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RESEARCH PAPER

Leaf margin analysis of Chinese woody plants and the constraints on its application to palaeoclimatic reconstruction

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ABSTRACT

Aim Leaf margin states have been found to be strongly related to temperature, and hence have been used to reconstruct palaeotemperatures. Here, we aimed to explore the uncertainties and conditions of use of this technique in China by testing the influences of plant life-form, precipitation and evolutionary history on the relationship between percentage of untoothed species and temperature.

Location China.

Methods Using distribution maps and leaf margin states (untoothed versus toothed) of 10,480 Chinese woody dicots and dated family-level phylogenies, we evaluated the phylogenetic signal of leaf margin state, and demonstrated the variation in the patterns of leaf margin percentage and the relationship with temperature across different life-forms (evergreen and deciduous; trees, shrubs and lianas), regions with different precipitation and species quartiles with different family ages.

Results Significant phylogenetic signals were found for the percentage of untoothed species within families. Relationships between leaf margin percentage and temperature were: (1) weak or insignificant for all woody dicots, shrubs, evergreen and deciduous dicots, but strong for trees and lianas; (2) significantly enhanced with increasing precipitation, and (3) significantly weakened for trees belonging to old families.

Main conclusions Our results showed the complete leaf margin spectrum found in China and revealed great uncertainties in its relationship with temperature induced by life-form, precipitation and evolutionary history. These findings suggest that analysis of leaf margins for palaeotemperature reconstruction should be done cautiously: (1) only dicot trees with a relatively young family age can be used and their leaf margin states are more strongly affected by winter cold than by mean annual temperature; (2) the transfer function between leaf margin percentage and temperature is only reliable in humid and semi-humid regions of China.

Keywords
Chinese woody dicots, family age, leaf margin, life-forms, palaeoclimate, phylogeny, temperature.

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INTRODUCTION

The morphological traits of plant leaves and their relationship with the environment is a central topic in plant ecology. Leaf morphological traits, such as leaf margin and leaf size, can directly influence the physiological and biochemical processes of plants (Feild *et al.*, 2005; Royer & Wilf, 2006; Ordoñez *et al.*, 2009) and are therefore indicators of the adaptation of the plant to climate (Baker-Brosh & Peet, 1997; Vendramini *et al.*, 2002). Because leaf morphological traits are usually strongly correlated with climate, they are frequently used for palaeoclimatic reconstruction (Wolfe, 1979; Wilf, 1997; Jacobs, 1999; Kowalski, 2002; Greenwood *et al.*, 2004; Peppe *et al.*, 2011; Chen *et al.*, 2014).

Of the various leaf morphological variables, leaf margin state (toothed versus untoothed) has been found to be strongly related to temperature, and hence this relationship has long been studied by botanists and palaeoecologists (Wolfe, 1979; Peppe *et al.*, 2011; Chen *et al.*, 2014). As an important leaf trait, the teeth of leaves affect plant functions directly or indirectly. First, leaf teeth influence leaf venation networks (Givnish, 1978), and shed water by guttation to release excess root pressure, hence affecting the efficiency of water transportation within leaves (Canny, 1990; Feild *et al.*, 2005). Second, leaf teeth are the energy exchange 'hotspots' of leaves. The open pores or hydathodes on toothed leaves can thin the boundary layer of leaves and enhance the rate of sap flow (Canny, 1990), thus improving gas exchange, allowing sufficient carbon to be obtained early in the growing season (Baker-Brosh & Peet, 1997; Royer & Wilf, 2006). Toothed leaves can also act as defensive structures to reduce herbivory (Brown *et al.*, 1991; Royer & Wilf, 2006). Due to the strong influence of leaf teeth on leaf physiology, the percentage of species with untoothed leaves has long been used as an indicator of local temperature (Bailey & Sinnott, 1915; Bailey & Sinnott, 1916). Indeed, previous studies found positive correlations between the percentage of untoothed dicot species and mean annual temperature in many regions, and transfer functions based on this relationship have been proposed to reconstruct palaeotemperature for many different regions: North America (Wolfe, 1979; Dolph & Dilcher, 1980; Wilf, 1997; Adams *et al.*, 2008), South America (Kowalski, 2002), southern Europe (Traiser *et al.*, 2005), tropical Africa (Jacobs, 1999), East Asia (Wolfe, 1979; Su *et al.*, 2010; Chen *et al.*, 2014), Australia (Greenwood *et al.*, 2004; Greenwood, 2005) and globally (Peppe *et al.*, 2011). However, the slopes and the quality of the fit of proposed transfer functions varies across different regions (Greenwood *et al.*, 2004; Adams *et al.*, 2008; Peppe *et al.*, 2011), limiting the generalizability of those models. The drivers of the variation of transfer functions remain controversial. Moreover, the conditions of leaf margin analysis (LMA) have rarely been explored quantitatively using systematic data at large spatial scales. Therefore, investigations of the response of leaf margin states to temperature and the underlying impact factors are needed to improve its application to palaeotemperature reconstruction.

It has been reported that plants with different life-forms adopt different environmental adaptations associated with leaf traits (Cornelissen *et al.*, 1999; Campanella & Bertiller, 2008), and may respond differently to environmental stress (Wright, 1992; Pausas & Austin, 2001). Therefore, species with different life-forms may differ in their leaf margin state and its response to environmental temperature, suggesting that life-form can cause uncertainties in LMA. For example, previous studies have indicated different leaf margin–temperature relationships for woody, herbaceous and ferny species. The leaf margin states of woody plants usually respond effectively to the macroenvironment because of the longer lifespan of such plants (Traiser *et al.*, 2005), whereas those of herbaceous and ferny species are often less affected by climate due to their distinct hydraulic conductivity and leaf venation networks (Royer *et al.*, 2012; Peppe *et al.*, 2014). Similarly, Bailey & Sinnott (1916) found different percentages of entire-leaved species among trees, shrubs and herbs, and these percentages varied in tropical and temperate regions. More recently, Royer *et al.* (2012) showed that the relationship between leaf margin state and temperature might differ between canopy and subcanopy plants, and this caused noise in the calibration of palaeotemperature. However, differences in the geographical patterns of leaf margin state and their relationship with climate across woody plant life-forms have rarely been evaluated in eastern Asia (Royer *et al.*, 2012).

Moreover, a recent study suggested that precipitation, interacting with temperature, could influence leaf physiognomic traits (Adams *et al.*, 2008; Peppe *et al.*, 2011). With limited data, Wolfe (1993) found that dry microthermal and subalpine samples behaved as outliers in LMA compared with samples from wet areas. Given its ecological function, leaf margin state is strongly related to the availability of water. In humid and semi-humid regions with sufficient precipitation, leaf margin state may respond primarily to temperature (Adams *et al.*, 2008), leading to a strong relationship between the percentage of untoothed species and temperature. In contrast, drought in arid regions may weaken the thermal impacts on leaf margins through adaptive changes in leaf size or type (Parkhurst & Loucks, 1972; Givnish & Vermeij, 1976). Therefore, quantifying how the interaction between temperature and precipitation influences leaf margin state could help to reduce the uncertainties in palaeoclimate reconstructions based on LMA.

