



Sensitivity of leaf size and shape to climate within *Acer rubrum* and *Quercus kelloggii*

Dana L. Royer¹, Jennifer C. McElwain², Jonathan M. Adams³ and Peter Wilf⁴

¹Department of Earth and Environmental Sciences, Wesleyan University, Middletown, CT 06459, USA; ²UCD School of Biology and Environmental Science, University College Dublin, Belfield Dublin 4, Ireland; ³Department of Biological Sciences, Rutgers University, Newark, NJ 07102, USA; ⁴Department of Geosciences, Pennsylvania State University, University Park, PA 16802, USA

Summary

Author for correspondence:

Dana L. Royer

Tel: +860 685 2836

Fax: +860 685 3651

Email: droyer@wesleyan.edu

Received: 24 March 2008

Accepted: 2 April 2008

• Variation in the size and shape (physiognomy) of leaves has long been correlated to climate, and paleobotanists have used these correlations to reconstruct paleoclimate. Most studies focus on site-level means of largely nonoverlapping species sets. The sensitivity of leaf shape to climate within species is poorly known, which limits our general understanding of leaf–climate relationships and the value of intraspecific patterns for paleoclimate reconstructions.

• The leaf physiognomy of two species whose native North American ranges span large climatic gradients (*Acer rubrum* and *Quercus kelloggii*) was quantified and correlated to mean annual temperature (MAT). *Quercus kelloggii* was sampled across a wide elevation range, but *A. rubrum* was sampled in strictly lowland areas.

• Within *A. rubrum*, leaf shape correlates with MAT in a manner that is largely consistent with previous site-level studies; leaves from cold climates are toothier and more highly dissected. By contrast, *Q. kelloggii* is largely insensitive to MAT; instead, windy conditions with ample plant-available water may explain the preponderance of small teeth at high elevation sites, independent of MAT.

• This study highlights the strong correspondence between leaf form and climate within some species, and demonstrates that intraspecific patterns may contribute useful information towards reconstructing paleoclimate.

Key words: *Acer rubrum* (red maple), climate, leaf physiognomy, leaf teeth, paleoclimate, *Quercus kelloggii* (California black oak).

New Phytologist (2008) **179**: 808–817

© The Authors (2008). Journal compilation © *New Phytologist* (2008)

doi: 10.1111/j.1469-8137.2008.02496.x

Introduction

Many plant traits are sensitive to climate (Woodward, 1987; Breckle, 2002) and paleobotanists commonly use plant–climate relationships to reconstruct ancient climates (Chaloner & Creber, 1990; Parrish, 1998). Most plant–climate studies focus on interspecific patterns, for example correlating leaf area to mean annual precipitation (MAP) across distinct biomes (Givnish, 1984; Wilf *et al.*, 1998). However, intraspecific patterns also provide useful information. Most critically, plant–climate relationships within species are affected by both ecotypic variation of plant traits (as in interspecific patterns)

and the plasticity of plant traits. In this study, we examined the strength of correlation between leaf size and shape (physiognomy) and climate for two North American species with broad climatic ranges, *Acer rubrum* (red maple, Sapindaceae) and *Quercus kelloggii* (California black oak, Fagaceae). We sampled *Q. kelloggii* across a large elevation gradient (146–2362 m) but restricted sampling of *A. rubrum* to lowland areas (< 250 m). Our results provide new information about the sensitivity of leaf traits to climate within species and demonstrate the potential for incorporating intraspecific physiognomic data from fossil plants in paleoclimatic reconstructions.

Leaf teeth and climate

It has long been noted that the percentage of woody dicot species in a flora that are toothed inversely correlates with mean annual temperature (MAT) (Bailey & Sinnott, 1916; Wolfe, 1979, 1993; Wilf, 1997; Jacobs, 1999, 2002; Gregory-Wodzicki, 2000; Kowalski, 2002; Greenwood *et al.*, 2004; Greenwood, 2005a; Royer *et al.*, 2005; Traiser *et al.*, 2005). More recently, Huff *et al.* (2003) and Royer *et al.* (2005) reported strong correlations among 17 sites (mostly from eastern North America) between MAT and site-level means of a suite of physiognomic variables, including number of teeth, tooth area and perimeter/area relationships. Compared with warmer sites, colder sites contained species whose leaves generally had more teeth, a larger tooth area and a higher perimeter-to-area ratio (Royer *et al.*, 2005).

The biological basis for these correlations may be related to the observed increases in rates of photosynthesis and transpiration within teeth early in the growing season (Baker-Brosh & Peet, 1997; Royer & Wilf, 2006). This increase in sap flow presumably enhances the delivery of solutes to young emerging leaves and to recently dormant leaves, which may confer an advantage to plants in progressively colder environments with shorter growing seasons (Royer & Wilf, 2006). Thus, leaves with many large teeth may be adaptive in cold climates. This proposed mechanism also provides an explanation for why at a given MAT toothed species are proportionately more abundant in physiologically wet environments (Bailey & Sinnott, 1916; MacGinitie, 1953; Wolfe, 1993; Burnham *et al.*, 2001; Kowalski & Dilcher, 2003; Greenwood, 2005b) because in these environments the impact of water costs associated with leaf teeth are less severe. Hydathodes in teeth may also serve to release excess root pressure via guttation, thereby preventing the flooding of intercellular airspaces; this process could be beneficial to plants in cold climates where freeze–thaw embolisms are more prevalent (Feild *et al.*, 2005).

Intraspecific patterns between leaf physiognomy and climate

Building on the interspecific work of Bailey & Sinnott (1916) and others described above, a currently unresolved question is how tooth size, shape, and number respond to climate within species. Royer *et al.* (2005) reported significant correlations between MAT and physiognomic variables related to tooth size, tooth number, and perimeter/area within four species in the eastern USA (*A. rubrum*, *Prunus serotina*, *Ostrya virginiana*, *Carpinus caroliniana*). However, because the level of sampling within this data set was low ($n \leq 12$ sites for all species), these patterns could only be considered preliminary.

Investigating the role of intraspecific variation in leaf–climate relationships is important for at least three reasons. First, such work bears directly on the broader issue of how

plasticity and genotype influence the sensitivity of leaf form to climate. Many studies have sought to tease apart how plasticity and genotype affect the relationships between plant traits and climate, and considerable progress has been made in the areas of physiology, growth, stomatal patterning and leaf size (Gurevitch, 1988; Williams & Black, 1993; Morecraft & Woodward, 1996; Beerling & Kelly, 1997; Cordell *et al.*, 1998; Oleksyn *et al.*, 1998; Imbert & Houle, 2000; Hovenden, 2001; Flann *et al.*, 2002; Hovenden & Vander Schoor, 2004). By contrast, very little is known about the impact of plasticity vs genetic determination on the relationships between leaf shape and climate. To rigorously test the role of leaf plasticity in leaf–climate relationships, transplant studies are required (Hovenden & Vander Schoor, 2004); however, measuring the physiognomic variability across the native ranges of individual species (which reflects both plasticity and differences in genotype) is an important first step.

