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ROLES OF CLIMATE AND FUNCTIONAL TRAITS IN CONTROLLING TOOTHED VS. UNTOOTHED LEAF MARGINS[1]

DANA L. ROYER,[2,6] DANIEL J. PEPPE,[3] ELISABETH A. WHEELER,[4] AND ÜLO NIINEMETS[5]

[2]Department of Earth and Environmental Sciences, Wesleyan University, Middletown, Connecticut 06459 USA; [3]Department of Geology, Baylor University, Waco, Texas 76798 USA; [4]Department of Forest Biomaterials, North Carolina State University, Raleigh, North Carolina 27695 USA; and [5]Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu 51014 Estonia

- *Premise of the study:* Leaf-margin state (toothed vs. untoothed) forms the basis of several popular methods for reconstructing temperature. Some potential confounding factors have not been investigated with large data sets, limiting our understanding of the adaptive significance of leaf teeth and their reliability to reconstruct paleoclimate. Here we test the strength of correlations between leaf-margin state and deciduousness, leaf thickness, wood type (ring-porous vs. diffuse-porous), height within community, and several leaf economic variables.
- *Methods:* We assembled a trait database for 3549 species from six continents based on published and original data. The strength of associations between traits was quantified using correlational and principal axes approaches.
- *Key results:* Toothed species, independent of temperature, are more likely to be deciduous and to have thin leaves, a high leaf nitrogen concentration, a low leaf mass per area, and ring-porous wood. Canopy trees display the highest sensitivity between leaf-margin state and temperature; subcanopy plants, especially herbs, are less sensitive.
- *Conclusions:* Our data support hypotheses linking the adaptive significance of teeth to leaf thickness and deciduousness (in addition to temperature). Toothed species associate with the "fast-return" end of the leaf economic spectrum, providing another functional link to thin leaves and the deciduous habit. Accounting for these confounding factors should improve climate estimates from tooth-based methods.

Key words: climate; deciduous; evergreen; leaf mass per area; leaf teeth; leaf thickness; temperature; wood anatomy.

Leaf teeth are vascularized projections along the leaf margin (Ellis et al., 2009). In most regions of the world, the proportion of toothed woody angiosperm species (exclusive of monocots) scales inversely with mean annual temperature (MAT) (e.g., Bailey and Sinnott, 1916; Wolfe, 1979, 1993; Greenwood, 2005a; Peppe et al., 2011). This relationship forms the basis of a popular technique for reconstructing MAT from fossil plants called leaf-margin analysis (e.g., Wolfe and Hopkins, 1967; Wilf, 1997). Leaf-margin state (toothed vs. untoothed) is also the variable with the most explanatory power in multivariate methods for reconstructing MAT from leaf morphology (Wolfe, 1993; Peppe et al., 2011).

Although tooth-based paleoclimate methods are widely applied (see literature compilation in Little et al., 2010), other environmental and functional traits affect tooth–MAT relationships. Perhaps the best-documented confounding factor is local water availability (Brenner, 1902; Bailey and Sinnott, 1915, 1916; Wolfe, 1971, 1977, 1993; Burnham et al., 2001; Kowalski and Dilcher, 2003; Greenwood, 2005b; Xu et al., 2008; Royer

et al., 2009; Steart et al., 2010; Peppe et al., 2011). At a given MAT, sites with higher water availability (e.g., shallow water table, low evaporative demand) often have proportionately more toothed species; in contrast, vegetation in physiologically dry sites (e.g., saline, arctic, and alpine environments) is typically characterized by a larger fraction of untoothed species. In most settings, this water availability factor introduces an error in MAT estimation of up to 4°C (Peppe et al., 2011). Phylogenetic history can also confound leaf–climate relationships (Jordan, 1997, 2011; Little et al., 2010; Burnham and Tonkovich, 2011; Hinojosa et al., 2011). Little et al. (2010) found that both MAT and phylogenetic history significantly impact tooth-related variables, implying that a quantitative accounting of evolutionary factors should improve climate estimations from tooth-based methods.

The potential influences of other factors, including leaf thickness and deciduousness, are less well known. The primary motivation of the current study is to investigate some of these lesser-known factors to better understand the adaptive significance of leaf teeth and to improve tooth-based paleoclimate methods.

Adaptive significance of leaf teeth and selection of traits—The covariation between leaf-margin state and MAT may reflect a tradeoff between carbon uptake and water loss. Teeth commonly are sites of vigorous photosynthesis and transpiration in young leaves early in the growing season, accelerating growth relative to an equivalent untoothed leaf (Baker-Brosh and Peet, 1997; Royer and Wilf, 2006). This pulse in gas-exchange activity may be increasingly adaptive in progressively colder climates because it may extend the season of potential growth; in contrast, in progressively warmer climates

the water cost of teeth may outweigh the diminishing benefit of extending the growing season (Wing et al., 2000; Royer and Wilf, 2006). This early-season gas-exchange function of leaf teeth can also explain the covariation with local water availability: leaf teeth require abundant water to maximize their carbon benefit to the plant, and thus are selected against in locally drier environments, even if it is cold (Bailey and Sinnott, 1916; Wolfe, 1993).