Leaf traits related to leaf margin indicate strong niche conservatism (Jordan, 1997), suggesting that evolutionary history may play a significant role in shaping patterns of leaf margin percentage (Dolph & Dilcher, 1980; Ackerly, 2004; Schmerler *et al.*, 2012) and its relationship with temperature (Jordan, 1997, 2011; Little *et al.*, 2010). Using 17 sampling sites in North America, Little *et al.* (2010) found significant phylogenetic signals in leaf margin traits, and demonstrated that uncertainty in LMA increased after accounting for the influence of evolutionary history on leaf margin states. Similarly, Jordan (2011) argued that uncertainties in the relationship between leaf margin and temperature might be time dependent and would be increasingly high for deep geological time. However, the effects of macroevolution on the leaf margin–temperature relationship have not been evaluated in eastern Asia.

China bridges climatic zones from tropical to subarctic and from humid to arid regions and is rich in woody flora (Fang *et al.*, 2011); it therefore provides a representative area for the study of leaf morphology and its relationship with climate. Up to now, only a few studies have explored the relationship between leaf margin and temperature in eastern Asia, and the proposed transfer functions are mainly based on incomplete or outdated databases from eastern China (Wolfe, 1979; Su *et al.*, 2010; Chen *et al.*, 2014). Limited species lists and restricted spatial coverage mean that these studies hardly reflect the complete leaf morphological spectrum in China and its relationship with temperature. Therefore, it is vital to explore leaf margin conditions of woody dicots with an improved database.

In this study, we studied the percentages of untoothed species using a morphological database of 10,480 Chinese woody dicotyledons. We aim to evaluate: (1) the geographical patterns of leaf margin percentages and their relationships with environmental temperature; (2) the influences of life-form, precipitation and evolutionary history on those relationships; (3) the conditions for using leaf margin state for reconstruction of palaeotemperature.

MATERIALS AND METHODS

Leaf margin data of Chinese woody dicotyledons

Previous studies on leaf margin percentage have mainly focused on dicotyledons because most monocotyledons have untoothed leaves. For comparison with previous studies, we only analysed leaf margin data for dicotyledons. We first established a species list of all native woody dicots in China (10,480 species in total). Then, we obtained the leaf margin state for each species from several data sources, including *Flora of China* (http://www.efloras.org/flora_page.aspx?flora_id=2), the Chinese Virtual Herbarium (CVH, http://www.cvh.org.cn/) and recent publication on leaf margins (Chen *et al.*, 2014). From these sources, we also obtained the maximum height, endemism in China and life-form (trees, shrubs versus lianas; deciduous versus evergreen) of each species. The data on species endemism were also supplemented by Huang *et al.* (2012). The scientific species names in different data sources were standardized according to *Atlas of woody plants in China: distribution and climate* (Wang *et al.*, 2009; Fang *et al.*, 2011). Leaf margin states for compound leaves are defined as those of their leaflets. Leaf margin information for varieties and subspecies was inferred from their protospecies when there were no clear records in any of the data sources. The final database contained data on leaf margin states for 10,151 out of the 10,480 woody dicots (97%).

In our analysis, trees are defined as woody species with main stems or trunks and heights of >5 m, and shrubs as those without main stems (for more details see Fang *et al.*, 2012). As recent studies on LMA in China (Adams *et al.*, 2008; Chen *et al.*, 2014) used trees and large shrubs (defined as shrub species with a maximum height >3 m), we reclassified all woody dicots accordingly for comparison. In the following analysis, trees and large shrubs together were called 'large woody dicots' for short. Finally, our database contains: (1) 2939 trees, 4934 shrubs (among which 3025 are large shrubs), 913 woody lianas and 1694 species with multiple life-forms; (2) 3475 deciduous, 4476 evergreen species and 2529 species with multiple leaf habits. In addition, 5964 large woody dicots were included, and 5832 of those have leaf margin information. Moreover, 5612 woody dicots were identified as endemic species and 5495 of those have leaf margin information.

The distribution of Chinese woody dicotyledons

We obtained species distribution maps from the *Atlas of woody plants in China: distribution and climate* (Wang *et al.*, 2009; Fang *et al.*, 2011). The species distributions in this atlas were compiled from all national-level floras published before 2009, a great number of provincial floras, local floras and inventory reports across the country. Then, 21 Chinese botanists were invited to check and supplement each species distribution in every region (for more details on species distribution data see Wang *et al.*, 2009, 2011). To eliminate the influence of area, we transformed the distribution maps into equal-area grids with a size of 50 km × 50 km.

Climate data

We selected two temperature variables, mean annual temperature (MAT) and mean temperature of the coldest quarter (MTCQ). Although MAT was previously used in LMA, recent studies have shown that MTCQ is the major determinant of species richness of woody plants (Wang *et al.*, 2011) and the leaf margin percentage of dicot trees (Chen *et al.*, 2014) in China. The effects of coldness on leaf margin percentage support the 'gas-exchange hypothesis' that toothed leaves were an adaptation to cold climate to accelerate carbon uptake (Baker-Brosh & Peet, 1997; Royer & Wilf, 2006).

Previous studies indicated that toothed leaves are not only an adaptation to cold climate but also important for releasing the stress of intracellular flow (Feild *et al.*, 2005). As a result, water availability may change the relationship between percentages of untoothed species and temperature. In our analysis, we used mean annual precipitation (MAP) to represent regional water availability. In China, the 400-mm isoline of MAP is the boundary of semi-arid and semi-humid regions and that of forests and grasslands. We used this isoline to evaluate the influence of precipitation on the response of leaf margins to temperature.

Climatic data with spatial resolution of 1 km × 1 km were obtained from the WorldClim website (http://www.worldclim.org/) (Hijmans *et al.*, 2005). The value for each 50 km × 50 km grid cell was calculated as the average of all 1 km ×

1 km cells within it. MAP was logarithmically transformed to account for its right-skewed distribution.

Phylogeny data

We obtained two dated family-level phylogenies from recent publications (Davies *et al.*, 2004; Zanne *et al.*, 2014). The phylogeny from Davies *et al.* (2004) included 443 angiosperm families and has been widely used in previous studies on large-scale patterns of species diversity (e.g. Hawkins *et al.*, 2011). The phylogeny from Zanne *et al.* (2014) contained over 31,000 species from 438 angiosperm families, and its family backbone was extracted.

To match with the tips of these two phylogenies, the family names of our database were adjusted following the Angiosperm Phylogeny Website (http://www.mobot.org/MOBOT/research/APweb/). The family ages were defined as their stem ages, i.e. the time when a family diverged from its sister clade, and were extracted from the two phylogenies separately. Then, all species were sorted following their family ages and divided into four quartiles (Q1, Q2, Q3 and Q4) ranging from the youngest to the oldest. All the following phylogenetic analyses were repeated with the two phylogenies, and the results were generally consistent. Therefore, we included the results based on the phylogeny of Davies *et al.* (2004) in the main text and those based on the phylogeny of Zanne *et al.* (2014) as an appendix (Appendix S5 in the Supporting Information).

Data analysis

In this study, a leaf tooth is defined as a projection along the leaf margin that usually contains vasculature, and having a length within 25% of the length from the midvein to the projection (Ellis *et al.*, 2009). When this length is greater than 25% the projection is called a lobe. Species with toothed leaves were given a score of 0, while those with untoothed leaves were scored with a 1. If a species has both toothed and untoothed leaves (in an individual plant or different individuals of the species), its score is 0.5 (Wolfe, 1993). Teeth with thorns were excluded because of the special function of thorns.