Second, the paleobotanical community has applied leaf–climate relationships to fossil leaf floras for nearly a century to quantitatively reconstruct climate (Bailey & Sinnott, 1915; Dilcher, 1973; Wolfe, 1978, 1993; Wolfe & Upchurch, 1987; Greenwood & Wing, 1995; Utescher *et al.*, 2000; Jacobs, 2002; Wilf *et al.*, 2003; Wing *et al.*, 2005; Miller *et al.*, 2006). Most of the methods used in these studies are heavily dependent on tooth characters. Implicit in these paleobotanical studies, particularly those at high temporal resolution, is that plant traits respond rapidly to climate change in a predictable fashion and that a given climate will always select for the same range in leaf physiognomy (Christophel & Gordon, 2004). Therefore, if intraspecific responses of tooth morphology to climate were shown to be broadly similar to the interspecific patterns, this would further emphasize the value and reliability of paleoclimatic reconstructions based on leaf physiognomy.

Third, the nature of intraspecific patterns may confer preference to one leaf–paleoclimate method over others. For example, the method that reconstructs paleotemperature from the percentage of toothed species in a flora ('leaf-margin analysis') is dependent on a single, binary character (presence vs absence of teeth) that is fixed in most species. By contrast, the method presented by Huff *et al.* (2003) and Royer *et al.* (2005) ('digital leaf physiognomy') is based on multiple continuous variables (for a method based on multiple categorical variables see Wolfe, 1993). As a result, digital leaf physiognomy more fully captures the spectrum of physiognomic variation: for example, a species with a large variability across its geographic range in tooth variables (e.g. tooth count and tooth area) would be scored identically with leaf-margin analysis, but could be differentiated with digital leaf physiognomy. Therefore, a potential advantage of digital leaf physiognomy is that intraspecific patterns can contribute to the site-level means of the variables, for example if a toothed fossil species had a greater number of teeth in the cold end of its natural range.

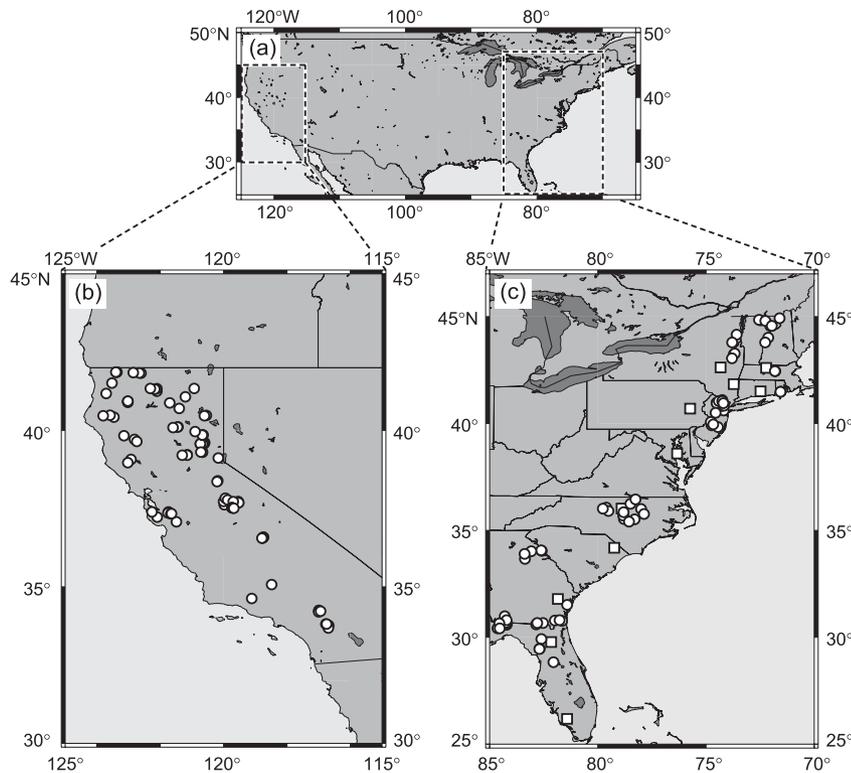


Fig. 1 (a) Overview map and maps showing the sampling locations for (b) *Quercus kelloggii* ($n = 147$ sites) and (c) *Acer rubrum* ($n = 77$ sites) data sets. The squares in (c) represent 11 additional *A. rubrum* sites from Royer *et al.* (2005). Maps generated using GMT software (<http://www.aquarius.ifm-geomar.de>; equidistant cylindrical projection).

Here, in an effort to more firmly ascertain the influence of climate on leaf physiognomy within species, we report results from a large data set that includes two North American woody plants (*A. rubrum* and *Q. kelloggii*) whose native ranges span large MAT gradients and are not closely related to each other. The results of our study provide a test for the importance of intraspecific patterns in leaf–climate relationships and for the usefulness of leaf–climate methods that are based on continuous physiognomic variables (e.g. digital leaf physiognomy) vs binary variables (e.g. leaf-margin analysis).

Materials and Methods

Collection of *A. rubrum*

Acer rubrum L. is a widely-distributed tree in eastern North America, ranging from Newfoundland to south Florida, and from the eastern seaboard to as far west as Texas and Oklahoma (USDA, 2007). During the summer of 2006, J.M.A. collected branches of *A. rubrum* containing phenologically equivalent leaves (computed from growing-degree-days and the phenological variation with latitude found by Borchert *et al.*, 2005) from 77 sites distributed from northern Vermont to central Florida (Fig. 1). Only sun leaves 3–4 m off the ground were collected. All samples were from edges along small tracks or sparsely traveled roads through forested areas. The MAT and MAP of the sites were determined from the model of New *et al.* (2002), which

interpolates climate station data at a 10-min spatial resolution. There is considerable range among the red maple sites in MAT (3.8–21.5°C) but less so in MAP (92–155 cm). Sampling was restricted to low elevation sites (< 250 m). Thus, the principal climatic gradient in this data set was temperature, which is primarily controlled by latitude.

