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RESEARCH PAPER

Leaf form–climate relationships on the global stage: an ensemble of characters

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ABSTRACT

Aim Early in their evolution, angiosperms evolved a diversity of leaf form far greater than that of any other group of land plants. Some of this diversity evolved in response to varying climate. Our aim is to test the global relationship between leaf form in woody dicot angiosperms and the climate in which they live.

Location We have compiled a data set describing leaf form (using 31 standardized categorical characters) from 378 natural or naturalized vegetation sites from around the world. Our data include sites from all continents except Antarctica and encompass biomes from tropical to taiga, over a range of elevations from 0.5 m to over 3000 m.

Methods We chose the Climate Leaf Analysis Multivariate Program sampling, scoring and analytical protocols to test the relationships between climate and leaf form, which is based on canonical correspondence analysis. Cluster analysis evaluates the role of historical factors in shaping the patterns, and pairwise Pearson correlations examine the relationships among leaf characters.

Results Woody dicot leaf characters form a physiognomic spectrum that reflects local climate conditions. On a global scale, correlations between leaf form and climate are consistent, irrespective of climate regime, vegetation type or biogeographic history. Relationships with temperature variables are maintained even when leaf margin characters, regarded as being particularly well correlated with mean annual temperature, are removed.

Main conclusions In natural woody dicot vegetation an integrated spectrum of leaf form has developed across multiple leaf character states and species. This spectrum appears more strongly influenced by prevailing climate than biogeographic history. The covariation of leaf traits across species suggests strong integration of leaf form. New methods of exploring structure in multidimensional physiognomic space enable better application of leaf form to palaeoclimate reconstruction.

Keywords
Climate change, CLAMP, ecosystems, leaf form, multivariate statistics, woody dicots.

INTRODUCTION

Angiosperms display tremendous variation in leaf form among species, within species and within individuals. Phenotypic plasticity accounts for some variation (Pigliucci, 2003) while some stems from flexibility in leaf developmental programmes (Falconer & Mackay, 1996; Juenger *et al.*, 2005; Rodriguez *et al.*, 2014). In all cases, variation in leaf form reflects a trade-off between resources invested and photosynthetic return (e.g. Givnish, 1984; Bloom *et al.*, 1985) constrained by the environ-

ment. That plants maintain a nearly constant leaf temperature (21.4 ± 2.2 °C) over a wide range of conditions (Hellicker & Richter, 2008) illustrates the homeostatic role of leaf and canopy form (Leuzinger & Korner, 2007). Yet this balance cannot be explained by leaf economic traits (e.g. leaf mass per unit area, photosynthetic capacity and elemental composition) alone (Wright *et al.*, 2004). Leaf form (physiognomy) must play an important role.

The leaf form–climate relationship has been explored for in the context of methods that use fossil woody dicot leaves to reconstruct palaeoclimate, palaeolatitude and palaeoaltitude. Initial work recognized a linear relationship between untoothed leaf margins in trees and mean annual temperature (MAT) (Bailey & Sinnott, 1915, 1916; Wolfe, 1979). This relationship has been applied extensively to reconstruct past climates (e.g. Wolfe, 1978; Upchurch & Wolfe, 1987; Wing & Greenwood, 1993) and palaeolatitude (Miller *et al.*, 2006). The method is popular because it is easy to apply (toothed versus untoothed margin is easy to assess, even in incomplete fossils), is simple and systematic error can be quantified (Wilf, 1997). However, the linear relationship (both slope and intercept) varies biogeographically (Wolfe & Upchurch, 1987; Wolfe, 1993; Greenwood *et al.*, 2004; Wright *et al.*, 2004; Little *et al.*, 2010; Steart *et al.*, 2010; Jacques *et al.*, 2011; Peppe *et al.*, 2011; Kennedy *et al.*, 2014). This creates problems when choosing a calibration set against which to compare a fossil assemblage.

Another attraction of the linear relationship between a single leaf feature and a single climate parameter is the potential to link form and environment through an ecophysiological function (Baker-Brosh & Peet, 1997; Royer & Wilf, 2006). Such mechanistic links would lend confidence to leaf form–climate correlations, but they remain elusive (Jordan, 2011). Leaves must optimize a variety of ecophysiological functions simultaneously and are developmentally integrated; it seems unlikely then that they would show single-character form–function–environment relationships. Moreover, the search for retrodictive links between leaf form and climate might be more successful when a range of traits are considered simultaneously.

Researchers have explored multivariate methods that identify relationships among a variety of leaf characters and environmental variables (Wing & Greenwood, 1993; Wolfe, 1993; Kovach & Spicer, 1995; Gregory-Wodzicki, 2000; Steart *et al.*, 2010; Peppe *et al.*, 2011; Teodoridis *et al.*, 2011; Kennedy *et al.*, 2014) and have successfully applied these methods to retrodicting palaeoclimate (e.g. Herman & Spicer, 1996; Forest *et al.*, 1999; Kennedy *et al.*, 2002; Uhl *et al.*, 2007; Godefroit *et al.*, 2009; Tomsich *et al.*, 2010; Velasco-de Leon *et al.*, 2010; Jacques *et al.*, 2011; Srivastava *et al.*, 2012; Khan *et al.*, 2014; Shukla *et al.*, 2014). However, the multivariate approach has been rightly criticized because practitioners have employed geographically limited calibration data sets (Jordan, 2011).

To date, the largest number of sites used in training sets that employ multivariate leaf form–climate relationships with standardized sampling protocols is 189 (Jacques *et al.*, 2011). This data set covered mostly North America and Asia. A larger data set (1835 sites) was regionally restricted (Europe only) and

derived from floral manuals (Traiser *et al.*, 2005) rather than field sampling that approximates how leaves enter the fossil record. Other calibration sets have been smaller (175 sites) and represented a wider geographic range (Wright *et al.*, 2004), or smaller still (92 sites) and restricted to North America and the Pacific rim (Peppe *et al.*, 2011). A very small data set (17 sites) was used to argue that the long-established leaf margin–temperature relationship (Bailey & Sinnott, 1915, 1916; Wolfe, 1979) has significant phylogenetic bias (Little *et al.*, 2010). Enlarging sample size while maintaining standardized sampling is a focus of our work.

Beyond simply enlarging sample size, important questions remain. For example, have regional floras developed individualistic solutions to optimize performance under specific climate regimes (Little *et al.*, 2010; Jordan, 2011)? This would be true if: (1) lineages found different solutions to leaf function in differing climates and (2) lineages, and radiations within them, are distributed in a geographically non-random way. The latter is true, but widespread convergence in leaf form calls the former assumption into question. Alternatively, has the shared ancestral developmental programme of angiosperms led to shared leaf form–climate relationships that repeat across regions (Bailey & Sinnott, 1916)? If this is the case we would expect to see convergent patterns of multivariate leaf form–climate relationships across regions.