Linear mixed-effects models (LMMs) were used to evaluate the influence of life-form and family age on the relationship between leaf margin state and temperature at species level. First, for each species we estimated the mean temperature within its distribution range ($\mathrm{MAT}_{\mathrm{sp}}$). Then we conducted LMMs using the leaf margin scores of all species as the dependent variable, $\mathrm{MAT}_{\mathrm{sp}}$ as the fixed effect and life-form (tree, shrub or liana) or family age quartile (i.e. Q1, Q2, Q3 and Q4) as random effects. Both random intercept models and random slope models were performed to test the effects of $\mathrm{MAT}_{\mathrm{sp}}$ on the leaf margin score. ANOVAs were used to compare different models and the one with the lowest Akaike information criterion was chosen as the best. As some woody dicots are not endemic to China, which will bias the estimation of $\mathrm{MAT}_{\mathrm{sp}}$, this analysis was repeated for all dicots and endemic woody dicots separately. LMMs were conducted using the lme4 package (Bates *et al.*, 2012) in R.

To evaluate the phylogenetic signal of leaf margin, we first calculated the percentage of untoothed species for each family, and then estimated Blomberg's K (Blomberg *et al.*, 2003) and Pagel's λ (Freckleton *et al.*, 2002) for all woody dicots, trees, shrubs and lianas separately. As polytomies tend to lead to flatter phylogenetic signals (Davies *et al.*, 2011), we estimated the phylogenetic signal of leaf margin by the family-level phylogeny rather than inserting all species as polytomies. Two R packages, phytools (Revell, 2012) and phylosignal (Keck *et al.*, 2016), were used and both generated similar results for phylogenetic signal (Appendix S5).

By combining species distribution and the leaf margin scores of each species, we calculated the percentage of untoothed species as the sum of the leaf margin scores of all eligible species within each grid cell divided by the corresponding species richness in that grid cell. The percentage of untoothed species was calculated separately for all woody dicots, and then for those with different life-forms (trees, shrubs, lianas; deciduous and evergreen dicots), respectively. In the analyses for life-form, species with multiple life-forms were excluded to avoid confusion. Moreover, we calculated the percentages of untoothed species for large woody dicots and large shrubs separately for comparison with previous studies (Chen *et al.*, 2014).

Ordinary least squares (OLS) regression was used to explore the relationship between the percentage of untoothed species and temperature variables for all woody dicots, for those with different life-forms and for different age quartiles (i.e. Q1, Q2, Q3 and Q4), respectively. We also explored the relationship between leaf margin percentage and family age using OLS regressions. Multiple regressions and three-dimensional plots were used to evaluate the effects of interactions between MAT and MAP on patterns in the percentage of untoothed species. Modified *t*-tests were applied to assess the significance of those relationships after taking spatial autocorrelation in percentages of untoothed species into account (Clifford *et al.*, 1989). Following previous studies (Wolfe, 1993; Adams *et al.*, 2008), we analysed our data after excluding grid cells with fewer than 20 species. For comparison, analyses based on all grid cells were also conducted and all results were consistent with those based on grid cells with more than 20 species (see Appendix S2).

All statistical analyses were conducted using R (http://www.r-project.org/).

RESULTS

The pattern of the percentage of untoothed species and its relationship with temperature

The percentage of untoothed species for all Chinese woody dicots decreases with latitude, especially in eastern China (Fig. 1a; see also Appendix S1). It is lowest in north-east China, where only 15% of the species are untoothed, and highest on the Qinghai-Tibetan Plateau.

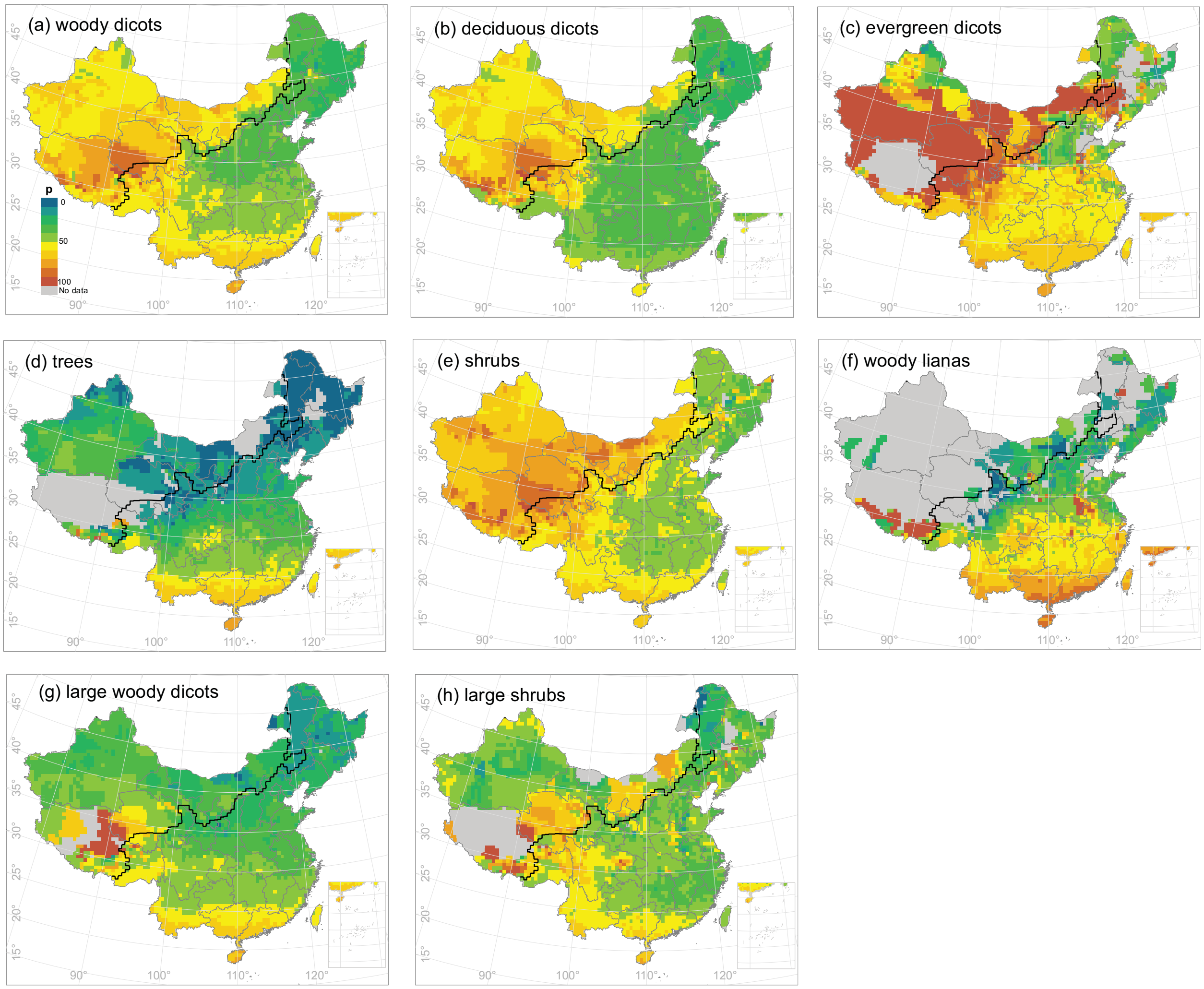

Figure 1 Patterns in the percentage of woody dicot species with untoothed leaves (p): (a) all woody dicots; (b) deciduous dicots; (c) evergreen dicots; (d) trees; (e) shrubs; (f) woody lianas; (g) large woody dicots (defined as trees and shrubs with a mature height >3 m); (h) large shrubs (defined as shrubs with a mature height >3 m). The solid black lines in the figures show the isoline at a mean annual precipitation of 400 mm. Grid cells without data are shown in grey.