Collection of *Q. kelloggii*

Quercus kelloggii Newberry is a common oak species in the foothills and mountains of California and Oregon, and it ranges from the Mexican border northward to near Eugene, OR; *Q. kelloggii* is also noted for its wide elevation range (< 100–2400 m) (McDonald, 1969). In August 2003, J.C.M. collected two branches and 10 individual leaves from trees at 147 sites distributed throughout California (Fig. 1). The following attributes from each leaf or branch sample were described: height, aspect (N, S, E, W) and degree of shading (full sun or shade). For each sampled tree, the following ecological attributes were described: tree habit (shrub/tree), forest habit (open/closed), and ability to resprout. The following variables for each site were also quantified: elevation, slope of land surface, aspect of slope and soil pH. Climate variables (MAT, MAP) for each tree locality were determined by interpolating the geographically closest climate station data and taking into account elevation differences between stations and tree localities using a digital elevation model and GIS software (ARCVIEW 8.3; ESRI, Redlands, CA, USA). Monthly mean wind speed

Fig. 2 Representative examples of *Acer rubrum* leaves from a cold climate (left column; site 44.48.215 near Irasburg, VT, USA; mean annual temperature (MAT) = 5.1°C), *A. rubrum* leaves from a warm climate (center column; site 43 near Meridian, FL, USA; MAT = 19.1°C), and *Quercus kelloggii* leaves (right column; site 37 near Susanville, CA, USA; MAT = 9.6°C). These images illustrate how leaves are prepared for analysis: the top row contains leaves with their petiole detached, shadows removed and minor imperfections along the leaf margin restored; the bottom row contains the same leaves with their teeth digitally detached following the rules of Huff *et al.* (2003) and Royer *et al.* (2005). Bar, 5 cm.

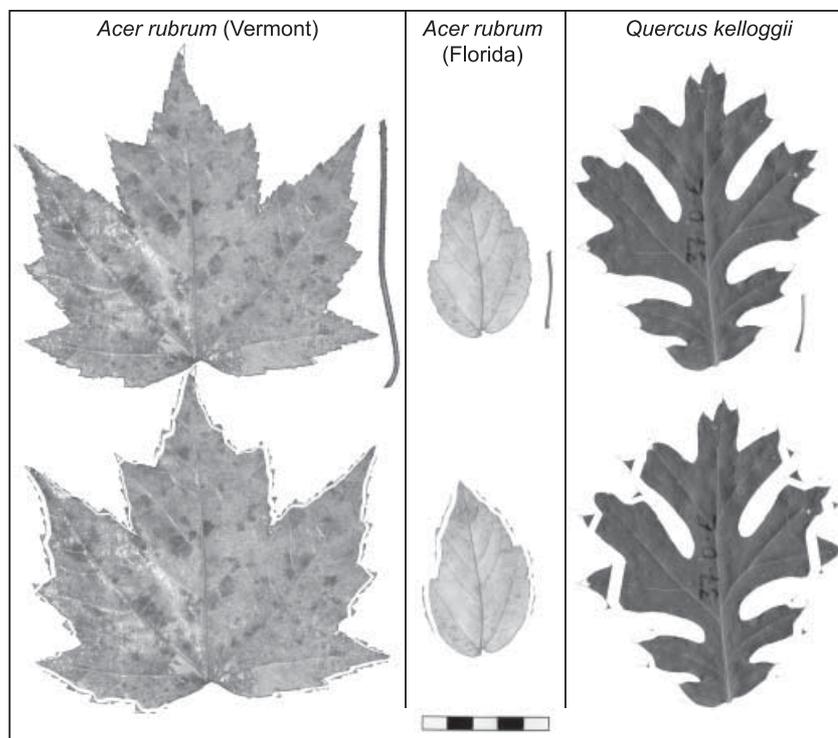


Table 1 Definitions of physiognomic variables used in study

	Physiognomic variable (abbreviation)	Definition (units)
1	Number of teeth (#Teeth)	Number of primary and secondary teeth
2	Blade area (BA)	Area of leaf blade (cm ²)
	Perimeter (Peri)	Blade perimeter (cm)
3	Number of teeth/blade area (#Teeth/BA)	(cm ⁻²)
4	Number of teeth/perimeter (#Teeth/peri)	(cm ⁻¹)
5	Shape factor	$4\pi \times \text{blade area}/\text{perimeter}^2$
	Internal Perimeter	Perimeter after teeth are removed (cm)
6	Perimeter ratio (Peri ratio)	Perimeter/internal perimeter (dimensionless)
7	Tooth area (TA)	Area of teeth (cm ²)
8	Tooth area/blade area (TABA)	(dimensionless)
9	Tooth area/perimeter (TA/peri)	(cm)
10	Average tooth area (AvgTA)	Tooth area/number of primary teeth (cm ²)

Numbers correspond to physiognomic variables in Tables 2 and 3.

was taken from the model of New *et al.* (2002). Among our sites, MAT ranges from 6.1 to 16.7°C, MAP from 29 to 179 cm and elevation from 146 to 2362 m. Compared with the *A. rubrum* sample, precipitation and elevation are much more important environmental gradients in the *Q. kelloggii* data set.

Digital processing of leaf images

The methodology of processing leaves for digital leaf physiognomy has been described elsewhere by Huff *et al.* (2003) and Royer *et al.* (2005). Briefly, groups of three

A. rubrum leaves were digitally photographed (3264 × 2448 pixel resolution) against a black velvet background; for *Q. kelloggii*, individual leaves were scanned with a flatbed scanner (5050 × 3860 pixel resolution). In Adobe Photoshop 9.0 (Adobe Systems, San Jose, CA, USA), shadows were removed and imperfections along the leaf margin were restored; leaf teeth were then digitally detached from the leaf following the rules of Huff *et al.* (2003) and Royer *et al.* (2005) (see Fig. 2 for representative examples). Leaf physiognomic variables related to tooth number, tooth area, and perimeter/area (see Table 1 for list and definitions) were then

Table 2 Correlations between physiognomic variables and mean annual temperature (°C) for *Acer rubrum*

	1	2	3	4	5	6	7	8	9	10
	#Teeth	Blade area (cm ²)	#Teeth/BA (cm ⁻²)	#Teeth/peri (cm ⁻¹)	Shape factor	Peri ratio	TA (cm ²)	TABA	TA/peri (cm)	Avg TA (cm ²)
<i>m</i>	–	–	–	–	+	–	–	+	–	+
<i>r</i> ²	0.54	0.38	0.07	0.27	0.24	0.38	0.29	0.04	0.01	0.10
<i>P</i>	< 0.0001	< 0.0001	0.02	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.07	0.47	0.006

m, slope of correlation; abbreviations of physiognomic variables are defined in Table 1; numbers in column headers correspond to numbers in Table 1; statistically significant correlations ($P < 0.05$) are tinted.

computed using IMAGE-J (<http://rsb.info.nih.gov/ij/>) for *A. rubrum* and SIGMA SCAN PRO 5.0 (SPSS Science, Chicago, IL, USA) for *Q. kelloggii*; a crosscheck between software revealed no significant differences (< 1%). For both data sets, three leaves were typically analysed per site ($n = 233$ *A. rubrum* leaves; $n = 299$ *Q. kelloggii* leaves); two sun leaves and one shade leaf per site were usually analysed for *Q. kelloggii*. All physiognomic, climatic, and ecological data are provided in the Supplementary Material, Table S1, and all unprocessed leaf images are available from D.L.R.'s website (<http://droyer.web.wesleyan.edu>).