A large, globally distributed, standardized data set is needed to address these questions. We analyse such a data set to assess: (1) whether suites of characters covary as predicted by the phenotypic integration model of leaf form evolution; (2) whether variation in leaf form segregates regionally as predicted by the hypothesis that historical factors (e.g. vicariance or phylogenetic radiation) produced patterns in leaf trait variation; and (3) which characters and models produce the most accurate and precise correlations with temperature-related climate variables. In addition, we use these data to evaluate the structure of leaf physiognomic space and ask whether this structure suggests other possible analytical tools.

We focus specifically on temperature-related variables because: (1) these have been widely investigated; (2) they are of paramount interest in a warming world; and (3) the potential for leaf form to reliably reflect precipitation variables is complicated by the buffering of the soil water reservoir. Nevertheless, we provide results for moisture-related variables in the Supporting Information (see Figs S9–S14).

MATERIAL AND METHODS

Sampling

All methods that reconstruct climate using leaf form assume that the assemblage of species present in a given location is in equilibrium with its prevailing climate at a local scale (Gottfried *et al.*, 1999; Gehlausen *et al.*, 2000). Therefore, accuracy requires field sampling and not herbarium, floral list or other spatially homogenized samples (Wolfe & Uemura, 1999). This study uses only data sampled directly from the field. Only natural vegeta-

tion, minimally disturbed by human activity and unmodified by exotics, was sampled. All visible variants in leaf form within each taxon were scored, including those from early and mid-successional communities and reproductive and sterile shoots (Corney *et al.*, 2012).

Our data set (378 sites) represents all continents except Antarctica. Compared with previously published data sets it enlarges the range of vegetation and climate types analysed and incorporates sites in cosmopolitan, highly endemic and depauperate floras. By combining a large number of sites with different regional histories we look for emergent structure in the data above that produced by the stochastic distribution of lineages. A geographical summary of the vegetation types samples is provided in Fig. S1.

Leaf physiognomic data

The Climate Leaf Analysis Multivariate Program (CLAMP) (Wolfe, 1993; Kovach & Spicer, 1995; Yang *et al.*, 2011) is widely used for palaeoclimatic reconstruction (Gregory-Wodzicki, 2000; Spicer *et al.*, 2003; Uhl *et al.*, 2007; Godefroit *et al.*, 2009; Tomsich *et al.*, 2010). Digital leaf physiognomy (DLP) also uses multiple variables to reconstruct climate (Royer *et al.*, 2005; Peppe *et al.*, 2011). While DLP has been discussed at professional meetings (e.g. Janesko *et al.*, 2004; Royer *et al.*, 2004, 2008; Cariglino *et al.*, 2005; Peppe *et al.*, 2009), work published in a peer-reviewed context remains developmental (Royer *et al.*, 2005; Peppe *et al.*, 2011). We chose CLAMP for this analysis because it is the multivariate method that is most widely accessible, has been used for the longest time and has been most thoroughly tested.

We captured the leaf physiognomic spectrum for each vegetation site using standard CLAMP categorical character scores. Each taxon present at a site was scored for 31 categorical leaf character states including lobing, margin characteristics, size, apex and base form, length-to-width ratio and shape. Scores were combined for all taxa at each site resulting in a 378 site × 31 character data array, PhysgGlobal378, which is provided together with location data and vegetation types in Table S1. Collecting and scoring protocols are provided on the CLAMP website (http://clamp.ibcas.ac.cn).

Many of the taxa scored remain unidentified. Collections were made when some or all taxa were not in flower and/or in regions where the flora has not been completely documented with modern methods. This reality prohibits phylogenetic analysis of leaf form patterns across the entire data set. Phylogenetic study of appropriate subsets is a goal of future research.

Climate data

In CLAMP's early development, climate data were taken from the nearest available weather station (e.g. Wolfe, 1993; Forest *et al.*, 1999; Gregory-Wodzicki, 2000). This may reduce accuracy when stations are far from sample sites, microclimates are variable or elevation differs between station and sample site. To improve accuracy, we derived climate data (GRIDMetGlobal378_HiRes) from gridded 0.16° × 0.16° latitude/longitude resolution climate data, which were derived from a single sampling interval (1961–90) and corrected for instrument bias (New *et al.*, 2002). Climate data were interpolated and corrected for the exact vegetation sample site location and elevation (Spicer *et al.*, 2009). Climate data are presented in Table S1.

Analysis

To explore the relationships between leaf characters across all sites, we calculated pairwise Pearson correlation values for all character pairs.

We explore the relationship between leaf form and geographic location using cluster analysis of leaf form data. This analysis evaluates the hypothesis that vicariance and the evolution of regional endemism produced leaf form/climate patterns. Clusters were generated using a complete linkage agglomerative algorithm upon Euclidean distance.

To investigate the relationships among characters and climate we used canonical correspondence analysis (CCA) (ter Braak, 1986) to position each sample in multidimensional space based on its physiognomic signature. Sites with similar leaf form spectra plot together, while dissimilar sites plot apart. The cloud of sites in multidimensional space makes up 'physiognomic space'. Climate data for each site are used to generate and calibrate temperature-related vectors [mean annual temperature (MAT), warm month (WWMT) and cold month (CMMT) mean temperatures, length of the growing season (LGS) and enthalpy, Table 1] within physiognomic space.

Plotting vector scores (the positions of sample sites along a climate vector) against observed climate, we developed a regression model summarizing the leaf form–climate relationship for that climate variable. This relationship can then be used to convert a vector score for a fossil leaf assemblage into a retrodiction of palaeoclimate. Regression models are derived from positional data in axes 1–4 space.

We investigated the structure of physiognomic space using a generalized additive model (Wood, 2011) with an integrated smoothness estimation obtained from the mgcv package for R (R Core Team, 2012). This method generates an infinite number of multidimensional regression surfaces through physiognomic space. Where the surfaces are flat planes, stacked parallel to one another, the trend can be summarized by a simple linear vector. In a more complex physiognomic space, the surfaces are curved and can fold back on themselves to form tunnels. In such cases, vectors poorly represent overall trends. In the generalized additive model, surfaces may pass near, but not through, all sample points, which produces smoothing. Surface smoothness is determined by the proportion of samples in the local area that are used to define the surface. In this exploratory study, we used default options to determine the smoothness. To fit a smooth surface, CCA axes 1–4 are smoothed equally in all directions and are rotation invariant. This is achieved by penalized thin plate regression splines (see Wood, 2003, for details) in the multidimensional smoothings.