Our results show that percentage of untoothed species for all woody dicots is not significantly correlated with temperature variables (Fig. 2, Appendix S2). In particular, MAT explains 11% of the variance in the percentage of untoothed species, while MTCQ explains almost none (Table 1). On the other hand, MAP explains 14% of this variance.

The influence of life-form on the percentage of untoothed species

Species with different life-forms tend to have different percentages of untoothed species. In particular, 64.5% of evergreen species have untoothed leaves while only 36.3% of deciduous species are untoothed, which suggests that evergreen dicots have higher tendency to be untoothed than deciduous ones (Appendix S3). Trees, shrubs and woody lianas have more untoothed than toothed species (Appendix S3), and this tendency is especially obvious for woody lianas (65.14% untoothed).

Species with different life-forms also differ in their patterns of leaf margin percentage (Fig. 1b–f). Compared with species having other life-forms, shrubs and deciduous dicots show more similar patterns to those of all woody dicots ($r = 0.89$; see Appendix S1). More interestingly, the differences among life-forms are mainly located in western China, whereas all life-forms show similar decreasing trends in percentages of untoothed species along latitude in eastern China (Fig. 1). Among all life-forms, the percentage of untoothed trees declines the fastest.

Analyses at species level and across space indicate that life-form significantly influences the relationship between leaf margin state and temperature. The life-form of species significantly affects the slope of the relationship between species-level leaf margin scores and MAT_{sp} (Appendix S3). Moreover, the relationship between the percentage of untoothed species

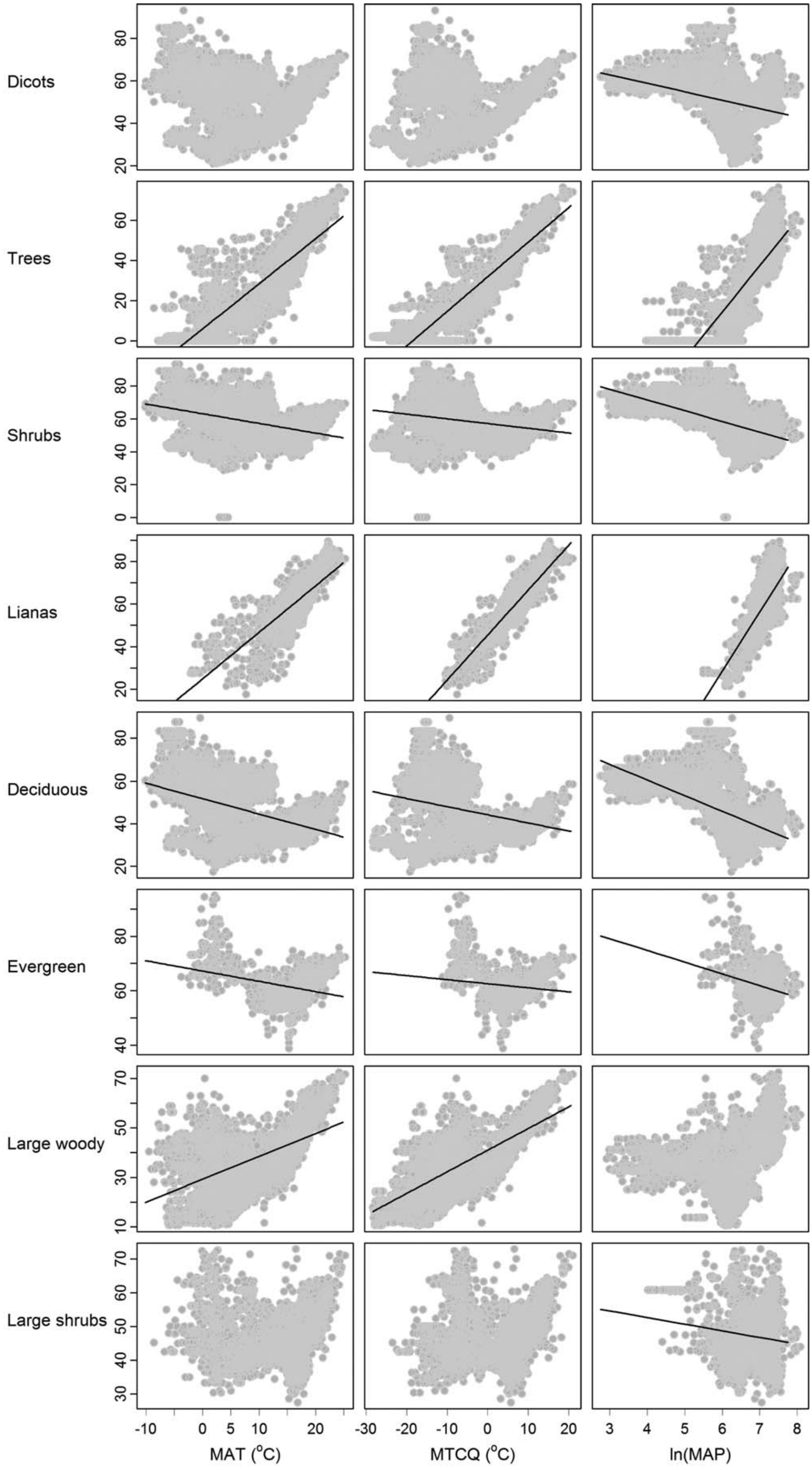

Figure 2 Relationships between climate variables and the percentage of untoothed species for all woody dicots and those with different life-forms. The *y*-axes represent the percentage of untoothed species. From top down: all woody dicots, trees, shrubs, woody lianas, deciduous dicots, evergreen dicots, large woody dicots and large shrubs. The *x*-axes represent climate variables. From left to right: mean annual temperature (MAT), mean temperature of the coldest quarter (MTCQ) and log-transformed mean annual precipitation (ln(MAP)). Linear regressions were conducted for all relationships, and the *P*-values of the regressions were calculated by a modified *t*-test. A black line is drawn when $P < 0.05$.

Table 1 R^2 of the relationships between the percentages of untoothed species and climate. *P*-values were calculated with a modified *t*-test (Clifford *et al.*, 1989).

	MAT		MTCQ		ln(MAP)	
Life-form	R^2	P	R^2	P	R^2	P
Woody dicots	0.011	0.263	0.006	0.509	0.137	0.001
Trees	0.737	0.000	0.833	0.000	0.622	0.000
Shrubs	0.128	0.000	0.053	0.044	0.348	0.000
Woody lianas	0.627	0.000	0.815	0.000	0.555	0.000
Deciduous dicots	0.167	0.000	0.081	0.009	0.406	0.000
Evergreen dicots	0.100	0.000	0.018	0.001	0.061	0.000
Large woody dicots	0.311	0.001	0.554	0.000	0.124	0.053
Large shrubs	0.000	0.631	0.019	0.065	0.020	0.026

MAT, mean annual temperature; MTCQ, mean temperature of the coldest quarter; MAP, mean annual precipitation.
Note: large woody plants are shrub and tree species with a maximum height over 3 m, and large shrubs are shrub species with maximum height >3 m.

within grid cells and the corresponding temperature differs significantly among different life-forms (Table 1, Fig. 2). Specifically, the percentages of untoothed trees and lianas are both significantly positively correlated with MAT and MTCQ (Fig. 2, rows 2 and 4; Table 1), which is consistent with previous studies (Royer *et al.*, 2012; Chen *et al.*, 2014). In contrast, the percentages of untoothed shrubs and deciduous and evergreen dicots are all weekly negatively correlated with temperature (Fig. 2, rows 3, 5 and 6; Table 1). The leaf margin percentage of large shrubs is insignificantly correlated with temperature, and hence the percentage of untoothed species for large woody dicots (i.e. trees plus large shrubs) is much more weakly correlated with temperature than those of just trees (Fig. 2, rows 7 and 8).