Results

Acer rubrum

There are many significant correlations in *A. rubrum* between MAT and leaf physiognomy (Table 2; Fig. 3). The leaves of red maples native to colder climates generally have more teeth, a higher tooth area, and are more highly dissected (Table 2; see also Fig. 2). An important confounding variable in this data set is leaf area: leaves are consistently larger at colder sites (Table 2; see also Fig. 2). Therefore, a contributing factor for why *A. rubrum* leaves from colder sites have more teeth and a higher tooth area is because they are larger. However, even after accounting for the influence of leaf size and leaf perimeter, significant correlations remain between number of teeth and MAT (see 'number of teeth/blade area' and 'number of teeth/perimeter' in Table 2; also, MAT is a significant variable in a multiple linear regression for tooth count that includes MAT and leaf area: $t_{[74]} = -5.57$, $P < 0.0001$). By contrast, there are no significant correlations between tooth area and MAT after accounting for leaf area and leaf perimeter (see 'tooth area/blade area' and 'tooth area/perimeter' in Table 2). Further, average tooth area in *A. rubrum* correlates positively with MAT (i.e. the average area of a single tooth is larger in warmer climates), despite tooth number correlating negatively (Table 2). After accounting for the influence of MAT, MAP is only weakly linked to leaf physiognomy ($P > 0.06$ for the

10 physiognomic variables listed in Table 2, with the exception of tooth area/blade area, 'TABA': $r = -0.266$; $P = 0.01$); these weak correlations are not surprising given the narrow MAP range in the *A. rubrum* data set (MAP = 92–155 cm).

Quercus kelloggii

The patterns for *Q. kelloggii* are more complex. Overall, the correlations between MAT and the leaf physiognomic variables are weaker (Table 3). Similar to the *A. rubrum* data set, the black oak leaves growing in warmer climates have fewer teeth per unit perimeter and a larger average tooth area, but leaf area is positively correlated with MAT, which is opposite to the pattern documented for red maple. Elevation, which varies considerably in the black oak data set (2200 m, vs < 250 m in the *A. rubrum* data set), correlates significantly with MAT ($r^2 = 0.19$, $P < 0.0001$); if elevation is controlled for using partial correlation, only the relationship between leaf area and MAT remains significant (Table 3).

For *Q. kelloggii*, elevation correlates significantly with more physiognomic variables than does MAT. Leaves from higher elevation are smaller, have more teeth per unit perimeter, less tooth area (both total tooth area and average tooth area) and less tooth area per unit perimeter (Table 3; Fig. 4). Most of these patterns remain even after removing the influence of MAT using partial correlation (Table 3).

Mean annual precipitation varies greatly within the *Q. kelloggii* data set (MAP = 28–179 cm) but it does not correlate significantly with any of the measured physiognomic variables ($P > 0.1$ for all comparisons). Also, other environmental and ecological variables (sun vs shade leaves, leaf collection height, shrub vs tree, ability to resprout, closed vs open habitat, slope of site, leaf and slope aspect, site elevation, soil pH) do not significantly confound the relationships between MAT and leaf physiognomy; three exceptions are that tree habit, forest habit and ability to resprout confound the relationship between MAT and leaf area (see the Supplementary Material, Table S2).

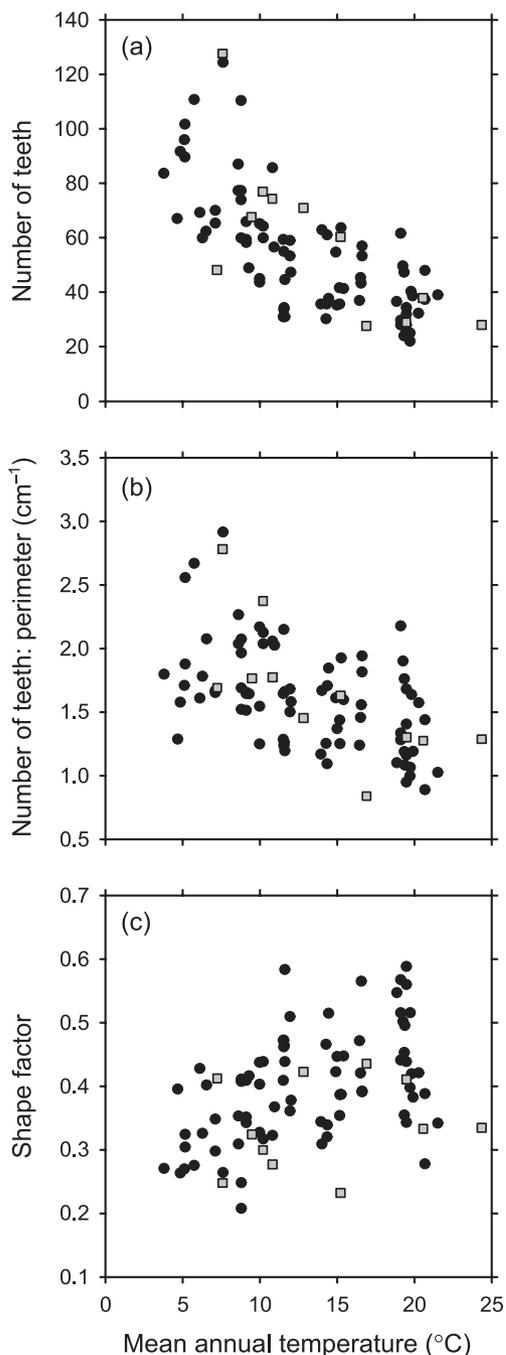


Fig. 3 Relationship between mean annual temperature (MAT) and some of the measured physiognomic variables for *Acer rubrum*. Circles, this study; squares, Royer *et al.* (2005). See Table 1 for definition of physiognomic variables and Table 2 for statistical information about the correlations.