The gas-exchange patterns of leaf teeth may help underpin the functional dependency between teeth and climate in some plants, but other hypotheses exist for why plants have teeth that do not invoke climate directly. Givnish (1978, 1979) proposed that thin leaves are more likely to be toothed because, for reasons of support, they are more likely to have strong secondary veins, often extending to the leaf margin. As a result, regions along the margin between major veins in thin leaves may lack sufficient structural support, causing a wavy or serrated (toothed) margin to form. Givnish (1978) provided some measurements of leaf thickness to support the hypothesis, but follow-up studies are lacking.

Leaf teeth also may be linked functionally to deciduousness. Baker-Brosh and Peet (1997) hypothesized that any tooth-driven pulse in early-season photosynthesis is maximized in an open canopy with abundant irradiance. Relative to an evergreen canopy, a deciduous canopy is more open early in the growing season. Similarly, Peppe et al. (2011) proposed that the early-season photosynthetic pulse is maximized in a deciduous canopy because it flushes synchronously. These hypotheses dovetail well with the early-season gas-exchange hypothesis, together predicting a maximal carbon benefit in cool, deciduous forests.

Alternatively, the link between leaf teeth and deciduousness may be underpinned by the leaf economics spectrum (Royer et al., 2005). The leaf economics spectrum is composed of strongly interrelated traits (Wright et al., 2004). A species with a fast photosynthetic rate will likely have a fast respiration rate and high leaf nitrogen and phosphorus concentration per mass to support its vigorous photosynthetic activity. Such a "fast-return" specialist will probably have short-lived leaves because the return on investment comes quickly, and have a low leaf mass per area because short-lived leaves do not require expensive, durable construction. These traits form a one-dimensional spectrum that describes how fast (or slow) a plant is processing its nutrient resources (Wright et al., 2004). If teeth boost carbon uptake early in the growing season, then it is possible that toothed species are more likely to be on the "fast-return" end of the leaf economics spectrum, which includes a short leaf lifespan (e.g., deciduous habit). There is limited support for this association: relationships between tooth-related variables and both leaf mass per area and leaf nitrogen concentration at 14 eastern North American sites are strong when expressed as site means but considerably weaker at the species level (Royer et al., 2005). Royer et al. (2009) directly assessed growth rate for several hundred Australian rainforest species and found no strong relationship with leaf-margin state. A larger survey of species is clearly needed to evaluate the hypothesis.

A growing number of studies based on qualitative (Bailey and Sinnott, 1916; Givnish, 1979) and limited quantitative analyses (Wolfe, 1971, 1993; Jordan, 1997) document the propensity for untoothed species to be evergreen and toothed species deciduous. Recently, Peppe et al. (2011) reported that the site-mean percentages of untoothed species at 29 sites from Asia and North and Central America are higher for evergreens than for deciduous species. Although these data are encouraging,

a larger species-based analysis is needed to adequately assess the proposed link.

The traits discussed—MAT, leaf thickness, and deciduousness—are important for understanding tooth ecology because they have been invoked to explain the adaptive significance of leaf teeth. Additional plant traits may also relate to leaf-margin state if they aid in the adaptive frameworks just discussed. For example, ring-porous wood contains vessels that are distinctly larger in the earlywood; in contrast, vessel diameters in diffuse-porous wood do not vary much within an annual cycle (IAWA Committee, 1989). Because conductivity scales with the fourth power of diameter, large-diameter earlywood vessels allow very high sap flow rates. These wide vessels typically embolize and generally cease to function within a few weeks; their function is therefore generally restricted to the early part of the growing season (Zimmermann and Brown, 1971; Hacke and Sperry, 2001). Ring-porous wood correlates strongly with the deciduous habit (Chowdhury, 1964; Boura and De Franceschi, 2007; Wheeler et al., 2007). If the early-season gas-exchange hypothesis is correct, ring-porous wood should also associate with toothed leaves because the increased sap flow rate demanded by toothed leaves can be more readily supplied by large-diameter earlywood vessels.

Nearly all leaf-climate work concentrates on woody dicots. This stems largely from the legacy of Bailey and Sinnott (1916), who on the basis of qualitative analyses concluded that the relationship between leaf-margin type and MAT is weaker for herbs than for woody taxa. Peppe et al. (2011) found that the percentages of untoothed woody and herbaceous species were similar in central Connecticut, opening the possibility for a similar sensitivity to MAT. This has direct relevance for paleoclimate reconstruction because in the fossil record herbs, although rare, can be difficult to distinguish from woody taxa. Clearly, a more robust database is needed to investigate the issue.

Goals of study—Here we construct a trait array for 3549 species to quantify the impact on leaf-margin state (toothed vs. untoothed) by climate, deciduous vs. evergreen habit, leaf thickness, leaf mass per area, wood type (ring-porous vs. diffuse-porous), leaf nitrogen concentration, and growth habit (herbaceous, shrub, liana, tree). We pay particular attention to the three traits that have been invoked to explain the adaptive significance of teeth—MAT, deciduousness, and leaf thickness—to evaluate the relative viabilities of the adaptive hypotheses. Finally, we investigate how our results may be used to improve leaf-climate methods.