The influence of precipitation on the relationship between the percentage of untoothed species and temperature

Multiple regressions indicate that the interaction of MAT and MAP significantly influences the relationship between the percentage of untoothed species and temperature (Appendix S4). In particular, woody dicots have a disproportionately high percentage of untoothed species when MAP is below 400 mm (Fig. 3). Moreover, the percentage of untoothed species increases with temperature when MAP is >400 mm.

It is noteworthy that the influence of precipitation on the relationship between the percentage of untoothed species and temperature varies among different life-forms. For trees and woody lianas, the relationships between the percentage of untoothed species and temperature variables are all much stronger in humid and semi-humid regions than in arid and semi-arid regions (Fig. 4, Table 2), which suggests that higher precipitation enhances the relationship between the percentage of untoothed species and temperature. In contrast, for all

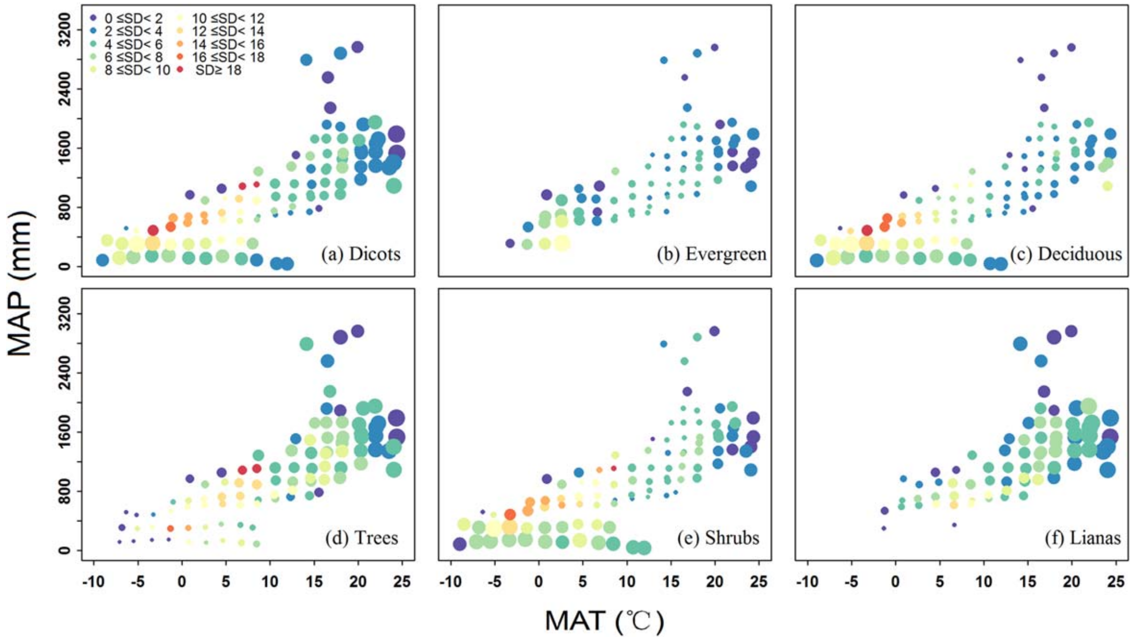

Figure 3 The interaction of mean annual temperature (MAT) and mean annual precipitation (MAP) on the percentage of untoothed species for all woody dicots and those with different life-forms. The size of the dots represents the mean value while dot colour represents the standard deviation of leaf margin percentages within grid cells for given temperature and precipitation bins (2 °C for MAT and 200 mm for MAP).