Discussion

Acer rubrum

The correlations between leaf physiognomy and MAT in *A. rubrum* largely match the site-mean trends observed by

Huff *et al.* (2003) and Royer *et al.* (2005): leaves from colder sites are toothier and more highly dissected (Table 2; Fig. 3). Recognition of these patterns within species emphasizes the tight link between leaf form and climate. For paleobotanists, this sensitivity of leaf form to climate within a species, combined with similar preliminary results from three other species (Royer *et al.*, 2005), provides additional support for using leaf physiognomy to reconstruct ancient climates.

The *A. rubrum* results also highlight the value of using continuous, not categorical, variables for paleoclimatic reconstructions (Royer *et al.*, 2005). For example, with leaf-margin analysis all red maple leaves receive the same score (toothed) even though in our data set the number of teeth per leaf ranges from 17 to 151. Incorporation of these types of additional information, which we have shown here to co-vary with MAT within a single species, should contribute towards more robust paleoclimate methods.

The average area of individual teeth increases with warmer temperatures in both species (Tables 2 and 3); this is opposite to the site mean observations of Royer *et al.* (2005). The reasons for this difference are unclear, however, it should be noted that the site-level correlation reported by Royer *et al.* (2005) was only marginally significant ($r^2 = 0.22$; $P = 0.054$). Nonetheless, the increase in average tooth area in warmer climates explains why MAT does not correlate significantly, in this species, with tooth area per unit perimeter or per unit leaf area, despite the fact that leaves have fewer teeth per unit perimeter and per unit leaf area in warmer climates.

Explanation for the weak temperature correlations in *Q. kelloggii*

The general lack of significant relationships between MAT and leaf shape in *Q. kelloggii* (Table 3) may result from an inherent insensitivity within the species or from the confounding effects of other environmental and/or ecological variables. The first option is difficult to analyse rigorously, although it is noteworthy that black oak leaves are less 'toothy' than red maple leaves. For example, the average number of teeth per black oak leaf in our data set is 15.6 but for red maple is 52.9 (and this despite black oak leaves being, on average, larger in area). Thus, black oaks have less tooth morphospace available to them, which may restrict the magnitude of any morphological change with climate. Also, the range in MAT in the black oak data set (10.6°C) is considerably smaller than in the red maple data set (17.7°C). It is therefore possible that the range in MAT in the *Q. kelloggii* data set was not sufficient to resolve weak but nonetheless real trends.

Almost all environmental and ecological factors considered in this study were found to have little to no influence on the correlations between leaf shape and MAT in *Q. kelloggii* (see the Supplementary Material, Table S2). We therefore

Table 3 Correlations between physiognomic variables and mean annual temperature (MAT), elevation, or mean annual wind speed for *Quercus kelloggii*

	1	2	3	4	5	6	7	8	9	10
	#Teeth	Blade area (cm ²)	#Teeth/A (cm ⁻²)	#Teeth/peri (cm ⁻¹)	Shape factor	Peri ratio	TA (cm ²)	TABA	TA/peri (cm)	Avg TA (cm ²)
MAT (°C)										
<i>m</i>	-	+	+	-	+	+	+	-	+	+
<i>r</i> ²	0.01	0.07	0.02	0.04	0.00	0.00	0.01	0.01	0.00	0.04
<i>P</i>	0.23	0.002	0.09	0.01	0.75	0.84	0.30	0.36	0.80	0.01
MAT; partial correlation, controlling for elevation										
<i>m</i>	+	+	+	-	-	-	+	-	-	+
<i>r</i> ²	0.00	0.10	0.00	0.04	0.00	0.00	0.00	0.02	0.00	0.02
<i>P</i>	0.99	0.003	0.53	0.05	0.80	0.75	0.59	0.23	0.64	0.18
Elevation (m)										
<i>m</i>	+	-	-	+	+	-	-	-	-	-
<i>r</i> ²	0.03	0.15	0.01	0.16	0.00	0.00	0.10	0.01	0.06	0.18
<i>P</i>	0.03	< 0.0001	0.28	< 0.0001	0.63	0.74	0.0001	0.26	0.004	< 0.0001
Elevation; partial correlation, controlling for MAT										
<i>m</i>	+	-	+	+	+	-	-	-	-	-
<i>r</i> ²	0.02	0.10	0.15	0.12	0.00	0.00	0.09	0.03	0.03	0.15
<i>P</i>	0.06	0.0001	< 0.0001	< 0.0001	0.46	0.79	0.0002	0.05	0.03	< 0.0001
Wind speed (m s ⁻¹); partial correlation, controlling for MAT										
<i>m</i>	+	-	+	+	+	-	-	-	-	-
<i>r</i> ²	0.00	0.00	0.00	0.00	0.00	0.01	0.05	0.07	0.05	0.04
<i>P</i>	0.99	0.74	0.60	0.68	0.85	0.25	0.008	0.002	0.007	0.018

m, slope of correlation; MAT, mean annual temperature. Abbreviations for physiognomic variables are defined in Table 1; numbers in column headers correspond to numbers in Table 1; statistically significant correlations ($P < 0.05$) are tinted.

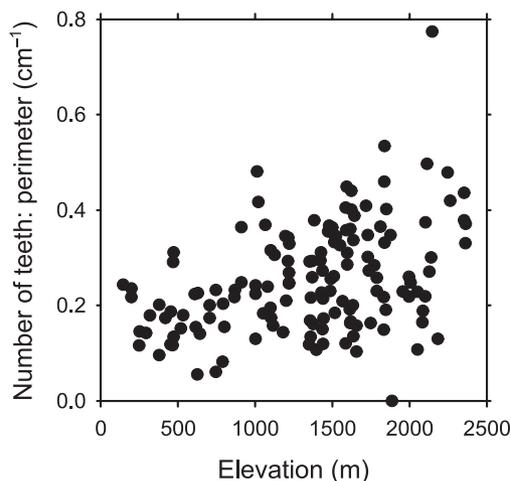


Fig. 4 Relationship between the number of teeth per unit perimeter and elevation for *Quercus kelloggii*. See Table 3 for statistical information about the correlation.

conclude that leaf shape in *Q. kelloggii* is probably not sensitive to MAT; however, it is possible that other environmental or ecological factors not considered in our study are confounding the relationship.