MATERIALS AND METHODS

We assembled a trait database from new and published sources (for raw data, see Appendix S1 with the online version of this article; Table 1 summarizes the data sources). All species in the study are nonmonocotyledonous angiosperms. Unknown species, even if known at the genus level, were excluded. Species means form the basis of all analyses ($N = 3549$ species). Figure 1 shows the geographic distribution of the 398 sites included in the analysis.

Newly compiled data for leaf-margin state come from species descriptions and freely available images (e.g., online herbaria). Toothed species were given a score of 0, untoothed species 1, and species with both margin states 0.5. Both margin states can co-occur in a single species either within individuals, for example due to ontogeny, or across individuals. In our scoring system, teeth need to be vascularized (e.g., thorns and spines are not teeth), and lobes are not considered teeth. Royer et al. (2005) described the criteria for differentiating lobes from teeth. In most cases, if the feature in question extends beyond one-quarter of the distance between the tip and midvein, it is a lobe, not a tooth.

TABLE 1. Sources for trait data.

Species list	N	Margin state	Climate	Ever.	Thick.	LMA	N	Wood	Growth
Peppe et al., 2011	2123	✓	✓	✓$_p$,+$_p$	+$_p$	+$_p$	✓$_p$		
Wright et al., 2004	1783	+	✓	✓		✓	✓$_p$		✓
InsideWood	1098	+		+$_p$				✓	
Niinemets	999	+	✓	✓$_p$,+$_p$	✓	✓$_p$			✓
Royer et al., 2009	702	✓	✓	✓					✓

Notes: See Appendix S1 for raw data. A check (✓) indicates that data come from source in first column. A plus sign (+) indicates that data were compiled for this study. The subscript "p" denotes that the sampling is partial. Sample size (*N*) is the number of species-site combinations. Climate = mean annual temperature and mean annual precipitation; Ever. = deciduous vs. evergreen; Thick. = leaf thickness; LMA = leaf mass per area; N = leaf nitrogen concentration by mass; Wood = ring porous vs. diffuse porous; Growth = herbaceous, shrub, liana (woody vine), tree. All species are scored for herbaceous vs. woody. InsideWood database (Wheeler, 2011) is stored at website http://insidewood.lib.ncsu.edu/. The Niinemets source contains data from Niinemets et al. (1999, 2003, 2007) and Niinemets (1999, 2001).

New data for deciduousness come from species descriptions (0 = deciduous; 1 = evergreen; 0.5 = species with both leaf habits). We consider species that lose all leaves for some part of the year as deciduous and species that persistently retain at least part of their canopy as evergreen; we scored semideciduous species as 0.5. New data on leaf thickness and leaf mass per area come from the same dried leaf vouchers used to score other traits in the Peppe et al. (2011) database. Leaf thickness was measured with digital calipers in two spots near the leaf base, avoiding major veins; leaf mass was determined with a digital balance and leaf area from digital images and ImageJ software (http://rsbweb.nih.gov/ij/) (see Peppe et al., 2011 for methodological details). For both thickness and leaf mass per area, typically three leaves per species-site pair were measured and averaged. For wood type, species were given a score of 0 for ring-porous wood, 1 for diffuse-porous wood, and 0.5 for semi-ring-porous wood or for species with multiple wood types.

To test the strength of association between two variables in cases where at least one is categorical, we computed Kendall's τ_b rank coefficient, a nonparametric correlation test that accounts for ties; in cases where both variables are quantitative, we calculated Pearson's correlation coefficient. We computed Pearson's partial correlation coefficient to quantify the importance of confounding factors on bivariate correlations in cases where one or both variables are quantitative; in cases where both variables are categorical, we calculated a logistic regression, excluding intermediate categorical scores of the dependent variable (i.e., scores of 0.5). We also used logistic regression to test differences in the regression slopes of relationships where margin state is the dependent variable.

We used multiple linear regression to test the potential of improving leaf-climate models via the incorporation of traits that our study identifies as functionally linked to leaf-margin state. A multiple linear regression approach, with climate as the dependent variable, has been used in previous studies (Wing and Greenwood, 1993; Peppe et al., 2011). We consider these analyses exploratory; they are not prescriptive and are meant only to identify a possible future direction. Importantly, most tooth-based paleoclimate methods are site based, whereas our preliminary model development is species-based. A species-based approach was necessary in our case because traits from all appropriate species at all sites were not collected. Any rigorous method development will need to account for this effect, as well as other factors such as phylogenetic history (Little et al., 2010).

