used the phylogenetic regression model of de Villemereuil et al. (2012) to account for phylogenetic structure in the model. Following the method of Grafen (1989), the ultrametric phylogeny was converted into a scaled (0–1) variance–covariance matrix (Σ), with covariances defined by shared branch lengths of species pairs, from the root to their most recent ancestor. Correlations were allowed to vary according to the phylogenetic signal of each continuous dependent variable, which was measured via Pagel's λ (Pagel, 1999). Values of λ close to 1 indicate large phylogenetic signal, with
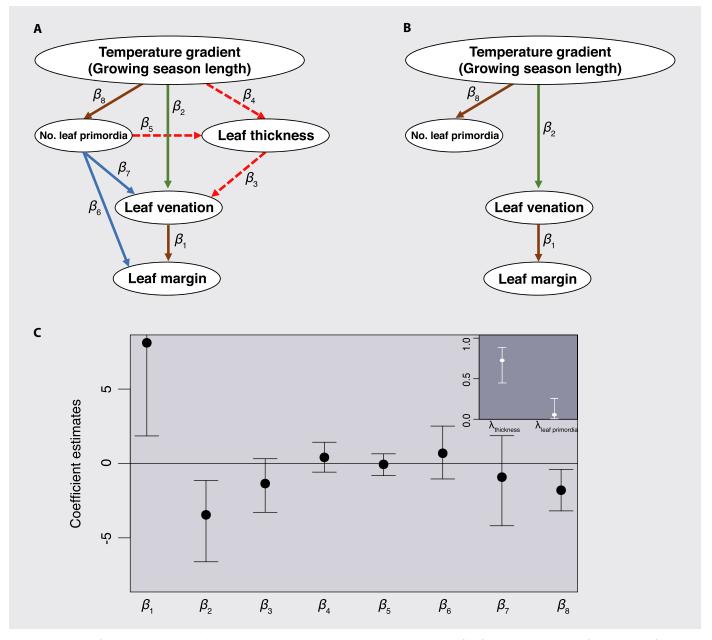
FIGURE 3. Testing for the hypothesized relationships between growing season length, number of leaf primordia in buds, leaf thickness, leaf venation, and leaf-margin type in deciduous woody species. (A) Conceptual models explaining the relationship between mean annual temperature and leaf margin types. Red arrows illustrate relationships expected from the support–supply model (Givnish and Kriebel, 2017); blue arrows illustrate relationships expected from the bud-packing model (Edwards et al., 2017); brown arrows illustrate relationships expected under both models; green arrows illustrate the alternative hypothesis that other factors explain the latitudinal gradient in leaf margin types. Beta coefficients (ß) refer to model parameters estimated in panel C. (B) Relationships that are supported by our Bayesian analysis of 144 deciduous woody species (panel C). (C) Estimated coefficient values for relationships in panel a, showing means and 95% credible intervals. Values reflect standardized data and can be interpreted as relative effect sizes. The inset shows fitted values of phylogenetic signal (Pagel's λ, mean and 95% credible intervals) for the dependent variables leaf thickness and number of leaf primordia in buds.

the trait behaving as expected under a Brownian motion model of evolution; values close to 0 indicate phylogenetic independence. Because λ estimation is not possible for binary variables, family-level random effects were included in the binary leaf venation and leaf teeth model equations to account for phylogenetic structure. The phylogeny came from Panchen et al. (2014), with missing species added manually by including them as polytomies based

on genus name. Branch lengths of the phylogeny were adjusted to match widely accepted family divergence times (Bell et al., 2010; Smith et al., 2010).