Elevation and *Q. kelloggii*

Although elevation cannot explain why the relationships between leaf shape and MAT are weak in *Q. kelloggii* (Table 3; Table S2), there are nonetheless many significant correlations between physiognomy and elevation. Even after removing the influence of MAT, leaves from higher elevation are smaller in area, have a greater number of teeth and less tooth area (Table 3; Fig. 4). Reductions in leaf size with elevation have been documented in many species (Webb, 1968; Körner *et al.*, 1986; Gurevitch, 1988; Halloy & Mark, 1996; Morecraft & Woodward, 1996; Cordell *et al.*, 1998; Hovenden & Vander Schoor, 2004). Some authors have attributed this response to temperature (Körner *et al.*, 1986; Gurevitch, 1988): our study demonstrates that temperature is important but that additional factors are also required (Table 3). Other possible factors include more intense solar radiation (Hovenden & Vander Schoor, 2004, 2006), decreased water and/or nutrient availability (Givnish, 1979; Roderick *et al.*, 2000; McDonald *et al.*, 2003), shorter growing season (Kouwenberg *et al.*, 2007) and reduced CO₂ partial pressure at high elevation (McElwain, 2004).

The relationship between leaf area and elevation is documented in many species, but we are unaware of any studies reporting on the relationship between tooth morphology and

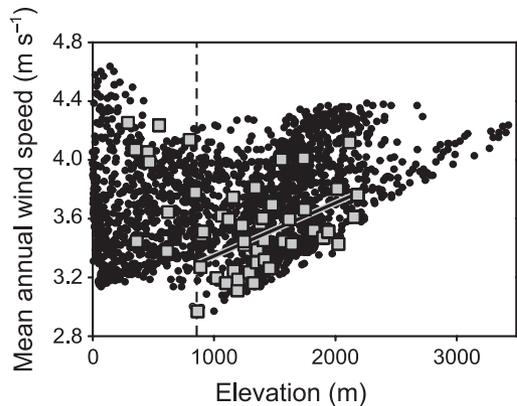


Fig. 5 Relationship between elevation and mean annual wind speed for areas within 33–42°N latitude and 116–124°W longitude (see Fig. 1 for geography). All data (circles) are from the model of New *et al.* (2002) (see the Materials and Methods section for details). Squares, data that are geographically closest to the *Quercus kelloggii* sites. The vertical dashed line corresponds to an elevation of 850 m. The least-squares regression line is for *Q. kelloggii* sites > 850 m elevation ($n = 127$; $r^2 = 0.28$; $P < 0.0001$).

elevation. In comparison with studies that have focused on MAT (Royer *et al.*, 2005), our results are paradoxical: at high elevation (and low MAT), leaves have more teeth but the individual teeth are smaller. Interestingly, our *A. rubrum* results are broadly similar (leaves in cold climates have more teeth but the individual teeth are smaller), but the underlying reasons must differ because the *Q. kelloggii* patterns hold true even after the effects of MAT are removed (Table 3).

Can wind speed explain the elevation patterns in *Q. kelloggii*? According to Royer & Wilf (2006), a function of leaf teeth is to enhance transpirational losses for the sake of carbon gain (see the Introduction); teeth facilitate this water loss probably because they are rich in vascular tissue and because their geometric construction leads to a local thinning of the leaf boundary layer (Canny, 1990; Schuepp, 1993; Wolfe, 1993). This latter point may be important with respect to wind because windy conditions also reduce leaf boundary layers and may therefore obviate the need for large teeth. Thus, small teeth may be functionally adequate in windy environments.

In the general geographic area of our *Q. kelloggii* data set, wind speed scales with elevation, but only for elevations > 850 m (Fig. 5; < 850 m: $n = 729$, $r^2 = 0.00$, $P = 0.12$; > 850 m: $n = 1252$, $r^2 = 0.19$, $P < 0.0001$); the same patterns hold for the areas closest to the *Q. kelloggii* sites (squares in Fig. 5). Importantly, after removing the influence of MAT using partial correlation, windy sites correlate with leaves with significantly smaller teeth (as measured by total tooth area, average area of individual teeth, and tooth area per unit leaf area and per unit perimeter; see Table 3); these patterns remain even if the influence of MAT is not removed, if the analysis is restricted to mean wind speed during the growing

season, or if the analysis is restricted to sites > 850 m elevation (results not shown). Overall, these data support the hypothesis that wind speed may be linked to tooth size.

We must look to other factors for explaining why leaves from high-elevation sites have more teeth than at low-elevation sites. We conjecture that water availability may be an important factor: elevation does not correlate with MAP at the *Q. kelloggii* sites ($n = 145$, $r^2 = 0.00$, $P = 0.49$), therefore the amount of water available to plants should increase with elevation because rates of evapotranspiration typically decline with elevation in climates such as those in California, with dry adiabatic lapse rates (Körner, 2003; Smith & Johnson, in press). Plants can better 'afford' leaf teeth in physiologically wet environments (Royer & Wilf, 2006; see also the Introduction), and this may provide a ready explanation for the abundance of teeth at high-elevation sites, independent of MAT. We hypothesize that the combined effects of higher wind speed and more plant-available water selects for *Q. kelloggii* leaves with an abundance of small teeth at high elevation.

Concluding remarks

Our results suggest that tooth size, shape, and number within some, but not all, species are sensitive to climate. This sensitivity provides added support for using continuous variables related to tooth number and leaf dissection to reconstruct ancient climates. *Acer rubrum*, a species sampled entirely from low-elevation habitats comparable to most fossil floras, produces toothier and more highly dissected leaves in colder environments. However, the leaf shape of *Q. kelloggii*, sampled from a broad elevation range, is largely insensitive to MAT, and instead correlates significantly with elevation; we hypothesize that wind speed and water availability underpin these elevation patterns. The underlying reasons for the insensitivity between tooth physiognomy and MAT in *Q. kelloggii* are unclear, but we suggest that the overall low numbers of teeth relative to leaf size in the species (compared with *A. rubrum*) may be a contributing factor.

Acknowledgements

We thank Rebekah Hines, Maddeleine McLeester and Michael Fortweglar for field assistance in California, and Mei Ling Lee and Yangjian Zhang for their considerable help in collecting *A. rubrum* leaves. We also thank Hanna Park for scanning *Q. kelloggii* leaves, Joshua Cummings, Margo Fernandez-Burgos, David Janesko, Sophia Kim, Crystal Kirby and Sage Trombulak for help in processing leaf images, and Margo Fernandez-Burgos for help in drafting Fig. 2. This work was supported in part by the Petroleum Research Fund of the American Chemical Society (grant 40546-AC8) and the National Science Foundation (grants EAR-0236489 and EAR-0207440).