RESULTS

Leaf-margin state among woody species correlates significantly with all traits (first data column in Table 2), with toothed species having significantly different mean trait values compared with untoothed species (Fig. 2; *P* < 0.001 for all comparisons). As expected, untoothed species are more common in warm climates (Fig. 2A). Untoothed species are also 57% thicker on average and 59% more likely to be evergreen than deciduous (Fig. 2B, C); 57% of all deciduous species are toothed
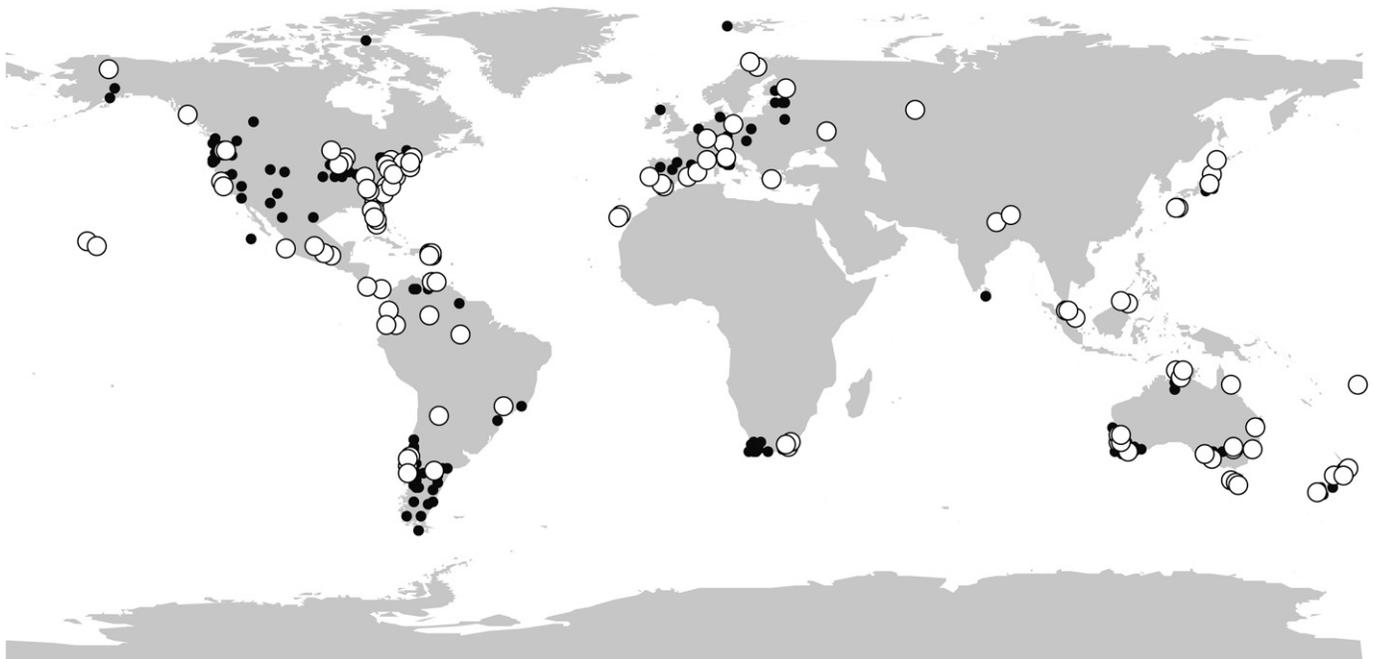

Fig. 1. Geography of sites. Sites represented by 10 or more species are denoted by open symbols (*N* = 153 sites; 4597 species-site pairs) and <10 species by filled symbols (*N* = 245 sites; 1010 species–site pairs). Many site symbols overlap because of the global-scale of the map. Species from the Inside-Wood database (Table 1) lack site information and are not included.

TABLE 2.   Correlation coefficients (r) and sample sizes (number of species) for pairwise trait comparisons.

Trait	Untoothed	Evergreen	Thickness	LMA	Nitrogen	D-P wood	MAT	MAP
Untoothed	3263	3006	667	1893	1083	1098	2916	2916
Evergreen	0.31**	3006	655	1815	1081	988	2758	2758
Thickness	0.29**	0.48**	667	474	340	239	667	667
LMA	0.20**	0.30**	0.84**	1893	1083	576	1893	1893
Nitrogen	−0.19**	−0.34**	−0.40**	−0.55**	1083	364	1083	1083
D-P wood	0.30**	0.42**	0.13*	0.08*	−0.16**	1098	768	768
MAT	0.27**	0.21**	0.15**	−0.05*	−0.05	0.35**	2916	2916
MAP	0.08**	0.21**	−0.16**	−0.31**	−0.003	0.28**	0.35**	2916

Notes: Correlation coefficients appear in lower left and sample size in upper right. We use Kendall's τ_b rank correlation coefficient for comparisons involving categorical variables (D-P wood, Untoothed, Evergreen) and Pearson's correlation coefficient for all other correlations. Herbaceous species are excluded. Categorical variables scored as Untoothed: 1 = yes, 0.5 = sometimes, 0 = no, toothed leaf margin; Evergreen: 1 = yes, 0.5 = sometimes, 0 = no, deciduous; D-P wood (diffuse-porous wood): 1 = yes, 0.5 = sometimes or semi-ring-porous, 0 = no, ring-porous wood. Other definitions: Thickness = leaf thickness; LMA = leaf mass per area; Nitrogen = leaf nitrogen concentration by mass; MAT = mean annual temperature; MAP = mean annual precipitation. * $P < 0.05$; ** $P < 0.01$