Model parameterization and prior choice in the HB model were analogous to Fridley and Craddock (2015) and Zohner et al. (2016), using the JAGS implementation (Plummer, 2003) of Markov chain Monte Carlo (MCMC) methods in the R package R2JAGS (Su and

Yajima, 2014). We ran three parallel MCMC chains for 20,000 iterations with a 5000-iteration burn-in and evaluated model convergence with the Gelman and Rubin (1992) statistic. Noninformative priors were specified for all parameter distributions, including normal priors for α and β coefficients (fixed effects; mean = 0; variance = 1000), and uniform priors between 0 and 100 for the variance of the genus and family random intercept effects, based on de Villemereuil and colleagues (2012). Model fit was assessed by comparing fitted versus observed values of dependent variables.

To support the results of our phylogenetic hierarchical hypothesis testing, we applied simple (generalized) linear models. A χ^2 test was performed to test for the effect of venation type on the probability of exhibiting leaf teeth. The effects of MAT, leaf thickness, and the number of leaves within winter buds on binary variables were tested by simple and mixed generalized linear models based on a quasi-binomial distribution. To estimate goodness of fit for the generalized linear models, we calculated a pseudo-R^2 according to Nagelkerke (1991) using the nagelkerkeR2 function of the fmsb R package.

All statistical analyses were conducted in R 3.4.3 (R Core team, 2018).

RESULTS

Overall, our Bayesian hierarchical model performed well in predicting species-specific leaf venation and leaf-margin types. Using a 50% probability threshold, a model that included MAT, primordium number, leaf thickness, family identity, and habit (Eq. 2) correctly distinguished between craspedodromous and noncraspedodromous venation in all but 12 species (92% accuracy; pseudo-R^2 = 0.74). A model that included leaf venation type, primordium number, family identity, and habit (Eq. 1) correctly predicted the presence/absence of leaf teeth in all but eight species (94% accuracy; pseudo-R^2 = 0.73).

The highest mean effect size was found for the correlation between venation type and the presence of leaf teeth, with craspedodromous leaf venation strongly increasing the probability of a species to exhibit leaf teeth (β_1). When modeling venation type, our Bayesian model revealed that the number of craspedodromous species decreases with increasing MAT (β_2). The mean effect size of MAT (β_2) was three to four times higher than that of leaf thickness (β_3) and the number of leaves preformed in winter buds (β_7). As such, in disagreement with both the support–supply and bud-packing hypotheses, neither leaf thickness nor the number of leaves preformed in winter buds influenced venation type and thus the presence of teeth (β_3 and β_7; Fig. 3C). Furthermore, inconsistent with the support–supply model, MAT had no effect on leaf thickness (see β_4 in Figs. 3C, 4C). MAT influenced the number of leaves preformed in winter buds (β_8), with the number of leaves preformed in winter buds decreasing, on average, by 0.3 leaves per each °C increase in MAT. The estimated λ values, a measure of phylogenetic signal, indicate that leaf thickness is conserved across the phylogeny, whereas bud-packing is phylogenetically more independent (inset Fig. 3C).

In a mixed generalized linear model, MAT, number of leaf primordia in buds, and leaf thickness together explained 27% of the total variation in leaf venation among species, with MAT and number of primordia, but not leaf thickness, having significant effects on leaf venation ($P < 0.001$, $P = 0.01$, and $P = 0.07$, respectively). Univariate analyses confirmed that MAT was the strongest predictor of leaf venation type. While MAT explained 18% of the between-species variation in leaf venation, number of primordia and leaf thickness only explained 8% and 5%, respectively (Fig. 4). Similarly, MAT explained 7% of the between-species variation in leaf-margin type, whereas number of primordia and leaf thickness explained <1% (Fig. 4G–I).

Our study of multiple herbarium specimens from each species' natural range confirmed that species with toothed leaf margins in the Munich Botanical Garden consistently produce such margins. Close-up images of each species' leaf margin in herbarium specimens are available at https://www.gfbio.org, accession 37fa1c25-5751-4894-941d-b87fc2435b9f (Materials and Methods).