References

- Bailey IW, Sinnott EW. 1915. A botanical index of Cretaceous and Tertiary climates. *Science* 41: 831–834.
- Bailey IW, Sinnott EW. 1916. The climatic distribution of certain types of angiosperm leaves. *American Journal of Botany* 3: 24–29.
- Baker-Brosch KF, Peet RK. 1997. The ecological significance of lobed and toothed leaves in temperate forest trees. *Ecology* 78: 1250–1255.
- Beerling DJ, Kelly CK. 1997. Stomatal density responses of temperate woodland plants over the past seven decades of CO₂ increase: a comparison of Salisbury (1927) with contemporary data. *American Journal of Botany* 84: 1572–1583.
- Borchert R, Robertson K, Schwartz MD, Williams-Linera G. 2005. Phenology of temperate trees in tropical climates. *International Journal of Biometeorology* 50: 57–65.
- Breckle S-W. 2002. *Walter's vegetation of the earth: the ecological systems of the geo-biosphere*, 4th edn. Berlin, Germany: Springer-Verlag.
- Burnham RJ, Pitman NCA, Johnson KR, Wilf P. 2001. Habitat-related error in estimating temperatures from leaf margins in a humid tropical forest. *American Journal of Botany* 88: 1096–1102.
- Canny M. 1990. What becomes of the transpiration stream? *New Phytologist* 114: 341–368.
- Chaloner WG, Creber GT. 1990. Do fossil plants give a climatic signal? *Journal of the Geological Society, London* 147: 343–350.
- Christophel D, Gordon P. 2004. Genotypic control and environmental plasticity—foliar physiognomy and paleoecology. *New Phytologist* 161: 327–329.
- Cordell S, Goldstein G, Mueller-Dombois D, Webb D, Vitousek PM. 1998. Physiological and morphological variation in *Metrosideros polymorpha*, a dominant Hawaiian tree species, along an altitudinal gradient: the role of phenotypic plasticity. *Oecologia* 113: 188–196.
- Dilcher DL. 1973. A paleoclimatic interpretation of the Eocene floras of southeastern North America. In: Graham A, ed. *Vegetation and vegetational history of Northern Latin America*. Amsterdam, the Netherlands: Elsevier, 39–53.
- Feild TS, Sage TL, Czerniak C, Iles WJD. 2005. Hydathodal leaf teeth of *Chloranthus japonicus* (Chloranthaceae) prevent guttation-induced flooding of the mesophyll. *Plant, Cell & Environment* 28: 1179–1190.
- Flann C, Ladiges PY, Walsh NG. 2002. Morphological variation in *Leptorhynchus squamatus* (Gnaphalieae: Asteraceae). *Australian Systematic Botany* 15: 205–219.
- Givnish TJ. 1979. On the adaptive significance of leaf form. In: Solbrig OT, Jain S, Johnson GB, Raven PH, eds. *Topics in plant population biology*. New York, NY, USA: Columbia University Press, 375–407.
- Givnish TJ. 1984. Leaf and canopy adaptations in tropical forests. In: Medina E, Mooney HA, Vázquez-Yanes C, eds. *Physiological ecology of plants of the wet tropics*. The Hague, the Netherlands: Dr W Junk, 51–84.
- Greenwood DR. 2005a. Leaf form and the reconstruction of past climates. *New Phytologist* 166: 355–357.
- Greenwood DR. 2005b. Leaf margin analysis: taphonomic constraints. *Palaios* 20: 498–505.
- Greenwood DR, Wilf P, Wing SL, Christophel DC. 2004. Paleotemperature estimation using leaf-margin analysis: is Australia different? *Palaios* 19: 129–142.
- Greenwood DR, Wing SL. 1995. Eocene continental climates and latitudinal temperature gradients. *Geology* 23: 1044–1048.
- Gregory-Wodzicki KM. 2000. Relationships between leaf morphology and climate, Bolivia: implications for estimating paleoclimate from fossil floras. *Paleobiology* 26: 668–688.
- Gurevitch J. 1988. Variation in leaf dissection and leaf energy budgets among populations of *Achillea* from an altitudinal gradient. *American Journal of Botany* 75: 1298–1306.
- Halloy SRP, Mark AF. 1996. Comparative leaf morphology spectra of plant communities in New Zealand, the Andes and the European Alps. *Journal of the Royal Society of New Zealand* 26: 41–78.
- Hovenden MJ. 2001. The influence of temperature and genotype on the growth and stomatal morphology of southern beech, *Nothofagus cunninghamii* (Nothofagaceae). *Australian Journal of Botany* 49: 427–434.
- Hovenden MJ, Vander Schoor JK. 2004. Nature vs nurture in the leaf morphology of Southern beech, *Nothofagus cunninghamii* (Nothofagaceae). *New Phytologist* 161: 585–594.
- Hovenden MJ, Vander Schoor JK. 2006. The response of leaf morphology to irradiance depends on altitude of origin in *Nothofagus cunninghamii*. *New Phytologist* 169: 291–297.
- Huff PM, Wilf P, Azumah EJ. 2003. Digital future for paleoclimate estimation from fossil leaves? Preliminary results. *Palaios* 18: 266–274.
- Imbert É, Houle G. 2000. Ecophysiological differences among *Leymus mollis* populations across a subarctic dune system caused by environmental, not genetic, factors. *New Phytologist* 147: 601–608.
- Jacobs BF. 1999. Estimation of rainfall variables from leaf characters in tropical Africa. *Palaeogeography, Palaeoclimatology, Palaeoecology* 145: 231–250.
- Jacobs BF. 2002. Estimation of low-latitude paleoclimates using fossil angiosperm leaves: examples from the Miocene Tugen Hills, Kenya. *Paleobiology* 28: 399–421.
- Körner C. 2003. *Alpine plant life*. Berlin, Germany: Springer-Verlag.
- Körner C, Bannister P, Mark AF. 1986. Altitudinal variation in stomatal conductance, nitrogen content and leaf anatomy in different plant life forms in New Zealand. *Oecologia* 69: 577–588.
- Kouwenberg LLR, Kürschner WM, McElwain JC. 2007. Stomatal frequency change over altitudinal gradients: prospects for paleoaltimetry. *Reviews in Mineralogy & Geochemistry* 66: 215–241.
- Kowalski EA. 2002. Mean annual temperature estimation based on leaf morphology: a test from tropical South America. *Palaeogeography, Palaeoclimatology, Palaeoecology* 188: 141–165.
- Kowalski EA, Dilcher DL. 2003. Warmer paleotemperatures for terrestrial ecosystems. *Proceedings of the National Academy of Sciences, USA* 100: 167–170.
- MacGinitie HD. 1953. Fossil plants of the florissant beds, Colorado. *Carnegie Institution of Washington Publication* 599: 1–198.
- McDonald PG, Fonseca CR, Overton JM, Westoby M. 2003. Leaf-size divergence along rainfall and soil-nutrient gradients: is the method of size reduction common among clades? *Functional Ecology* 17: 50–57.
- McDonald PM. 1969. *Silvical characteristics of California black oak (Quercus kelloggii Newb.)*. USDA Forest Service Research Paper PSW-53. Berkeley, CA, USA: Pacific Southwest Forest and Range Experiment Station.
- McElwain JC. 2004. Climate-independent paleoaltimetry using stomatal density in fossil leaves as a proxy for CO₂ partial pressure. *Geology* 32: 1017–1020.
- Miller IM, Brandon MT, Hickey LJ. 2006. Using leaf margin analysis to estimate the mid-Cretaceous (Albian) paleolatitude of the Baja BC block. *Earth and Planetary Science Letters* 245: 95–114.
- Morecraft MD, Woodward FI. 1996. Experiments on the causes of altitudinal differences in the leaf nutrient contents, size and $\delta^{13}\text{C}$ of *Alchemilla alpina*. *New Phytologist* 134: 471–479.
- New M, Lister D, Hulme M, Makin I. 2002. A high-resolution data set of surface climate over global land areas. *Climate Research* 21: 1–25 (data available at <http://www.cru.uea.ac.uk/cru/data/tmc.htm>).
- Oleksyn J, Modrzyński J, Tjoelker MG, Zytkowski R, Reich PB, Karolewski P. 1998. Growth and physiology of *Picea abies* populations from elevational transects: common garden evidence for altitudinal ecotypes and cold adaptation. *Functional Ecology* 12: 573–590.
- Parrish JT. 1998. *Interpreting pre-Quaternary climate from the geologic record*. New York, NY, USA: Columbia University Press.
- Roderick M, Barry S, Noble I. 2000. A framework for understanding the relationship between environment and vegetation based on the surface area to volume ratio of leaves. *Functional Ecology* 14: 423–437.