Table 1 Summary of regression statistics, which are R^2 and standard deviation (SD), for five climate variables: mean annual temperature (MAT), warm month mean temperature (WMMT), cold month mean temperature (CMMT), length of the growing season (LGS) and enthalpy. Models explored include all characters and the sequential removal of character suites. The probability that the relationships are the product of chance is extremely low for all regressions ($P < 2.2 \times 10^{-16}$).

	MAT (°C)	WMMT (°C)	CMMT (°C)	LGS (months)	Enthalpy ($\times 0.1$ kJ kg^{-1})
All characters					
R^2	0.72	0.44	0.64	0.67	0.73
SD	4.0	3.8	6.7	1.9	1.1
No margin					
R^2	0.59	0.38	0.52	0.57	0.64
SD	4.9	4.0	7.8	2.1	1.2
No size					
R^2	0.67	0.45	0.60	0.65	0.66
SD	4.3	3.8	7.1	1.9	1.2
No apex					
R^2	0.70	0.39	0.60	0.67	0.68
SD	4.2	4.0	7.1	1.9	1.1
No base					
R^2	0.72	0.44	0.65	0.67	0.71
SD	4.0	3.8	6.6	1.9	1.1
No L/W ratio					
R^2	0.72	0.43	0.64	0.68	0.71
SD	4.0	3.9	6.7	1.9	1.1
No shape					
R^2	0.70	0.43	0.61	0.67	0.73
SD	4.2	3.98	7.0	1.9	1.1

L/W ratio, length/width ratio.

RESULTS

Figure 1 shows the global distribution of the 378 sites included in this study. While this data set does not comprehensively sample all of the world's woody vegetation, it offers improved coverage compared with that of the most commonly used version of CLAMP (173 sites, mostly from the Northern Hemisphere). Figure S1 presents the percentages of vegetation types represented in the 378 sites. Vegetation classification followed that of Olsen *et al.* (2001).

Covariation of physiognomic character states

Pairwise Pearson correlation coefficients for leaf characters (Table S2) are visualized in Figure 2. Almost all leaf characters have statistically significant correlations with other characters (Table S2). Some correlations (e.g. 'no teeth' and all tooth characters) are trivial because of the mutually exclusive nature of such scoring categories. Other correlations reveal genuine patterns within the data. A pattern of linkages in which many lines cross between leaf character groups shows that leaf characters tend to change in coordination.

Analysis of historical factors

Cluster analysis of leaf form data groups samples with similar leaf form irrespective of geographic origin. Six clusters emerged (Fig. S2), with patterns related to latitude (Groups 1–4, 6), monsoon climates (Group 5) and altitude (Group 3). Adaptations to high altitude, which may be reflective of low temperatures, appear to override adaptations to monsoon climates in the same region. When plotted on a global map (Fig. 3), sites in Group 1 plot at about 40 °N latitude across the Northern Hemisphere. Sites in Group 2 tend to plot between 40 °N and the Arctic Circle. Sites in Group 3 plot at similar latitudes (40° and above) across both hemispheres, with the exception of a group of sites at elevation in China. Sites in Group 4 are distributed between about 20° and 40° in both hemispheres. Sites in Group 5 tend to straddle the tropics in both hemispheres, and sites in Group 6 are found in subtropical latitudes in both hemispheres. Highly endemic Gondwanan floras (e.g. South Africa and New Zealand) share trait combinations with each other and with other sites that are broadly distributed across the Northern Hemisphere. Taken together, this shows that the combinations of character states that produce these clusters have aggregated across widely separated regions, rather than grouping by region or floristic similarity as would be predicted if physiognomic patterns were produced primarily by vicariance or evolutionary history.

Accuracy and precision in temperature variable reconstruction

A strong, positive relationship between the proportion of entire margin species in the vegetation and MAT emerged (Fig. 4; $R^2 = 0.51$, SD = 5.3, $P < 0.001$). This result echoes previous studies with fewer samples (Wolfe, 1979; Wilf, 1997). As expected (e.g. Wolfe & Upchurch, 1987; Wolfe, 1993; Greenwood *et al.*, 2004; Wright *et al.*, 2004; Little *et al.*, 2010; Steart *et al.*, 2010; Jacques *et al.*, 2011; Peppe *et al.*, 2011; Kennedy *et al.*, 2014), differences in the slope and intercept of the regional regressions also appeared. The positive correlation between entire margin and MAT breaks down in New Zealand (Gregory-Wodzicki, 2000; Kennedy, 2001; Kennedy *et al.*, 2014) and in the cold climates (MAT < +4 °C) of Asiatic Russia (Siberia) (Spicer *et al.*, 2004) (Fig. 4). When cold Siberian sites were removed, the regression improved ($R^2 = 0.59$, SD = 3.8, $P < 2.2 \times 10^{-16}$).

Figure 5(a) shows the multivariate regression model for MAT. When all 31 leaf form variables are considered together, predictive power (as measured by R^2) increased by approximately 20% over the univariate regression (Fig. 4) and precision increased by over 25% as quantified by a reduction in standard deviation. In the multivariate models, regional variants, such as New Zealand and Siberia, conform more nearly to the pattern seen in other areas. Removing sites with MAT values below 4 °C also improved the regression statistics for MAT in the multivariate analysis ($R^2 = 0.73$, SD = 3.1, $P < 2.2 \times 10^{-16}$).