vs. 22% for evergreen species. Because many of the traits are intercorrelated (Table 2; e.g., thick leaves are more likely to be evergreen), the strength of any pairwise correlation may be partly driven by covariation with other traits. If the subset of data scored for all four traits is analyzed using logistic regression ($N = 600$ species), with margin state as the dependent variable,
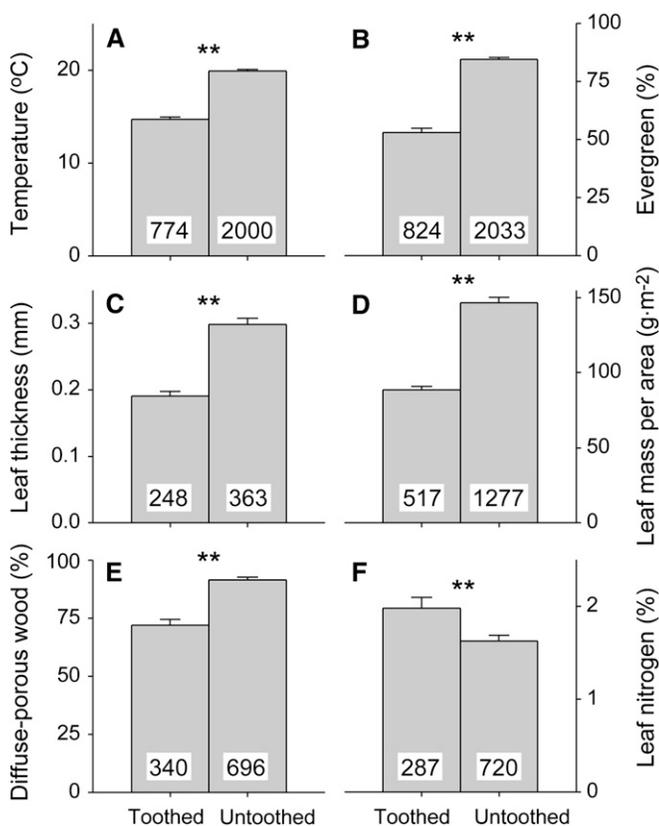

Fig. 2.   Differences between toothed and untoothed species in mean annual temperature and functional traits. In each panel, mean values of a variable for both leaf-margin types are plotted on the y-axis. Number of species used and standard errors of the mean are shown. All pairwise differences are significant at the α = 0.001 level (**) (z-test for proportional comparisons and t test for all other comparisons). Herbaceous species and species with a leaf-margin score of 0.5 are excluded.

the contributions of MAT, thickness, and deciduousness are all significant ($P < 0.002$ for each trait).

The data set for leaf thickness is smaller than for most other traits (Table 2) and so may not be capturing the full range of trait coordination. Leaf mass per area, defined as density × thickness, correlates strongly with thickness (Fig. 3; see also Niinemets, 1999) and can be used as a thickness proxy. In our database, the mean leaf mass per area of untoothed species is 66% higher than that in toothed species (Fig. 2D). Untoothed species are also more likely to have a lower leaf nitrogen concentration and be diffuse-porous (Fig. 2E, F); 69% of all ring-porous species are toothed vs. 26% for diffuse-porous species. Untoothed species are more common at high MAP, but this correlation, although statistically significant, is considerably weaker than the other tooth-related correlations (Table 2). Indeed, after controlling for the influence of MAT, the correlation between leaf-margin state and mean annual precipitation is not significant ($r = −0.03$; $P = 0.08$).

Although leaf-margin state correlates significantly with MAT in woody species (Figs. 2A, 4A), no significant relationship is seen in herbaceous species (Fig. 4B; $N = 235$ species; $P = 0.40$). If the woody taxa are further divided according to growth form as shrubs, lianas (woody vines), and trees, a clear pattern emerges whereby the strongest tooth–MAT relationship (i.e., steepest regression slope) occurs among trees, followed by lianas, shrubs, and finally herbs (Table 3).

DISCUSSION

Assessing the adaptive significance of leaf teeth—Mean annual temperature, leaf thickness, and deciduousness have each been proposed to control leaf-margin state (see introduction). Our data support all three adaptive hypotheses: MAT, leaf thickness, and deciduousness all independently affect leaf-margin state (Table 2).