DISCUSSION

The well-documented latitudinal increase in toothed leaf margins (Bailey and Sinnott, 1915, 1916; Wolfe, 1971, 1993; Peppe et al., 2011) has been difficult to explain because of the multiple correlations between leaf-margin type, venation, the deciduous habit, and regional climate. Using species range information and focusing on deciduous species (which by definition have winter buds), our phylogenetically informed models revealed three independent correlations, the strongest between venation and margin type (β_1 in Fig. 3C), another between growing season length (our proxy being MAT) and leaf venation (β_2 in Fig. 3C), and the third between growing season length and the number of leaves preformed in winter buds (β_8 in Fig. 3C). Mean annual temperature and growing season length are highly correlated with one another (Appendix S2), and a previous study showed that the correlation of leaf-margin type and growing season length was the same as that with MAT (Royer et al., 2005). A short growing season selects for preformation of leaves at the end of the previous growing season because species from cold regions (with short growing seasons) have a limited time for tissue formation, maturation, and photosynthesis and hence need to be photosynthetically active as soon as their leaves emerge. Neither leaf thickness nor the number of leaves preformed in winter buds had significant effects on leaf venation and leaf-margin type (Fig. 3), rejecting both the support–supply hypothesis and the bud-packing hypothesis, if the number of leaf primordia formed in buds (which is what we counted) correlates with packing density. Other factors, such as the relative sizes of leaves in the bud, phyllotaxis, and ptyxis, must also influence the density of packing in buds, but we know of no robust measure to assess all of these features within overwintering buds across a large sample of plant species (here 151).

Previous studies have analyzed the presence of leaf teeth in samples that included both evergreen and deciduous species and temperate and (sub)tropical species (e.g., Royer et al., 2012; Edwards et al., 2016, 2017; Givnish and Kriebel, 2017). This mixing introduces confounding effects (cf. Royer, 2012a, b), so that causation and correlation cannot be disentangled (introduction, Fig. 2). Evergreen trees and shrubs have longer mean leaf life spans, longer growing seasons, and thicker leaves than deciduous species. Any analysis of the effect of leaf thickness on leaf-margin type therefore needs to control for life span as we did in this study by excluding evergreens. Similarly, if the goal is to test for effects of the preformation of leaf primordia in buds on leaf-margin type, then deciduous and evergreen species need to be tested separately.
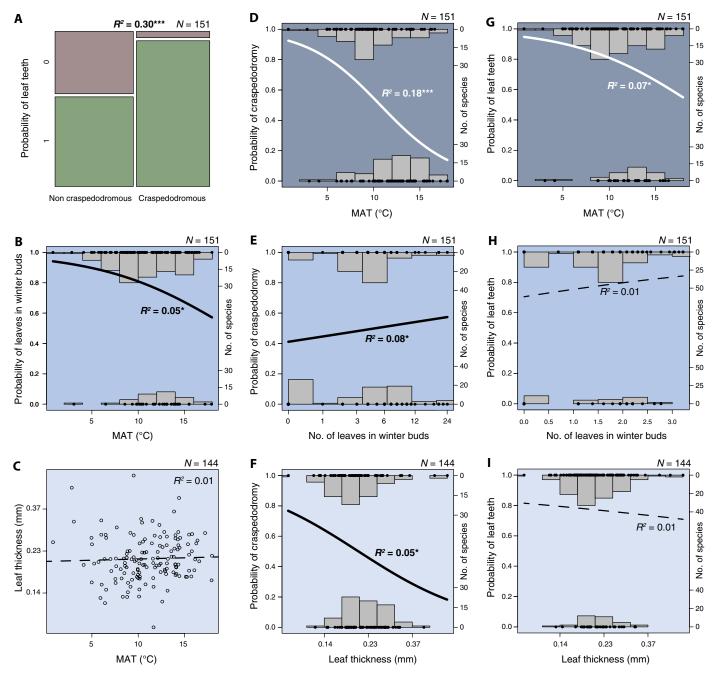
FIGURE 4. Univariate testing for the hypothesized relationships between mean annual temperature (MAT), number of leaf primordia in buds, leaf thickness, leaf venation, and leaf-margin type in deciduous woody species. (A) Percentage of species with leaf teeth in craspedodromous and non-craspedodromous species. (B, C) The effect of MAT in species' native ranges on number of primordia (B) and leaf thickness (C). Number of leaf primordia was included as a binary variable (leaves preformed in winter buds or not); leaf thickness was included as a continuous, log-transformed variable. (D–F) The univariate probability of craspedodromous venation in relation to MAT in species' native ranges (D), number of leaves preformed in winter buds (E), and leaf thickness (F). (G–I) The univariate probability of developing leaf teeth in relation to MAT in species' native ranges (G), number of leaf primordia (H), and leaf thickness (I). N above each plot gives the number of species included in the analysis.