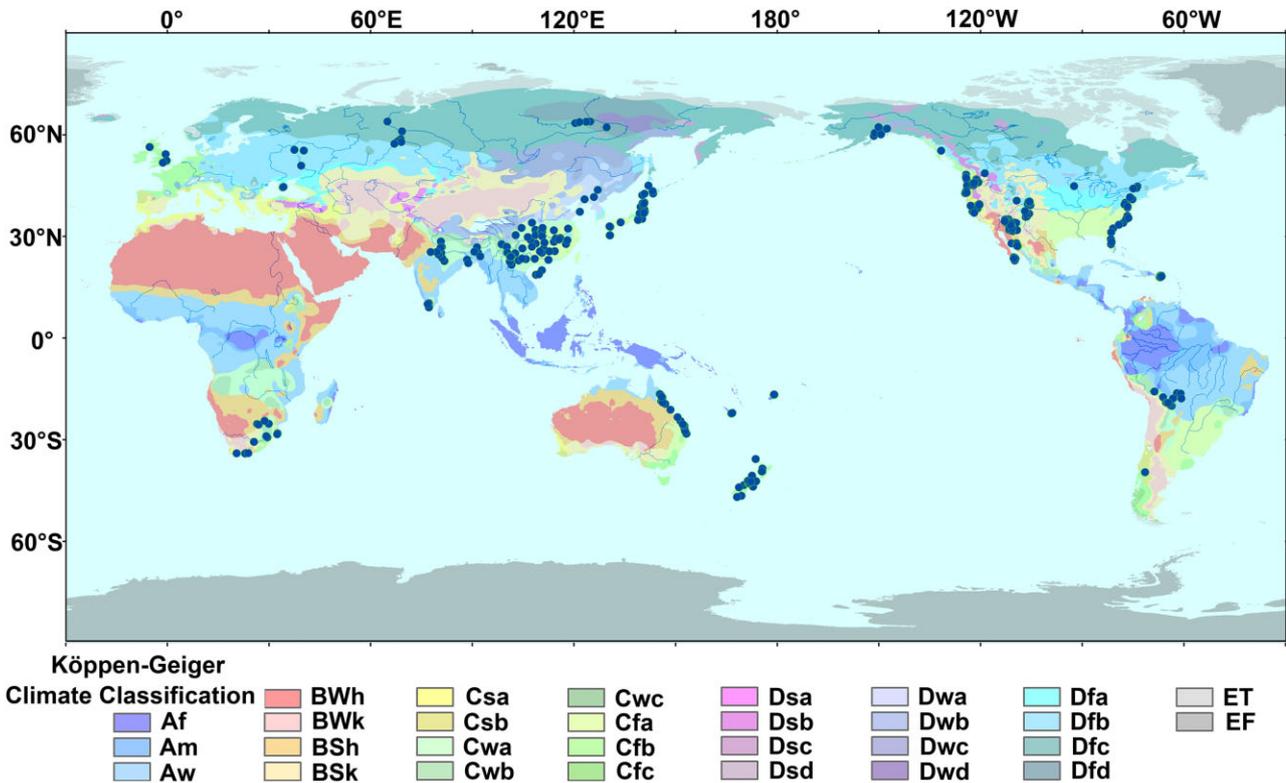Unlike leaf margin analysis, CLAMP returns temperature variables in addition to MAT. The WMMT plot (Fig. S6) shows

Figure 1 Map showing the distribution of 378 Climate Leaf Analysis Multivariate Program sample sites in relation to the Köppen–Geiger climate classification (Peel *et al.*, 2007). Samples are from vegetation growing in climates ranging from cool temperate to subtropical in both hemispheres, tropical to Taiga in the Northern Hemisphere, and dry (precipitation < 240 mm year⁻¹) to monsoonal (precipitation maximum of 6410 mm year⁻¹). A total of 355,942 character states were scored across 11,482 species occurrences. Sites averaged 30.4 species per site; the minimum number of taxa scored per site was 17 (three sites). Only 16 sites had fewer than the recommended 20 species per site. The first letter of Köppen–Geiger climate symbols represents main climate: A, tropical; B, arid; C, temperate; D, cold; E, polar. The second letter is precipitation: f (in Af), rainforest; m, monsoon; W, desert; S, steppe; s, dry summer; w, dry winter; f (in Cfa, Cfb, Cfc, Dfa, Dfb, Dfc and Dfd), without dry season; T, tundra and F, frost. The third letter is temperature: h, hot; k, cold; a, hot summer; b, warm summer; c, cold summer; d, very cold summer. Additional information on the included forest types is given as F ig. S1.

more scatter (SD = 3.9 °C) than the CMMT (SD = 6.7 °C) (Fig. S7). This is probably an artefact of a smaller range of temperatures over which WMMT (*c.* 24 °C) is measured compared with CMMT (*c.* 65 °C), which includes the very cold (*c.* −40 °C) Siberian CMMTs. Where the CMMT is above 10 °C the predictive power of leaf form diminishes and the regression trends towards horizontal.

In CLAMP the length of the growing season is the time in months when the average daily temperature is above 10 °C. The CLAMP regression (Fig. S8) shows Asiatic Russia plotting with the main trend but, with New Zealand, below the regression line.

Enthalpy, a thermodynamic property of the atmosphere, is used in palaeoaltimetry studies (Forest *et al.*, 1999; Spicer *et al.*, 2003) and is a function of both temperature and moisture:

$$H = C_p T + L_v q$$

where H is enthalpy, C_p is the specific heat capacity of air at a constant pressure, T is temperature, L_v is the latent heat of vaporization and q is specific humidity. The multivariate regression model for mean annual enthalpy is given in Fig. 5(b).

Of all the temperature-related variables in our analysis, MAT and enthalpy showed the strongest relationships with leaf form ($R^2 > 0.72$ when all characters are included). This pattern persists when individual character groups are removed (Table 1). Regression models for other climate variables are supplied in Figs S6–S14.

To test the extent to which the multivariate relationships between leaf form and climate depend on margin characters, we removed these (i.e. 'no teeth', teeth regular/irregular, teeth close/distant, teeth round, teeth acute, teeth compound) from the data set. Table 1 summarizes the regression statistics for MAT, WWMT, CMMT, LGS and enthalpy when different characters are removed. Even in the absence of all leaf margin information our analysis still produced a strong correlation between leaf form and MAT, enthalpy (Fig. 6a, b), CMMT and LGS (Figs S16 & S17), albeit with increased variance in all cases. Thus, non-margin characters also contain information on temperature-related climate variables. In the multivariate context, New Zealand sites plot close to the regression line, showing that temperature-related information is not coded by leaf margin in these floras but resides in non-margin characters.
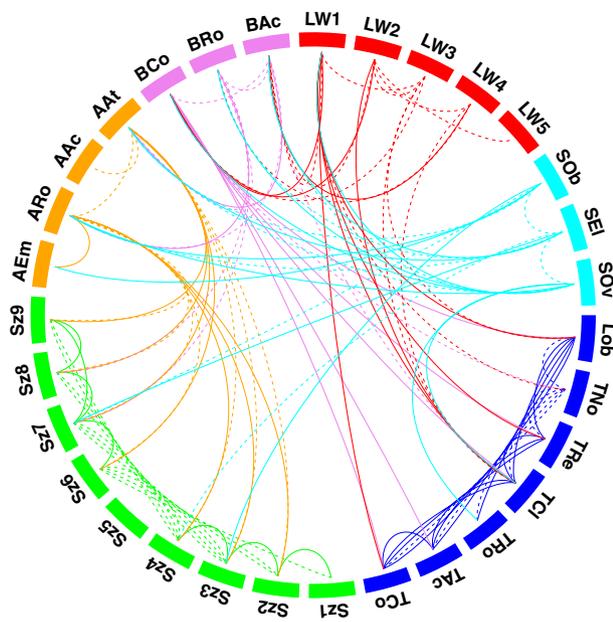
Figure 2 Linkage diagram illustrating the relationships between leaf characters shown by the pairwise Pearson correlation coefficients given in Table S2. The 31 leaf characters are grouped: lobing and tooth form, leaf size, apex form, base form, length-to-width ratio and shape. Leaf character groups are indicated by different colours: red, length-to-width ratios; light blue, leaf shape; dark blue, margin characters; green, leaf size; orange, apex characters; pink, base characters. The solid lines represent values of pairwise Pearson correlation above $\alpha \leq 0.5$ (a significant positive correlation), while the dashed lines indicate correlations $\alpha \geq -0.5$ (a significant negative correlation). Some correlations are trivial in that they arise from the scoring regime (e.g. 'no teeth' is negatively correlated with all tooth characters because leaves without teeth will not be scored for tooth characters) and are shown as links within leaf character groups. Meaningful correlations are those that link different character groups. For example the cordate base condition is positively correlated with several tooth characters. Abbreviations are as follows: Lob, lobing; TNo, no teeth; TRe, regularity of tooth spacing; TCl, closeness of teeth; TRo, teeth rounded and (or) appressed; TAc, teeth acute; TCo, teeth compound; Sz1, leaf size, nanophyll; Sz2, leaf size, leptophyll 1; Sz3, leaf size, leptophyll 2; Sz4, leaf size, micro 1; Sz5, leaf size, micro 2; Sz6, leaf size, micro 3; Sz7, leaf size, mesophyll 1; Sz8, leaf size, mesophyll 2; Sz9, leaf size, mesophyll 3; AEm, apex emarginate; ARo, apex round; AAc, apex acute; AAt, apex attenuate; BCo, base cordate; BRo, base round; BAc, base acute; LW1, length to width less than 1:1; LW2, length to width 1–2:1; LW3, length to width 2–3:1; LW4, length to width 3–4:1; LW5, length to width greater than 4:1; SOb, shape obovate; SEl, shape elliptic; SOv, shape ovate.