Leaf thickness and deciduousness tend to correlate only weakly with MAT. For example, both traits are aligned with the leaf economics spectrum (via leaf mass per area and leaf lifespan), which in turn is only moderately impacted by climate (Wright et al., 2004, 2005). In our data set, deciduousness correlates significantly with MAT after controlling for the covariation with leaf-margin state ($r = 0.22$; $P < 0.001$), but leaf thickness does not ($r = 0.04$; $P = 0.29$). In general, this means that thickness and deciduousness can explain some of the residual
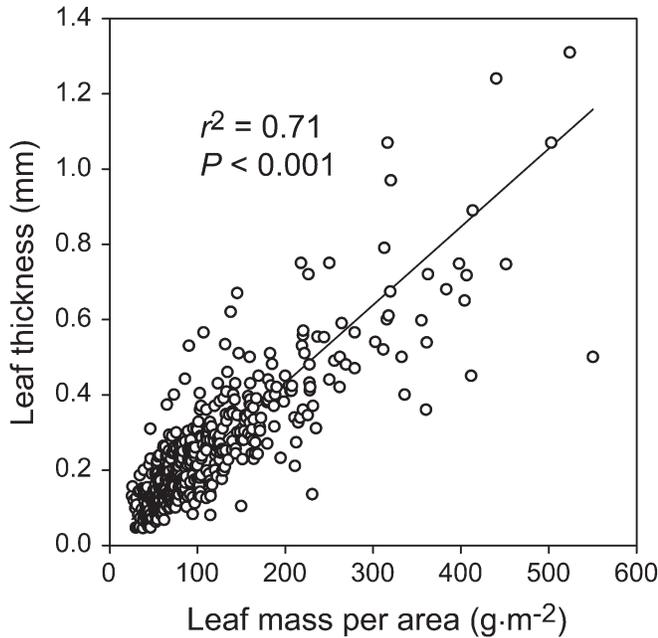
Fig. 3. Relationship between leaf mass per area and leaf thickness. Standardized major axis regression is shown ($r^2 = 0.71$; $P < 0.001$). Herbaceous species are excluded.

information in data sets used to calibrate leaf-margin analysis, but very little of the overall slope in the associated regressions. Australia provides a good case study. Vegetation along the east coast is nearly all evergreen, and the corresponding site-mean proportions of untoothed species is offset to higher values relative to most of the rest of the world (Greenwood et al., 2004). This offset in the *y*-intercept can be partly explained by the dominance of evergreen taxa (see also Peppe et al., 2011). However, the overall slope in the percentage untoothed–MAT regression broadly matches what is observed elsewhere in the world (Greenwood et al., 2004). Because the vegetation is nearly all evergreen, deciduousness cannot explain the slope.

The leaf-margin state in Australian vegetation probably reflects deciduousness and MAT, as well as other factors such as phylogenetic history, local water availability, and soil nutrient status (see also Greenwood et al., 2004; Jordan, 2011).

The association between untoothed species and ring-diffuse wood (Table 2, Fig. 2E) is consistent with the early-season gas-exchange hypothesis: the wide vessels in the earlywood of ring-porous species can better supply the sap needed to boost transpiration rates often observed in toothed species at the beginning of the growing season. This previously undocumented association provides a functional link between leaf and wood traits. Ring-porous species also correlate with the deciduous habit (Table 2; Boura and De Franceschi, 2007), but even if habit is accounted for, the relationship with leaf-margin state remains significant ($P < 0.001$, based on logistic regression).

Both synchronous leaf flushing and the leaf economics spectrum have been proposed to underpin the functional relationship between leaf-margin state and deciduousness (see the introduction). Our data can evaluate whether leaf-margin state is linked to the leaf economic spectrum because we measured two core economic traits (leaf nitrogen and leaf mass per area) as well as two closely aligned traits (leaf thickness and deciduous vs. evergreen habit). We find that toothed species have thinner leaves, a lower leaf mass per area, a higher nitrogen concentration, and are less likely to be evergreen than untoothed species (Table 2, Fig. 2). These associations are concordant with toothed species falling on the "fast-return" end of the leaf economics spectrum and untoothed species on the "slow-return" end. Importantly, this not only functionally links leaf-margin state to deciduousness, but also to leaf thickness because both thickness and deciduousness are aligned with the spectrum. Ring-porous wood correlates with both toothed species (Table 2, Fig. 2E) and the deciduous habit (Table 2; Boura and De Franceschi, 2007), implying another functional link between leaf-margin state and deciduousness that is underpinned by rapid growth.

The coordination among traits can be visualized in a principal axes ordination (Fig. 5). Here, untoothed species tend to associate with traits linked to the "slow-return" end, especially low leaf nitrogen concentration and evergreen habit. This provides further support that leaf-margin state is functionally linked to
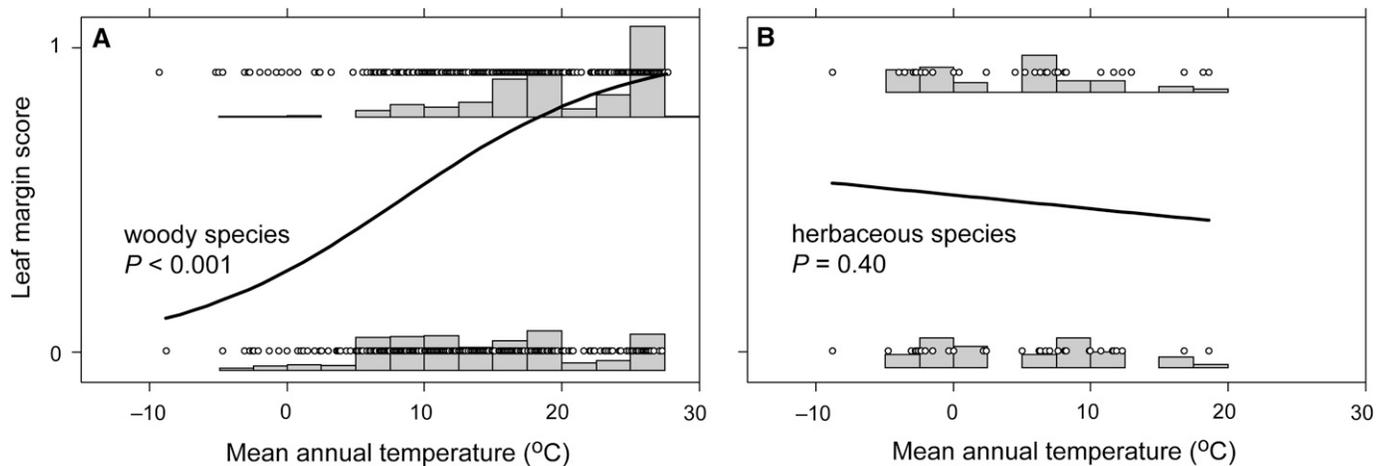
Fig. 4. Comparison of the relationships between leaf-margin state and mean annual temperature between woody and herbaceous species. A logistic regression for (A) woody species ($P < 0.001$) is much stronger than for (B) herbaceous species ($P = 0.40$), and the regression slope is much steeper (0.122 vs. −0.018; 95% confidence intervals do not overlap). Because the individual data points (open symbols) often overlap, normalized histograms are shown to help illuminate patterns. Leaf-margin score: 0 = toothed species; 1 = untoothed species; species with a score of 0.5 are excluded from the analysis.