Within deciduous species, there is substantial variation in leaf life span (e.g., Matsuki and Koike, 2006), but this variation is small relative to the life span differences between evergreen and deciduous leaves. Our results clearly show that, while a species' native climate (growing season length and MAT; Appendix S2) is linked to leaf venation and margin, its leaf thickness is not (Figs. 3 and 4). In other words, within temperate-zone deciduous species, thickness of leaves does not predict venation and teeth, contradicting the assumptions of the supply–support hypothesis (Givnish and Kriebel, 2017), and leaf primordium number in buds does not predict leaf-margin type (β_6; Fig. 3C), challenging the assumptions of the bud-packing hypothesis (Edwards et al., 2016, 2017). Instead, growing season length explains most of the variation in leaf venation and margin type among species. We know of no published evidence

that bud packing per se has any effect on leaf teeth. The study of Couturier et al. (2011), that is cited in support of such an effect by Edwards et al. (2017), used computer-aided "numerical folding" of leaves of *Acer pseudoplatanus*, *Tetrapanax papyriferum*, and a species of *Phoenix*, and concerned lobes, not teeth, as stated in the 2011 paper.

What factors might explain why short growing seasons favor leaf teeth in temperate zone woody plants? One possibility is a direct benefit of teeth, which enhance rates of carbon uptake at the beginning of the growing season when temperatures are limiting but moisture and nutrient availability are not (Royer and Wilf, 2006; Royer, 2012a). Evidence for such a direct benefit comes from the seasonal patterns of leaf-margin photosynthesis and transpiration in 60 woody species from Pennsylvania and North Carolina (Royer and Wilf, 2006). Physiological activity at leaf margins was greatest early in the growing season (first 30 days), with photosynthesis per unit area 3–5 times higher in toothed margins than in the rest of the leaf, and toothed margins were more active with respect to photosynthesis and transpiration than untoothed margins. Teeth also produce photosynthates while the leaves are still expanding and while shading is minimal (Baker-Brosh and Peet, 1997). Notably, Givnish and Kriebel (2017) estimated that for a few days in early spring, toothed leaf margins might increase 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. The cause may be the earlier maturation of the teeth (ends of secondary veins) and adjacent lamina than tissue in the core of the leaf or along untoothed leaf margins. A benefit of teeth via increased transpiration and photosynthate production early in the season implies that teeth should be most beneficial when growing seasons are short, matching the results of our Bayesian model that the number of craspedodromous (toothed) species increases with decreasing MAT. On the other hand, leaf teeth represent a strong water cost and only provide a benefit as long as water is not limiting (Royer and Wilf, 2006; Royer et al., 2009; Royer, 2012a). The prevalence of species with leaf teeth in any local flora therefore will depend on a combination of local season length, precipitation, and soil humidity (Wing et al., 2000).