Structure of physiognomic space

Figures S3–S5 show CCA plots of physiognomic space. To test whether leaf form–climate relationships arise as artefacts of the CCA methodology, we substituted random numbers for the physiognomic scores in the data matrix. The results for all climate variables were similar to that for MAT (Fig. S15),

which shows no relationship. The absence of relationships in random data demonstrates that leaf form–climate patterns are products of structure within the data rather than a methodological artefact.

Compared with similar analyses of regional data sets, the 378 site compilation produces regression models with greater scatter and thus lower precision. This loss of precision suggests that linear climate vectors may not adequately reflect increased complexity in the global data set. We demonstrate this by plotting isotherms of WMMT onto the distribution of sites on axis 1 versus axis 2 of physiognomic space (Fig. 7). Hypothetical samples X and Y have the same WMMT vector score as indicated by the point (Z), where the line connecting them crosses the WMMT vector. However, sample X lies on the 24 °C isotherm while Y lies on the 20 °C isotherm. In this instance, a single vector score is associated with a 4 °C difference in WMMT because the physiognomic space is curved.

To better visualize the complex structure of physiognomic space, we plotted isopleth surfaces for MAT, enthalpy and WMMT (Figs 8, S18, & S19). MAT (Fig. 8) approximates the simplest condition where parallel plate-like surfaces are stacked normal to the MAT vector. This underlying structure may explain why the linear vectors used in CLAMP produce meaningful results. A small amount of curvature may also explain the discord in leaf form–MAT relationships in some regions. Enthalpy (Fig. S19) displays a similar stacked plate structure, which explains why both MAT and enthalpy exhibit small increases in uncertainty as samples sites are added. In contrast the WMMT plot (Fig. S18) shows a distinct curvature, which suggests that a simple vector will not adequately capture the climate trends present in physiognomic space.

Building on the suggestion that local regressions may offer better climate reconstructions for fossil leaf assemblages (Stranks & England, 1997), we used surfaces to predict climate for each modern vegetation sample. Figures 9, S20 & S21 compare the relationships between the predicted and observed MATs, WMMTs and enthalpies produced by conventional CLAMP (Figs 9a, S20a & S21a) and multidimensional local regression (Figs 9b, S20b & S21b). For MAT, multidimensional local regression increases predictive power by 10% (as summarized by R^2) and improves precision by 22% (as measured by SD). Similar improvements were seen with WMMT and enthalpy (Figs S20 & S21). Figure 9(a, b) also shows that multidimensional local regression better accommodates the leaf form patterns found in Asiatic Russia.

DISCUSSION

Distribution of sample sites

Expanding data sets for more complete biogeographic and climate coverage is a goal of leaf form–climate research. The original CLAMP data set included 106 sites, mostly from North America, Puerto Rico and Japan. Although it captured a wide range of climates it had limited geographic range. Subsequently, regional floras outside the Holarctic floristic province (e.g.

Figure 3 Map showing the locations of samples divided into six groups based on cluster analysis of leaf physiognomic data (PhysgGlobal378) alone. Clusters were calculated using a complete linkage agglomerative algorithm with Euclidean distance similarity metric. Clusters show a symmetrical distribution about the equator and primarily reflect latitude and thus climate.
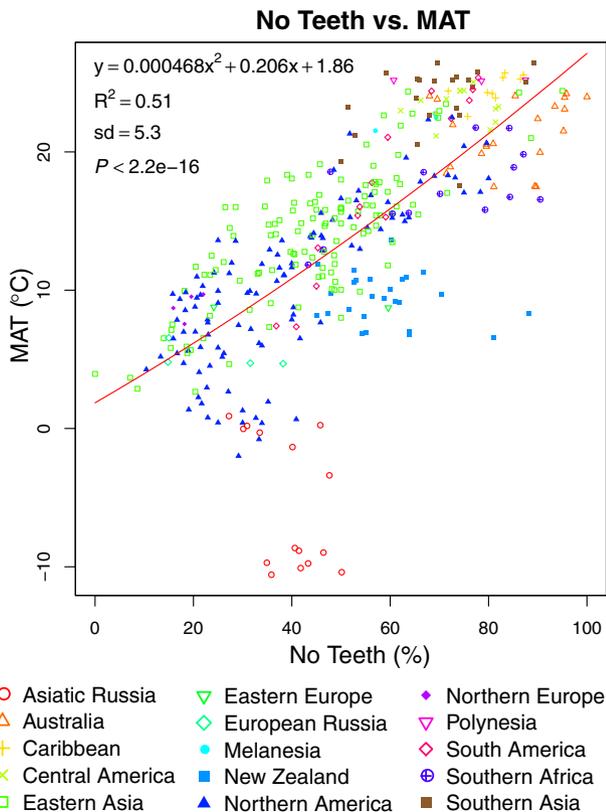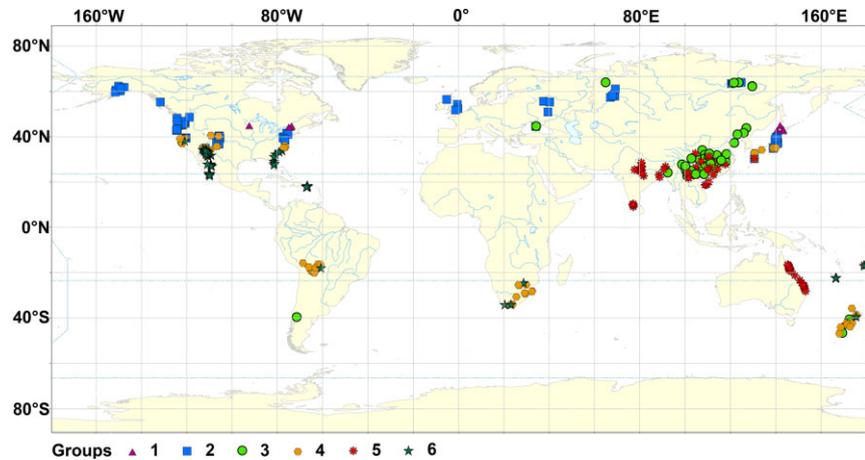