TABLE 3. Comparison across growth forms of the relationship between leaf-margin state and mean annual temperature.

Statistic	Tree	Liana	Shrub	Herb
N	878	85	582	235
Slope	0.22	0.15	0.058	−0.018
P	<0.001	0.02	<0.001	0.40

Notes: Analysis is based on logistic regression, where logit(leaf-margin proportion) = slope × (mean annual temperature) + constant. Species with multiple growth forms are excluded, as are species with the intermediate category for the dependent variable leaf-margin state (0.5). Herbaceous species are plotted in Fig. 4B and all woody species in Fig. 4A.

the leaf economics spectrum, including both deciduous vs. evergreen habit and leaf thickness. We have focused here primarily on how two traits related to the spectrum (deciduousness and leaf thickness) affect tooth-MAT relationships, but other elements of the spectrum may be important too (e.g., Greenwood et al., 2004; Steart et al., 2010; Jordan, 2011).

A strong coordination between leaf-margin state and the leaf economics spectrum is not present across all species (e.g., Royer et al., 2009) and alternative functional explanations are important too. The first principal axis in Fig. 5 only explains 45.2% of all trait variation or 59.7% if restricted to leaf-margin state and the traits most closely aligned with the economics spectrum (nitrogen, leaf mass per area, leaf thickness, deciduousness). In either case, this leaves considerable unexplained variance in the ordination. By comparison, the first principal axis in the leaf economic data set of Wright et al. (2004) explains 74.4% of trait variation.

Herbaceous vs. woody vegetation—Leaf-margin state in herbaceous species is insensitive to MAT (Fig. 4B), supporting
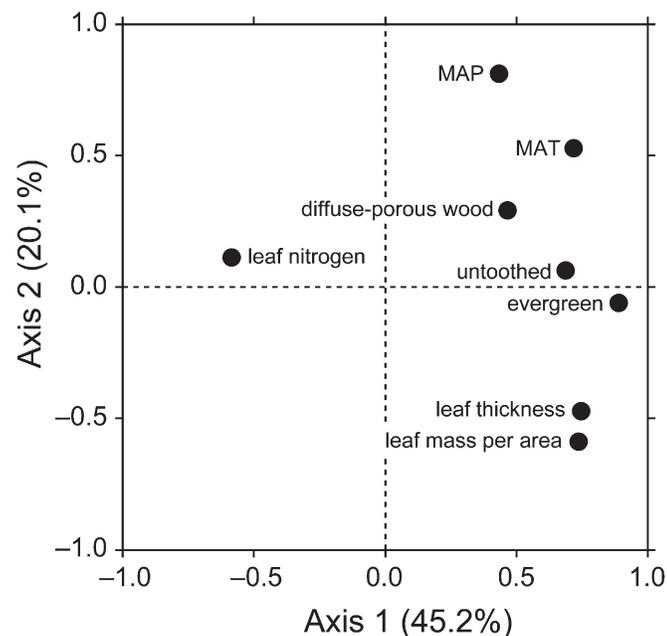

Fig. 5. Principal component analysis for measured traits. Axes 1 and 2 explain 45.2% and 20.1% of the trait variation, respectively. Herbaceous species are excluded. Untoothed species are most closely associated with low leaf nitrogen and the evergreen leaf habit.

the observations of Bailey and Sinnott (1916). In hindsight, the similarity in percentage untoothed between herbaceous and woody taxa in central Connecticut (Peppe et al., 2011) is fully expected, but not because leaf-margin state in herbs and woody taxa are equally sensitive to MAT: solving the two regression equations in Fig. 4 simultaneously yields a MAT of 7.8°C, which is close to the MAT at the site (10.2°C).