A direct selective benefit of leaf teeth under short growing seasons (i.e., in cold climates) as proposed by Royer and Wilf (2006) would validate the use of leaf margins for paleotemperature estimations. Little et al. (2010) criticized using leaf physiognomy for inferring paleotemperatures because the approach disregards the phylogenetic composition of the particular flora that is being studied. In this study, we accounted for phylogeny and found that the relationship between growing season length (i.e., MAT, Appendix S2) and toothed leaf margins holds even when controlling for species' evolutionary history, supporting the universal applicability of the leaf teeth–MAT–growing-season-length relationship (as argued by Royer, 2012a, b).

A caveat of our analysis is that we quantified bud traits in deciduous species growing in a botanical garden (with added checking in herbarium sheets that represented the species' natural range), meaning that our data do not address within-species variation. This problem is compounded by climatic ranges for each species coming from GBIF distribution data, with the medians being used to represent each species. We therefore cannot address local adaptation. Nevertheless, craspedodromous vs. noncraspedodromous leaf venation (which most strongly determines leaf tooth presence; Hickey, 1979; Roth et al., 1995; our Fig. 3C) is a species-level trait that is readily scored, and we therefore suspect that our results will hold.

AUTHOR CONTRIBUTIONS

C.M.Z. designed the study and conducted the analyses; S.S.R. provided conceptual context on the three hypotheses; E.R. carried out measurements and reviewed the relevant literature; C.M.Z. and S.S.R. co-wrote the paper.

DATA AVAILABILITY

Data file 1 (Appendix S1) is available as online supporting material.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. The investigated species with their taxonomic family, number of leaf primordia in bud, bud type, teeth per cm, venation, thickness (mm), habit (t = tree, s = shrub), and coded mean annual temperature (MAT). See Materials and Methods for details on MAT assignments.

Photographed leaf margins in randomly selected specimens from the natural range of each species are accessible at https://www.gfbio.org, accession 37fa1c25-5751-4894-941d-b87fc2435b9f.

APPENDIX S2. Across the geographic position system (GPS) coordinates of the 151 species analyzed here (Materials and Methods), the relationship between mean annual temperature (MAT) and growing season length holds.

LITERATURE CITED

Bailey, I. W., and E. W. Sinnott. 1915. A botanical index of Cretaceous and Tertiary climates. *Science* 41: 831–834.

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.

Bell, C., D. E. Soltis, and P. S. Soltis. 2010. The age and diversification of the angiosperms re-revisited. *American Journal of Botany* 97: 1296–1303.

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.

De Villemereuil, P., J. A. Wells, R. D. Edwards, and S. P. Blomberg. 2012. Bayesian models for comparative analysis integrating phylogenetic uncertainty. *BMC Evolutionary Biology* 12: 102.

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.

Edwards, E. J., D. S. Chatelet, E. L. Spriggs, E. S. Johnson, C. Schlutius, and M. J. Donoghue. 2017. Correlation, causation, and the evolution of leaf teeth: a reply to Givnish and Kriebel. *American Journal of Botany* 104: 509–515.

Fick, S. E., and R. J. Hijmans. 2017. Worldclim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* 37: 4302–4315.

Fridley, J. D., and A. Craddock. 2015. Contrasting growth phenology of native and invasive forest shrubs mediated by genome size. *New Phytologist* 207: 659–668.

Gelman, A., and J. Hill. 2007. Data analysis using regression and multilevel/hierarchical models. Cambridge University Press, Cambridge, UK.

Gelman, A., and D. B. Rubin. 1992. Inference from iterative simulation using multiple sequences. *Statistical Science* 7: 457–472.

Givnish, T. J. 1978. Ecological aspects of plant morphology: leaf form in relation to environment. *Acta Biotheoretica* (supplement *Folia Biotheroretica* 7) 27: 83–142.

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

Givnish, T. J., and R. Kriebel. 2017. Causes of ecological gradients in leaf margin entirety: evaluating the roles of biomechanics, hydraulics, vein geometry, and bud packing. *American Journal of Botany* 104: 354–366.