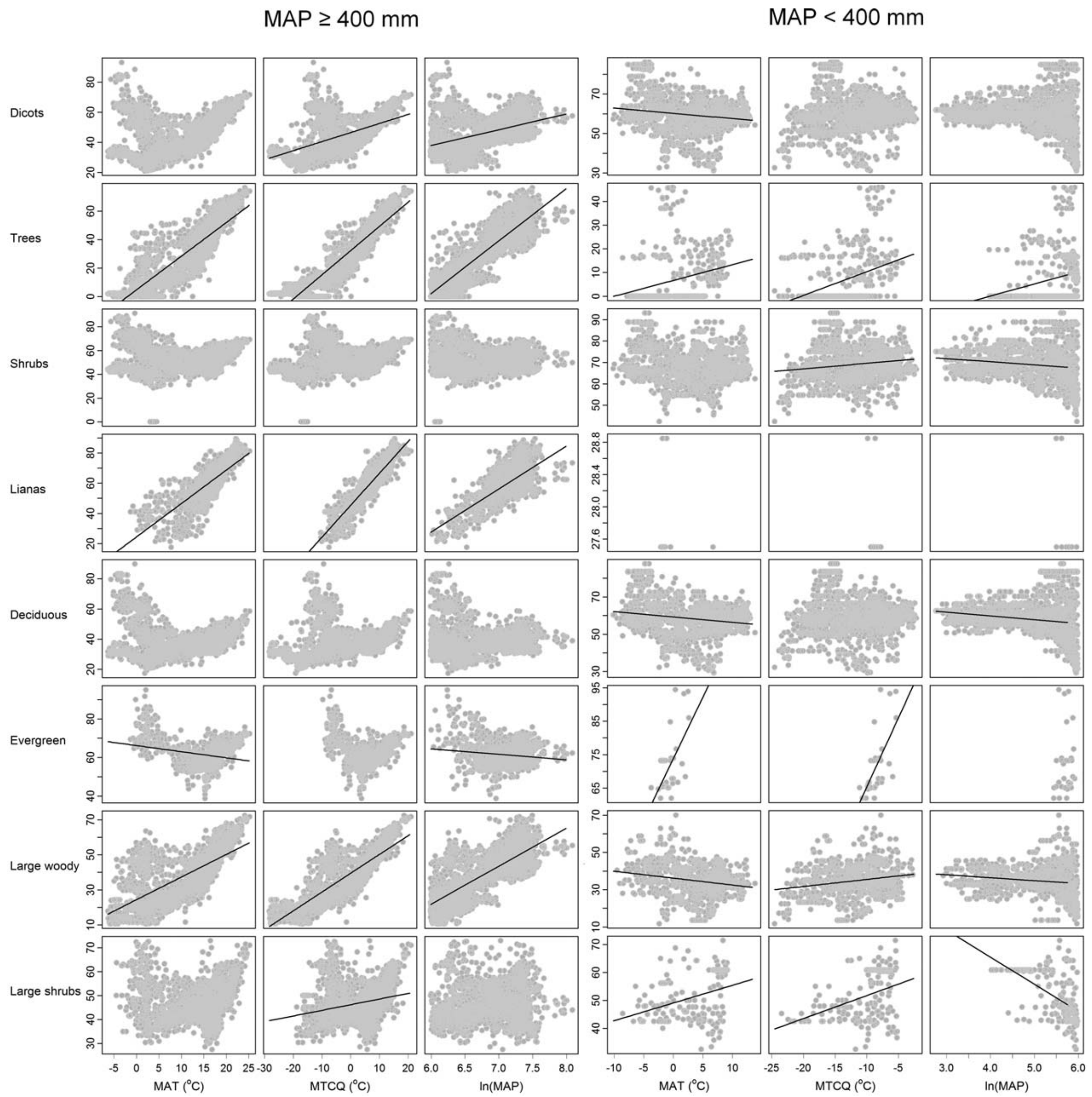

Figure 4 The influence of precipitation on the relationship between the percentage of untoothed species and temperature for all woody dicots and those with different life-forms. The three columns on the left show the relationship between the percentage of untoothed species and temperature in humid regions with mean annual precipitation (MAP) > 400 mm, while the three columns on the right show the relationship in arid regions with MAP < 400 mm. See the caption to Fig. 2 for the meaning of *x* and *y*.

woody dicots, shrubs and deciduous and evergreen species, increase in MAP does not significantly improve those relationships (Fig. 4, Table 2).

The influence of evolutionary history on the percentage of untoothed species

The percentages of untoothed species within families vary significantly (Fig. 5, Appendix S5). Some families are dominated by entire-leaved species (e.g. Asclepiadaceae, Cornaceae, Fabaceae, Lythraceae and Rubiaceae), while others contain primarily toothed species (e.g. Adoxaceae, Betulaceae, Clethraceae and Eupteleaceae). Both Blomberg's *K* and Pagel's λ indicate significant phylogenetic signals in the within-family percentage of untoothed species for all species, trees and shrubs (Fig. 5, Table S1 in Appendix S5), suggesting that the leaf margin states of these life-forms are phylogenetically conserved, and closely related families tend to have similar leaf margin states. In contrast, the leaf margins of lianas do not show a significant phylogenetic signal (Fig. 5).

Species-level analysis indicates that the slope of the relationship between the leaf margin scores of all species and MAT_{sp} is significantly influenced by family age (Table S2 in

Appendix S5). Moreover, family age also significantly influences the relationship between patterns of leaf margin percentages and temperature for trees and lianas (Table 3, Table S4 and Fig. S4 in Appendix S5). In particular, with the increase of mean family age (from Q1 to Q4), the relationship between the percentage of untoothed trees and temperature is weakened (Table 3, Appendix S5). In contrast, for shrubs, the percentage of untoothed species is weakly correlated with temperature across the age quartiles (Table 3).

DISCUSSION

Based on extensive data on leaf margin states and distribution maps for all Chinese woody dicots, we have demonstrated the complete leaf margin spectrum across China and revealed great uncertainties in its relationship with temperature induced by life-form, precipitation regime and evolutionary history. We found that the relationship between the percentage of untoothed species and temperature is stronger for trees and lianas than for shrubs, and stronger in humid than in arid regions. Moreover, significant phylogenetic signals are found in leaf margin states. Therefore, our findings suggest that caution is needed when using LMA: only dicot trees in humid regions should be used for palaeotemperature reconstruction and the transfer function may be unreliable for reconstruct palaeotemperature in deep geological time.

It is noteworthy that previous LMA mainly focused on the reconstruction of MAT (Royer *et al.*, 2005; Traiser *et al.*, 2005; Chen *et al.*, 2014). However, our analyses indicate that mean winter temperature (i.e. MTCQ) is more strongly correlated with leaf margin percentage than MAT. Similarly, Wang *et al.* (2011) found that winter coldness was the primary determinant of species diversity patterns of Chinese woody plants. Those findings suggest that winter coldness determines not only woody plant distributions but also the variation in leaf margin state. Therefore, the reconstruction of ancient MTCQ based on the percentage of untoothed species should be better than that of MAT.

Previous LMA studies at large scales in China generally suffered from limited or unreliable data (Wolfe, 1993; Su *et al.*, 2010; Chen *et al.*, 2014), leading to low accuracy and universality of the built transfer functions. The recent study in eastern China by Chen *et al.* (2014) included only 53% (3166 out of 5964 species) of Chinese large woody dicots, and covered only 39% of the area of Chinese humid regions (732 out of 2082 counties) and 21% of the whole of China. Due to the incomplete species lists and distribution data, county-level species diversity in Chen *et al.* (2014) was much lower than the actual value (Appendix S6). In contrast, our study covered the whole of China and analysed the leaf margin percentage using all Chinese woody dicots and a synthetic-site approach. Moreover, our study quantified the relationship between leaf margin state and temperature in arid regions and for different life-forms, and analysed the effects of evolutionary history on these relationships in eastern Asia for the first time.

Table 2 R^2 of the relationships between the percentages of untoothed species and temperature variables in regions with different precipitation. *P*-values were calculated with a modified *t*-test (Clifford *et al.*, 1989).

	MAT				MTCQ				ln(MAP)			
	MAP > 400 mm		MAP < 400 mm		MAP > 400 mm		MAP < 400 mm		MAP > 400 mm		MAP < 400 mm	
Trait	R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*
Woody dicots	0.088	0.065	0.289	0.004	0.167	0.020	0.033	0.002	0.009	0.167	0.020	0.099
Trees	0.744	0.000	0.845	0.000	0.749	0.000	0.075	0.000	0.131	0.000	0.056	0.000
Shrubs	0.009	0.258	0.024	0.123	0.002	0.614	0.008	0.134	0.025	0.008	0.026	0.006
Woody lianas	0.615	0.000	0.808	0.000	0.542	0.000	0.047	0.610	0.197	0.371	0.260	0.207
Deciduous dicots	0.018	0.100	0.014	0.239	0.004	0.511	0.035	0.000	0.002	0.493	0.040	0.010
Evergreen dicots	0.065	0.000	0.003	0.269	0.021	0.001	0.361	0.004	0.405	0.002	0.022	0.442
Large woody dicots	0.488	0.000	0.721	0.000	0.557	0.000	0.047	0.000	0.042	0.001	0.021	0.001
Large shrubs	0.000	0.857	0.052	0.010	0.000	0.827	0.114	0.000	0.160	0.001	0.298	0.000

MAT, mean annual temperature; MTCQ, mean temperature of the coldest quarter; MAP, mean annual precipitation.