Bailey and Sinnott (1916) speculate that the lack of sensitivity to regional climate in herbs is because the subcanopy microclimate is more divorced from regional climate than is the canopy microenvironment, especially in the tropics. As a result, subcanopy microclimate varies less across latitudes than does canopy microclimate. Alternatively, Feild et al. (2005) observed that leaf teeth in the herb *Chloranthus japonicus* release excess positive root pressure, which prevents the flooding of leaf intercellular spaces. Low-statured plants should be more sensitive to root-pressure damage, providing another explanation for why herbaceous leaf-margin state does not vary with MAT. Our data do not allow us to distinguish between these hypotheses, but we nonetheless observe a strong height gradient: not only are herbs less sensitive than woody species, but shrubs are less sensitive than trees (Table 3). Clearly, relative height within the whole community is an important covariate in the relationship between leaf-margin state and MAT.

Implications for paleoclimate reconstruction—We identified three factors that impact leaf-margin state independently from MAT: leaf thickness, deciduousness, and relative height within the community. Ideally, these should be accounted for in tooth-based paleoclimate reconstructions. Herbs do not pose a large problem because their fossilization potential is low (Spicer, 1981), but distinguishing lianas, shrubs, and trees from fossil leaves can be difficult. Extant calibration data for tooth-based paleoclimate methods typically include lianas, shrubs, and trees, meaning that part of the noise in these calibrations is likely due to variation in the shrub–liana–tree ratio across sites. As a result, the error introduced by growth form in leaf-climate models is already partly accounted for, but it probably cannot be reduced.

The inclusion of leaf thickness and deciduousness into leaf-climate models improves their predictive power. For the subset of species that include thickness and deciduousness information (N = 655), the r^2 for the relationship between leaf-margin state and MAT is 0.14. Including leaf-margin state and both thickness and deciduousness in a multiple linear regression improves the r^2 to 0.26, and the standard error of the regression for estimating MAT drops from 5.7°C to 5.3°C. These coefficients are considerably weaker than most calibrations for leaf-margin analysis (e.g., 0.63°C and 4.1°C for the 535 site compilation of Peppe et al., 2011) because our analysis is species-based while leaf-margin analysis is site-based. As a result, our data set provides only a sense of the relative improvements that could be expected. To assess the multiple linear regression model of Peppe et al. (2011), we first restricted our data to species with values for thickness, deciduousness, and the variables used in their model (leaf-margin state, feret diameter ratio, and number of teeth / internal perimeter) (N = 134). Similar to leaf-margin analysis, inclusion of thickness and deciduousness improved the r^2 from 0.26 to 0.35, and the standard error dropped from 4.2°C to 4.0°C. As with leaf-margin analysis, the digital leaf physiognomy approach of Peppe et al. (2011) was optimized for sites, not for individual species, and so the relative changes presented here are more important than the absolute values.

Unfortunately, leaf thickness and deciduousness cannot be reconstructed reliably from fossils. However, a proxy for leaf mass per area from petiole dimensions is available ([petiole width]^2/[leaf area], or W_p^2/A) (Royer et al., 2007). Leaf mass per area is related not only to leaf thickness (Fig. 3) but also to deciduousness via the leaf economics spectrum. When our data was limited to species with W_p^2/A information ($N = 1424$), incorporation of this proxy for leaf mass per area into a leaf-margin analysis framework improves the r^2 from 0.18 to 0.26, and the standard error of MAT estimation drops from 6.5°C to 6.2°C. Similarly, including W_p^2/A as an additional variable in the digital leaf physiognomy model of Peppe et al. (2011) ($N = 1424$ species) raises the r^2 from 0.22 to 0.30, and standard error drops from 6.3°C to 6.0°C. Thus, our preliminary calculations suggest that petiole width information may improve leaf-climate methods (see also Jordan, 2011). However, an expanded analysis is needed before any confident conclusions can be drawn because our analyses here are species-based, not site-based, and we did not quantitatively account for phylogenetic history (Little et al., 2010).

Summary—The correlation between leaf-margin state and MAT is a strong ecological signal observed in most vegetation types worldwide. This functional relationship has been and will continue to be useful for paleoclimate reconstruction. Both leaf thickness and deciduousness have been previously identified to explain the adaptive significance of leaf teeth, but these hypotheses have been largely lacking empirical support. Using a large, worldwide trait database, we have confirmed that leaf-margin state is strongly linked to leaf thickness and deciduousness. Our data also support the hypothesis that leaf-margin state is aligned with the leaf economics spectrum, with toothed species typically having a low leaf mass per area and high nitrogen concentration. This in turn provides one functional explanation for why leaf-margin state is linked to leaf thickness and deciduousness because these two traits also align with the spectrum.

We observed that the sensitivity of leaf-margin state to MAT is affected by canopy position of a given plant growth form. Trees are the most sensitive, followed by lianas, shrubs, and herbs, the latter of which show no sensitivity.

When applying tooth-based climate methods to fossils, it is important to consider the potential influence deciduousness, leaf thickness, and canopy position. Preliminary evidence suggests that the incorporation of a proxy for leaf mass per area (Royer et al., 2007), which is related to both thickness and deciduousness, may improve leaf-climate models.

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