Grafen, A. 1989. The phylogenetic regression. *Philosophical Transactions of the Royal Society, B, Biological Sciences* 326: 119–157.

Hickey, L. J. 1979. A revised classification of the architecture of dicotyledonous leaves. *In* C. R. Metcalfe and L. Chalk [eds.], the anatomy of the dicotyledons, 25–39. Oxford University Press, Oxford, UK.

Hijmans, R. J., S. Phillips, J. Leathwick, and J. Elith. 2011. Package 'dismo'. Website http://cran.r-project.org/web/packages/dismo/index.html [accessed 20 December 2018].

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.

Matsuki, S., and T. Koike. 2006. Comparison of leaf life span, photosynthesis and defensive traits across seven species of deciduous broad-leaf tree seedlings. *Annals of Botany* 97: 813–817.

Nagelkerke, N. J. D. 1991. A note on a general definition of the coefficient of determination. *Biometrika* 78: 691–692.

Pagel, M. 1999. Inferring the historical patterns of biological evolution. *Nature* 401: 877–884.

Panchen, Z. A., R. B. Primack, B. Nordt, E. R. Ellwood, A.-D. Stevens, S. S. Renner, C. G. Willis, et al. 2014. Leaf out times of temperate woody plants are related to phylogeny, deciduousness, growth habit and wood anatomy. *New Phytologist* 203: 1208–1219.

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

Plummer, M. 2003. JAGS: a program for analysis of Bayesian graphical models using Gibbs sampling. *In* K. Hornik, F. Leisch, and A. Zeileis [eds.], Proceedings 3rd International Workshop on Distributed Statistical Computing (DSC). Technische Universität Wien, Vienna, Austria.

R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.R-project.org/ [accessed 20 November 2018].

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

Royer, D. L. 2012a. Climate reconstruction from leaf size and shape: new developments and challenges. *Paleontological Society Papers* 18: 195–212.

Royer, D. L. 2012b. Leaf shape responds to temperature but not CO_2 in *Acer rubrum*. *PLoS ONE* 7: e49559.

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.

Royer, D. L., P. Wilf, D. A. Janesko, E. A. Kowalski, and D. L. Dilcher. 2005. Correlations of climate and plant ecology to leaf size and shape: potential proxies for the fossil record. *American Journal of Botany* 92: 1141–1151.

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.

Smith, S. A., J. M. Beaulieu, and M. J. Donoghue. 2010. An uncorrelated relaxed-clock analysis suggests an earlier origin for flowering plants. *Proceedings of the National Academy of Sciences, USA* 107: 5897–5902.

Spriggs, E. L., S. B. Schmerler, E. J. Edwards, and M. J. Donoghue. 2018. Leaf form evolution in *Viburnum* parallels variation within individual plants. *American Naturalist* 191: 235–249.

Su, Y.-S., and M. Yajima. 2014. R2jags v 0.04-03. Available at http://CRAN.R-project.org/package=R2jags [accessed 20 January 2019].

Wing, S. L., H. Bao, and P. L. Koch. 2000. An early Eocene cool period? Evidence for continental cooling during the warmest part of the Cenozoic. *In* B. T. Huber, K. G. MacLeod, and S. L. Wing [eds.], Warm climates in earth history, 197–237. Cambridge University Press, Cambridge, UK.

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

Wolfe, J. A. 1993. A method of obtaining climatic parameters from leaf assemblages. *U.S. Geological Survey Bulletin* 2040: 1–71.

Zohner, C. M., B. M. Benito, J.-C. Svenning, and S. S. Renner. 2016. Day length unlikely to constrain climate-driven shifts in leaf-out times of northern woody plants. *Nature Climate Change* 6: 1120–1123.

Zohner, C. M., B. M. Benito, J. D. Fridley, J.-C. Svenning, and S. S. Renner. 2017. Spring predictability explains different leaf-out strategies in the woody floras of North America, Europe and East Asia. *Ecology Letters* 20: 452–460.