Figure 4 Percentage of entire margined leaves for each vegetation site plotted against mean annual temperature (MAT). Note that here MAT is the dependant variable. This reflects the historical use of this relationship in leaf margin analysis (Wolfe, 1979; Wilf, 1997) to infer MAT from the measured proportion of leaf species without teeth found in a fossil assemblage.

endemic floras from Australia and New Zealand. That these floras conform to patterns observed in the Holarctic shows that there are meaningful patterns in the response of leaf form to climate that are shared among woody dicots, whether the result of symplesiomorphy or convergence.

Despite significant increases in geographic coverage provided by these new data, Fig. 1 reveals regions that remain poorly sampled. Most notable are voids in the wet, tropical equatorial regions of Africa, South America and Oceania. Subtropical regions of Central and South America are also poorly covered, as are high latitudes in Australia and South America. The depauperate floras of Europe and Central Asia are also poorly represented in the current data set. An emphasis of future work will be to fill in these remaining gaps.

A second focus of leaf form–climate research is the visualization of physiognomic space through improved computational techniques. In his original analysis, Wolfe (1993) aligned vectors for the first two correspondence analysis axes by eye, which limited the accuracy and complexity of the relationships that could be applied. Kovach & Spicer (1995) used CCA to regress climate variables onto physiognomic space, improving the objectivity and precision of the physiognomy/climate relationships, and the complexity of the relationships that could be explored. Subsequently, Spicer and colleagues (e.g. Spicer *et al.*, 2003) developed regression models for four-dimensional space. Our work takes an additional step forward by exploring curvature within physiognomic space.

Correspondence analysis of the dominantly Holarctic data set (e.g. Wolfe, 1993) revealed large lacunae in physiognomic space. This pattern could mean that significant discontinuities exist in the response of leaf form to climate. Such disjunct distributions in physiognomic space would be predicted if evolution had explored a variety of solutions to optimize function under diverse climates. However, the addition of more samples from different regions has now filled those lacunae to produce a continuous and overlapping distribution of sites. Notable exceptions are samples from extremely cold climates of Siberia (Figs 4–6 & 9a), which consistently plot apart from other regions. The Siberian samples may actually represent an alternative solution to the leaf form–climate problem. However, the solution is not marked by the
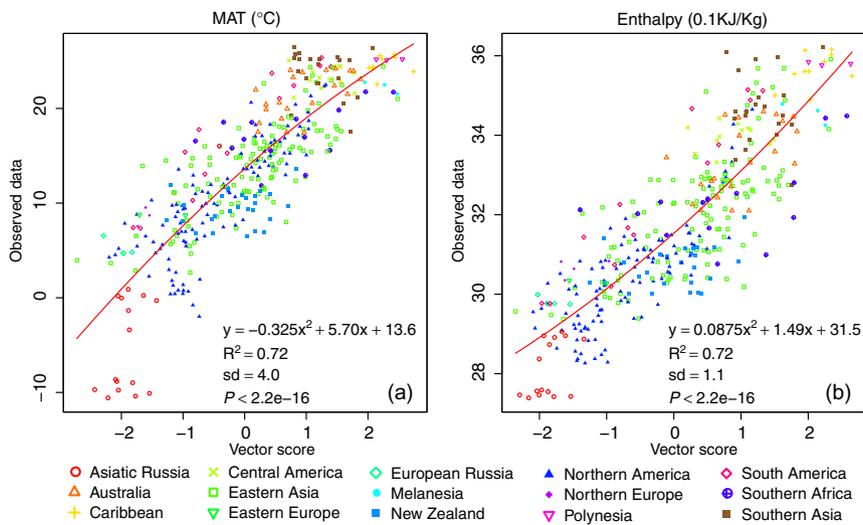
southern Africa; Steart *et al.*, 2010) and those experiencing different climate patterns (e.g. Asian Monsoon; Jacques *et al.*, 2011; Srivastava *et al.*, 2012) were added. Our work contributes many new sites from the Southern Hemisphere including Australia, New Zealand and a handful of sites from South America. These enhance representation of the Gondwanan flora and add
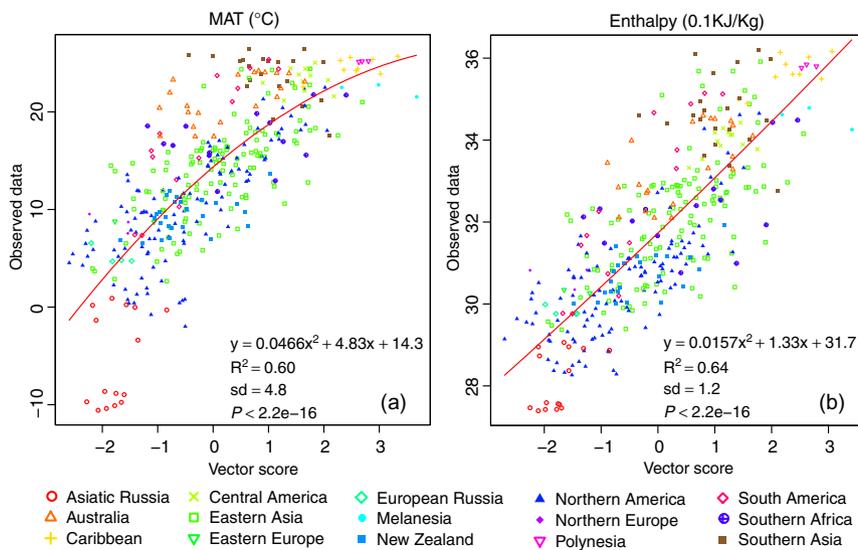
Figure 5 (a) Mean annual temperature (MAT) and (b) enthalpy. Climate Leaf Analysis Multivariate Program (CLAMP) regression models using all 31 leaf character states. The climate observed for a given sample is derived from $0.16° \times 0.16°$ latitude/longitude global gridded data (New *et al.*, 2002) interpolated for the location of the sample site and corrected for elevation (Spicer *et al.*, 2009). The vector score of a sample is its position along the climate vector aligned in four-dimensional space (canonical correspondence analysis axes 1–4). Note that as for Fig. 4 observed climate is the dependent variable reflecting the historical use of leaf physiognomy to infer climate parameters.