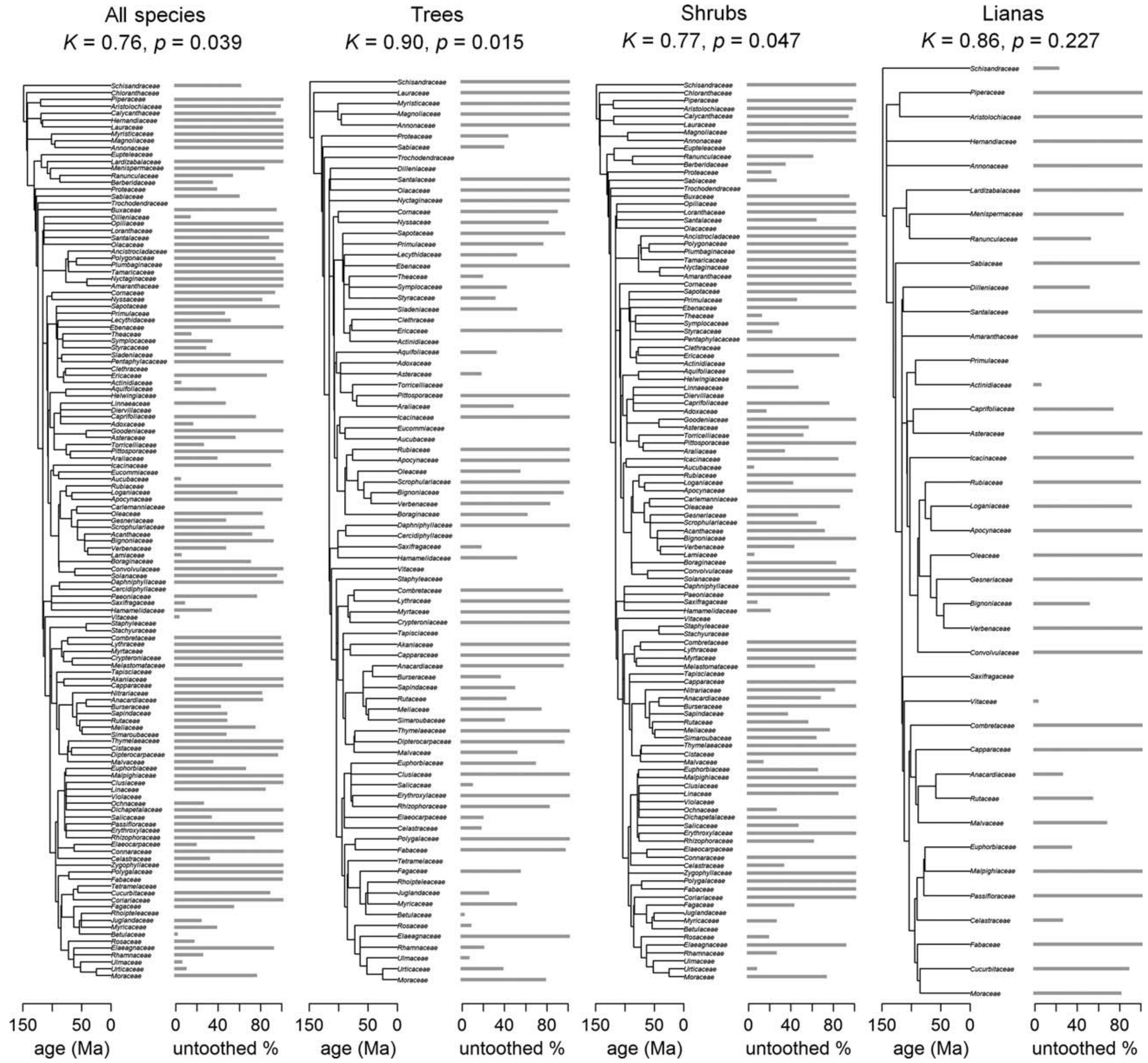

Figure 5 Proportion of untoothed dicot species within families mapped onto the family-level phylogeny (Davies *et al.*, 2004). Each tip on the phylogeny represents a family, and the grey bar to the right hand of each family name represents the proportion of untoothed woody dicot species found in China within that family.

Previous studies have hypothesized that life-form may affect leaf margin states and their relationship with temperature (e.g. Bailey & Sinnott, 1916), although quantitative studies remain deficient (but see Royer *et al.*, 2012). With comprehensive trait and distribution data for all woody plants in China, we quantified the uncertainties in LMA caused by plant life-form. We found significant positive relationships between the percentage of untoothed trees and temperature, especially in humid regions, which is partly consistent with previous studies (Adams *et al.*, 2008; Chen *et al.*, 2014). However, compared with trees, the percentage of untoothed shrubs and large shrubs shows a much weaker, or even opposite, trend along temperature gradient. These findings show that the inclusion of shrubs, as normally done in previous studies (Adams *et al.*, 2008; Chen *et al.*, 2014), would reduce the explanatory power of temperature on the spatial variation in percentage of untoothed species, and hence weaken the transfer functions for palaeotemperature reconstruction. Therefore, the life-form of woody dicots should be considered when using leaf margin analysis in the future.

The weak relationships for shrubs may be due to their habitats and physiological structure. In the forests of eastern China shrubs tend to grow in the underlayer and hence are mainly influenced by the under-canopy microclimate (Gomez-Aparicio *et al.*, 2005; D'Odorico *et al.*, 2010). In contrast, shrubs dominate the shrublands and deserts in western China, and the leaf morphology of shrubs is mainly

Table 3 R^2 of the relationships between the percentage of untoothed species and temperature variables for species quartiles with different family ages. *P*-values were calculated with a modified *t*-test (Clifford *et al.*, 1989).

	Q1		Q2		Q3		Q4	
	R^2	P	R^2	P	R^2	P	R^2	P
All species								
MAT	0.287	0.320	0.003	0.183	0.003	0.130	0.313	0.009
MTCQ	0.474	0.785	0.164	0.428	0.153	0.780	0.508	0.009
Trees								
MAT	0.732	0.000	0.403	0.257	0.391	0.003	0.155	0.003
MTCQ	0.845	0.000	0.669	0.606	0.667	0.001	0.313	0.006
Shrubs								
MAT	0.000	0.143	0.003	0.271	0.000	0.014	0.002	0.052
MTCQ	0.001	0.355	0.009	0.439	0.003	0.567	0.000	0.007
Woody lianas								
MAT	0.171	0.011	0.443	0.002	0.694	0.000	0.102	0.000
MTCQ	0.103	0.082	0.738	0.000	0.461	0.000	0.010	0.000

MAT, mean annual temperature; MTCQ, mean temperature of the coldest quarter.
Family ages were obtained from Davies *et al.* (2004). Q1, Q2, Q3 and Q4 represent the four species quartiles ranging from the youngest to the oldest (see Appendix S5 for details).

limited by water availability (Skarpe, 1990; Stephenson, 1990). Moreover, shrubs with a low height tend to suffer more from root pressure than from heat (Royer *et al.*, 2012). Therefore, the leaf margin states of shrubs may be less sensitive to environmental temperature than those of trees (Bailey & Sinnott, 1916; Royer *et al.*, 2012), which further supports our finding that only dicot trees are suitable for building transfer functions for palaeotemperature reconstruction. However, in fossils, trees can be hardly distinguished from shrubs. Building models linking the percentages of untoothed species for trees and those for woody dicots might reduce the 'noise' in palaeotemperature reconstruction caused by shrubs and help solve this contradiction.