- Royer DL, Wilf P. 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 DL, Wilf P, Janesko DA, Kowalski EA, Dilcher DL. 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.
- Schuepp PH. 1993. Leaf boundary layers. *New Phytologist* 125: 477–507.
- Smith WK, Johnson DM. (in press). Biophysical effects of altitude on plant gas exchange. In: De la Cabrera E, Smith WK, eds. *Biophysical plant ecology: perspectives and trends*. Los Angeles, CA, USA: University of California Press.
- Traiser C, Klotz S, Uhl D, Mosbrugger V. 2005. Environmental signals from leaves – a physiognomic analysis of European vegetation. *New Phytologist* 166: 465–484.
- USDA, NRCS. 2007. *The PLANTS database* (<http://plants.usda.gov>, accessed November 2007). Baton Rouge, LA, USA: National Plant Data Center.
- Utescher T, Mosbrugger V, Ashraf AR. 2000. Terrestrial climate evolution in northwest Germany over the last 25 million years. *Palaios* 15: 430–449.
- Webb LJ. 1968. Environmental relationships of the structural types of Australian rain forest vegetation. *Ecology* 49: 296–311.
- Wilf P. 1997. When are leaves good thermometers? A new case for leaf margin analysis. *Paleobiology* 23: 373–390.
- Wilf P, Johnson KR, Huber BT. 2003. Correlated terrestrial and marine evidence for global climate changes before mass extinction at the Cretaceous–Paleogene boundary. *Proceedings of the National Academy of Sciences, USA* 100: 599–604.
- Wilf P, Wing SL, Greenwood DR, Greenwood CL. 1998. Using fossil leaves as paleoprecipitation indicators: an Eocene example. *Geology* 26: 203–206.
- Williams DG, Black RA. 1993. Phenotypic variation in contrasting temperature environments: growth and photosynthesis in *Pennisetum setaceum* from different altitudes on Hawaii. *Functional Ecology* 7: 623–633.
- Wing SL, Harrington GJ, Smith FA, Bloch JI, Boyer DM, Freeman KH. 2005. Transient floral change and rapid global warming at the Paleocene–Eocene boundary. *Science* 310: 993–996.
- Wolfe JA. 1978. A paleobotanical interpretation of Tertiary climates in the northern hemisphere. *American Scientist* 66: 694–703.
- Wolfe JA. 1979. Temperature parameters of humid to mesic forests of Eastern Asia and relation to forests of other regions of the Northern Hemisphere and Australasia. *US Geological Survey Professional Paper* 1106: 1–37.
- Wolfe JA. 1993. A method of obtaining climatic parameters from leaf assemblages. *US Geological Survey Bulletin* 2040: 1–71.
- Wolfe JA, Upchurch GR. 1987. North American nonmarine climates and vegetation during the Late Cretaceous. *Palaeogeography, Palaeoclimatology, Palaeoecology* 61: 33–77.
- Woodward FI. 1987. *Climate and plant distribution*. Cambridge, UK: Cambridge University Press.

Supplementary Material

The following supplementary material is available for this article online:

Table S1 Physiognomic and environmental data for all leaves used in this study

Table S2 Correlations between physiognomic and ecological variables in *Quercus kelloggii* data set

This material is available as part of the online article from: <http://www.blackwell-synergy.com/doi/abs/10.1111/j.1469-8137.2008.02496.x>
(This link will take you to the article abstract).

Please note: Blackwell Publishing are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the journal at *New Phytologist* Central Office.



About *New Phytologist*

- *New Phytologist* is owned by a non-profit-making **charitable trust** dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at www.newphytologist.org.
- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as-ready' via *OnlineEarly* – our average submission to decision time is just 29 days. Online-only colour is **free**, and essential print colour costs will be met if necessary. We also provide 25 offprints as well as a PDF for each article.
- For online summaries and ToC alerts, go to the website and click on 'Journal online'. You can take out a **personal subscription** to the journal for a fraction of the institutional price. Rates start at £135 in Europe/\$251 in the USA & Canada for the online edition (click on 'Subscribe' at the website).
- If you have any questions, do get in touch with Central Office (newphytol@lancaster.ac.uk; tel +44 1524 594691) or, for a local contact in North America, the US Office (newphytol@ornl.gov; tel +1 865 576 5261).