Figure 6 (a) Mean annual temperature (MAT) and (b) enthalpy. Climate Leaf Analysis Multivariate Program (CLAMP) regression models with all leaf margin (no teeth, teeth regular/irregular, teeth close/distant, teeth round, teeth acute, teeth compound) character states removed, otherwise as for Fig. 5. Although the standard deviations (sd) are larger than those generated by the full data set, the relationships between the remaining (non-margin) characters and MAT and enthalpy remains similar to that shown in Fig. 5. The same is true for the removal of other characters suites with respect to all 11 climate variables used in this study.

presence of endemic taxa. Siberian samples are rich in genera such as *Ribes*, *Vaccinium*, *Salix* and *Alnus* that are common to cool temperate sites throughout the Holarctic. Therefore, differential distribution of taxa alone cannot explain the variation in these leaf form–climate relationships.

New sites added in this analysis reveal that disparate regions (e.g. Australia, North America and eastern Asia) plot in broadly overlapping clouds (Figs S3–S5), suggesting a continuum of leaf form across hemispheres, latitudes and climatic zones. Moreover, cosmopolitan Northern Hemisphere sites plot in continuum with isolated, endemic Gondwanan floras (e.g. South Africa, New Zealand and Australia), suggesting that the evolutionary roots of the characters expressed in this analysis are shared deep in the history of dicots. Cluster analysis provides additional support for this interpretation. Latitudinal clusters mirror each other on opposite sides of the equator, even when the floras themselves have been out of evolutionary communi-

cation for many millions of years. This provides strong evidence that commonalities of environment – particularly climate – drove broad convergence in leaf form even in floras that are widely separated by both space and time.

The endemic flora of New Zealand provides an interesting example. Although debate continues about the origin and antiquity of the New Zealand flora (e.g. Pole, 1994; Cooper & Cooper, 1995; Stöckler *et al.*, 2002; Knapp *et al.*, 2005, 2007; Landis *et al.*, 2008) it remains among the most isolated in the world and has evolved, at least since the Oligocene (*c.* 34 Ma), under the unusual circumstance of a selective landscape free of terrestrial mammals. In univariate analysis, the New Zealand flora lacks the typical positive relationship between proportion of entire-margin species and MAT (Gregory-Wodzicki, 2000; Kennedy, 2001; Kennedy *et al.*, 2014), suggesting that these lineages have explored an alternative solution to optimizing function in a given climate. However, when all leaf form characters are con-
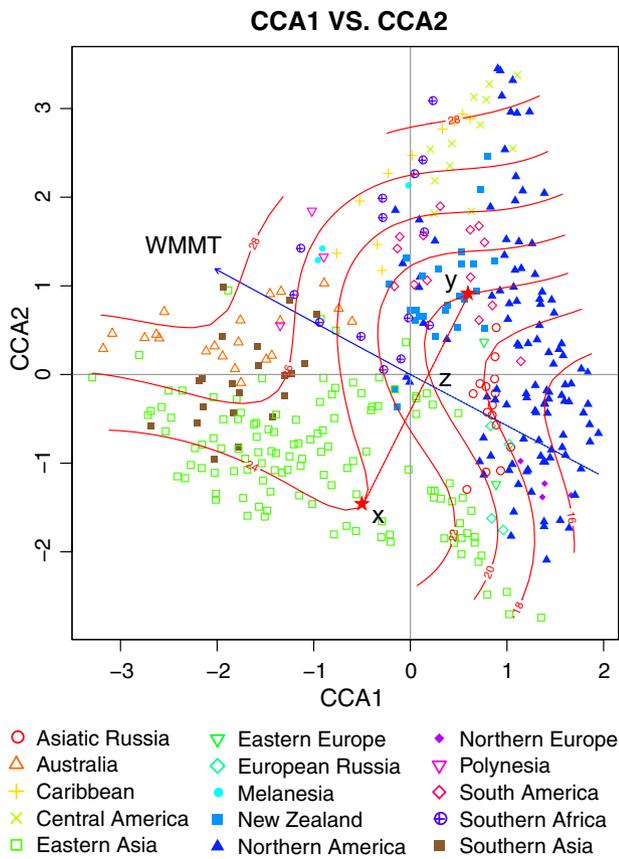
CCA1 VS. CCA2

sidered, New Zealand sites cluster with Holarctic and other regional data sets of similar latitude and climate. This shows that single characters may experience variation because of colonization and *in situ* evolutionary radiation, but that the underlying integrated response to climate emerges in multivariate analysis.

Distribution of characters

In developing CLAMP, Wolfe (1993) assumed that climate response was encoded in many leaf characters that varied in coordination as lineages adapted to climate. The idea that correlations among characters vary with environmental conditions is not new (e.g. Marshall *et al.*, 1986; Schlichting, 1989a; Stearns *et al.*, 1991). Moreover variation among traits may be functionally integrated (Schlichting, 1989b; Pigliucci, 2003). In our analysis, some correlations among characters are spurious, whilst others contain information. For example, we observed strong, positive correlations among lobing, closely spaced teeth and compound teeth with a length-to-width ratio of less than 1 (Table S2). Similarly, ovate shape is negatively correlated with acute apices and elliptic shape with round apices. Some relationships may be constrained by leaf developmental programmes, whilst others may be functionally linked. In either case, the
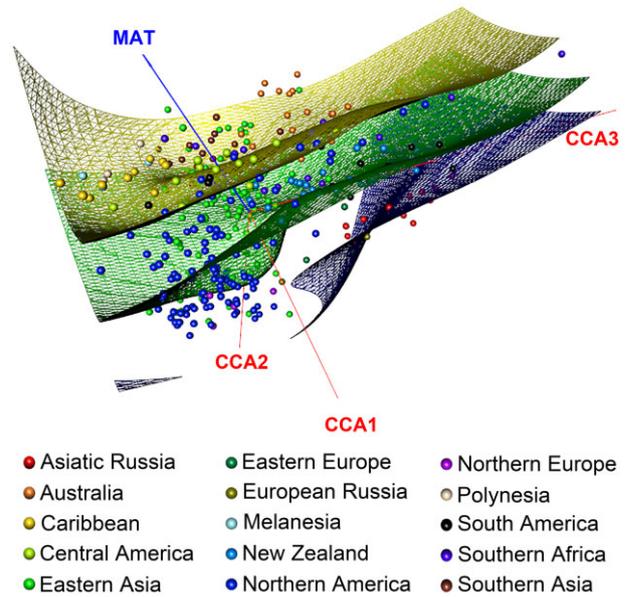
integrated nature of leaf form would suggest that a change in one character will produce changes in others. This integration implies that individual character–climate correlations may be less stable than relationships derived from many characters in combination. However, our analysis cannot distinguish among three working hypotheses for the origin of the observed integration: (1) climate information is encoded directly by non-margin characters through some unexplored functional mechanism; (2) non-margin characters covary with margin (perhaps through as yet undescribed developmental linkages) in ways that allow the temperature parameters to emerge from non-margin data; and (3) non-margin characters correlate with other climate variables that, in turn, are related to temperature and humidity by the laws of thermodynamics.