Our results indicate that deciduous species tend to have toothed leaves while evergreen ones are untoothed. Royer *et al.* (2012) analysed the leaf margin states of 3549 species and found that toothed species were more likely to be deciduous, and that this tendency was independent of temperature. Compared with evergreen species, deciduous species tend to invest less biomass in their short-lived leaves and usually have thinner leaves than evergreen species (Givnish, 1978). Therefore, deciduous species occupy the 'fast return' end of the leaf economics spectrum (Wright *et al.*, 2004), and tend to be toothed to improve physical support for the leaf blades and to avoid tearing from strong winds (Baker-Brosh & Peet, 1997). Moreover, the strong correlation between deciduousness and toothed leaves may be attributed to the leaf gas-exchange hypothesis (Royer & Wilf, 2006). Recent studies found that leaf teeth tend to enhance early season gas exchange in cold climates and thus may be a strategy of deciduous plants for efficient use of resources (Peppe *et al.*, 2011; Royer *et al.*, 2012). Furthermore, the difference in leaf margin state between deciduous and evergreen species is more pronounced for trees than for shrubs (Appendix S3), which may explain why the leaf-margin state of species with a higher maximum height and longer longevity is more sensitive to temperature than that of species with a lower height and shorter longevity (e.g. canopy trees versus shrubs) (Royer *et al.*, 2012). It is noteworthy that the impact of leaf habit on leaf margin state may covary with the effects of climate, because evergreen species are more common in warm regions than in cold regions. Further studies are needed to evaluate the effects of leaf habit on leaf margin after eliminating the influence of climate.

As predicted, precipitation significantly affects the relationship between the percentage of untoothed species and temperature. Specifically, our results suggest that high precipitation tends to enhance the influence of temperature on the leaf margin state. Moreover, our results show for the first time that the relationship between the percentage of untoothed species and temperature for different life-forms is weak in arid regions of China. Similarly, Wolfe (1993) found that dry microthermal and subalpine samples behaved as outliers in LMA. However, it is noteworthy that the dry samples in his study were only located in North America and their data differed from ours. The strong variation in the relationship between the percentage of untoothed species and temperature across different precipitation regimes may reflect different adaptations of plants to water and energy stress (Chaves *et al.*, 2002). Drought may weaken thermal impacts on plants through adaptive changes in morphology at both species and vegetation levels (Stephenson, 1990; Yordanov *et al.*, 2000). These results are consistent with previous findings for large-scale species diversity patterns. For example, Xu *et al.* (2016) found that the influence of energy on global oak species diversity strongly depends on the availability of water. It is noteworthy that the enhancement of the leaf margin–temperature relationship induced by precipitation is negligible for shrubs. These findings suggest that caution is required when using leaf margin data to reconstruct palaeotemperature because the transfer functions are strongly influenced by plant life-form and precipitation.

It is notable that the Qinghai-Tibetan Plateau has higher percentages of untoothed species than other regions, especially deciduous dicots and shrubs. This pattern may show the severe selection pressure in the unique environment of the Qinghai-Tibetan Plateau characterized by high altitude, high radiation, aridity and a cold climate (Liu *et al.*, 2002). It has been reported that alpine plants may have different leaf morphology spectra (Halloy & Mark, 1996). For example, plants in alpine areas tend to have small, thick leaves (Dolph & Dilcher, 1980; McDonald *et al.*, 2003; Traiser *et al.*, 2005). Untoothed small, thick leaves might be the strategies adopted by plants in the Qinghai-Tibetan Plateau. This unique pattern may explain the small effect of temperature on leaf margin percentage in arid regions found in our analysis. Moreover, the low species richness of woody plants in the Qinghai-Tibetan Plateau may also increase the uncertainties around the estimation of leaf margin percentages.

Our results indicate a significant phylogenetic signal in leaf margin states and suggest that the acquisition or loss of leaf teeth is phylogenetically conserved, further supporting the findings of Little *et al.* (2010). Moreover, our study found that evolutionary history significantly influences patterns of leaf margin percentage and their relationship with temperature, which is consistent with the hypothesis proposed in previous studies (Jordan, 1997; Schmerler *et al.*, 2012). Jordan (1997, 2011) argued that as toothed leaves are an adaptation to cool climate (Royer & Wilf, 2006), present-day leaf margin–temperature relationships may have resulted from the effects of environmental filtering on leaf morphology during the cold Quaternary. Therefore, he hypothesized that the uncertainties in leaf margin–temperature relationships increase from the Quaternary to further back in geological time. Similarly, we found that family age has a significant influence on the relationship between the leaf margin state of species and temperature, especially for endemic species. Species from older families tend to have a longer evolutionary history, and thus more chance of suffering from historical events unrelated to climate (Little *et al.*, 2010), which might impair the response of leaf margin to temperature. Moreover, relationships between percentages of untoothed trees and temperature become weak with increasing family age. Only tree species from young families show strong relationships between leaf margin percentages and temperature, and hence might be useful for palaeotemperature reconstruction. In contrast, uncertainties in the leaf margin–temperature relationship tend to be high for old families. Our results and previous findings suggest that the basic assumption that the leaf margin–temperature relationship does not change through time, although widely employed in previous studies, may not hold true.

Three data sources on leaf margins have been used in previous studies on LMA: (1) data measured in controlled lab experiments, (2) data from local forest plots, and (3) data recorded in regional floras (Traiser *et al.*, 2005; Adams *et al.*, 2008; Su *et al.*, 2010). Floras provide access to large numbers of data and reduce the effects of local-scale processes, such as microclimate and edaphic factors, on leaf morphology (Traiser *et al.*, 2005). However, floristic data ignore the differences in species abundances and intraspecific morphological differences within a region. As most individuals in a community are generally from a few common species, estimates of the percentage of untoothed species based on floristic data might underestimate the contribution of dominant species, but overestimate the effects of rare species, leading to a high uncertainty in palaeotemperature reconstruction. Therefore, further studies are needed to evaluate the potential influences of species abundance on the relationship between the percentage of untoothed species and environmental temperature (Bailey & Sinnott, 1916). Moreover, the recent method of digital leaf physiognomy measures the number and area of teeth on individual leaves to provide more details about the leaf margin (Huff *et al.*, 2003; Royer *et al.*, 2005; Peppe *et al.*, 2011), enabling further possible studies. Additionally, although we find evolutionary signals in the leaf margin state, the relative importance of environment and macroevolution on leaf traits remains to be explored in future (Little *et al.*, 2010).

CONCLUSIONS

LMA has been widely used for palaeotemperature reconstruction based on the relationship between the percentage of untoothed species and temperature. Here we present the first systematic analysis for the whole of China using a complete dataset of woody plant distribution and leaf margin states. We found that the relationship between the percentage of untoothed species and temperature is significantly influenced by plant life-form, precipitation and evolutionary history. These findings suggest that although LMA can be well applied, great uncertainties exist in this method, and the conditions for its use are: (1) only the percentage of untoothed species for dicot trees and lianas can be used; (2) the transfer function in LMA of China is only reliable in humid and semi-humid regions; (3) it cannot be used to reconstruct palaeotemperature in deep geological time.

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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:

Appendix S1 The geographical patterns and correlation coefficients of the percentage of untoothed species with different life-forms.
Appendix S2 Relationships between the percentage of untoothed species and climatic variables based on all grid cells with species richness > 0.
Appendix S3 The influence of life-form on leaf margin state.
Appendix S4 The influence of precipitation on the relationship between the percentage of untoothed species and temperature.
Appendix S5 The influence of family age on the pattern of leaf margin percentage and its relationship with temperature.
Appendix S6 Summary of our database and its comparison with recent publications.

BIOSKETCH

Yaoqi Li is a PhD student whose research focuses on patterns of plant morphology and their relationships with climate at large spatial scales.

The macroecology research group contributing this article is interested in the mechanisms underlying large-scale patterns of species diversity and functional traits. Y.L. and Z.W. conceived the idea, collected the data and conducted the analysis. All authors contributed to the writing.

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