Improvements in analytical approach also further our understanding of the integrated leaf/environment relationship. Greater scatter in the global regressions compared with regional data seems to be due to the increased complexity of physiognomic space. Greater precision may be obtainable by using local regressions (Stranks & England, 1997) within the global data set. The trade-off is uncertainty in the choice of local calibration for a given ancient vegetation and climate regime.

Reconstruction of temperature variables

Taken together, these results highlight four important points. First, climate information encoded in leaf form is an emergent property of multiple characters changing in coordination. There-
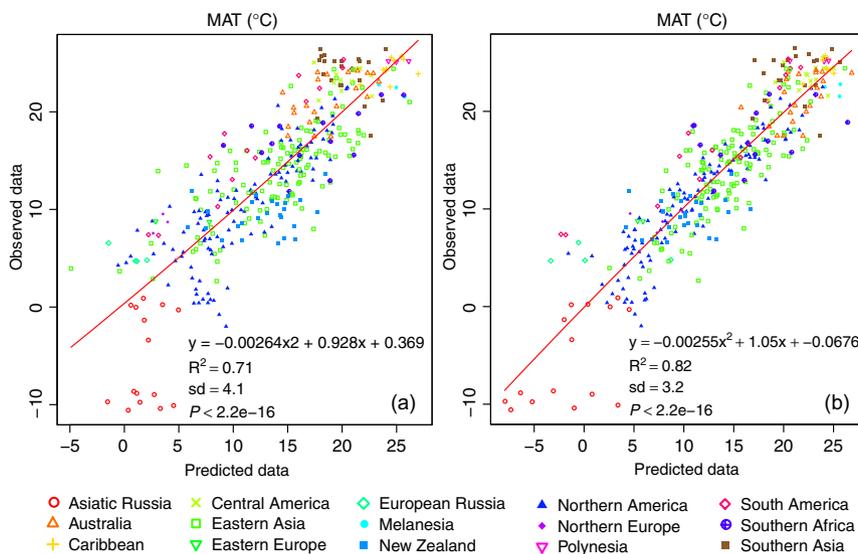
Figure 9 Mean annual temperature (MAT) predictions with multidimensional local regression. The relationship between the predicted and observed MAT (a) with a conventional vector-based Climate Leaf Analysis Multivariate Program analysis and (b) with multidimensional local regression. Using the surfaces generated by multidimensional local regression to predict MAT improves both accuracy and precision over the vector approach.

fore, mechanisms underlying leaf form–climate relationships must consider multiple characters. Some mechanistic hypotheses have linked margin type and temperature (e.g. Baker-Brosh & Peet, 1997; Feild *et al.*, 2005; Royer & Wilf, 2006), but they have not yielded convincing relationships that apply across taxa. Our results suggest that single-trait studies are unlikely to reveal the mechanisms that connect leaf form, function and climate. Second, fossil leaf assemblages commonly preserve species that are represented only by incomplete specimens. The ensemble nature of the leaf form–climate relationship means that the loss of individual characters does not undermine the utility of the resulting palaeoclimate reconstruction methods (Spicer & Yang, 2010). Third, continuity of leaf form–climate relationships across all the regions sampled so far provides a more secure basis for the application of this approach to reconstructing ancient climates using leaves from extinct taxa. And fourth, the development of new analytical methods such as multidimensional local regressions will increase accuracy and precision by exploiting complexities in physiognomic space.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Figure S1 Pie chart showing the percentages of different vegetation types represented in the 378 foliar physiognomic sampling sites used in the analysis.

Figure S2 Cluster analysis tree of CLAMP vegetation sites using leaf physiognomic data (PhysgGlobal378).

Figure S3 Canonical correspondence analysis plot showing vegetation sites positioned on axes 1–2 space using their physiognomic spectrum based on 31 leaf characters.

Figure S4 Canonical correspondence analysis plot showing vegetation sites plotted in axes 2–3 space using 31 leaf characters.

Figure S5 Canonical correspondence analysis plot showing vegetation sites plotted in axes 1–3 space using 31 leaf characters.

Figure S6 Warm month mean temperature regression model using 31 leaf characters.

Figure S7 Cold month mean temperature regression model using 31 leaf characters.

Figure S8 Length of the growing season regression model using 31 leaf characters.

Figure S9 Growing season precipitation regression model using 31 leaf characters.

Figure S10 Mean monthly growing season precipitation regression model using 31 leaf characters.

Figure S11 Precipitation during the three consecutive wettest months regression model using 31 leaf characters.

Figure S12 Precipitation during the three consecutive driest months regression model using 31 leaf characters.

Figure S13 Relative humidity regression model using 31 leaf characters.

Figure S14 Specific humidity regression model using 31 leaf characters.

Figure S15 Regression model for mean annual temperature when all observed leaf character scores are replaced by random numbers.

Figure S16 Cold month mean temperature regression model excluding all leaf margin characters (25 characters states).

Figure S17 Length of the growing season regression model excluding all leaf margin characters (25 characters states).

Figure S18 Isotherm surfaces for warm month mean temperature in physiognomy/climate space.

Figure S19 Isotherm surfaces for enthalpy in physiognomy/climate space.

Figure S20 Warm month mean temperature predictions with CLAMP and multidimensional local regression.

Figure S21 Enthalpy predictions with CLAMP and multidimensional local regression.

Table S1 Site locations, altitude, physiognomic scores, climate data and regions.

Table S2 Pairwise Pearson correlations between leaf characters.

BIOSKETCH

Jian Yang is a research scientist in the Institute of Botany, Chinese Academy of Sciences. He studies historical climatic changes, plant evolution and vegetation succession based on fossils, and the relationships between modern leaf characters and climate.

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