

## LEAF MARGIN ANALYSIS: A NEW EQUATION FROM HUMID TO MESIC FORESTS IN CHINA

TAO SU,<sup>1,2,3</sup> YAO-WU XING,<sup>1,2,3</sup> YU-SHENG (CHRISTOPHER) LIU,<sup>4\*</sup> FRÉDÉRIC M.B. JACQUES,<sup>1</sup> WEN-YUN CHEN,<sup>1,2</sup>  
YONG-JIANG HUANG,<sup>1,2</sup> and ZHE-KUN ZHOU<sup>1\*</sup>

<sup>1</sup>Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China; <sup>2</sup>Graduate University of the Chinese Academy of Sciences, Beijing 100049, China; <sup>3</sup>State Key Laboratory of Paleobiology and Stratigraphy, Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, Nanjing 210008, China; <sup>4</sup>Department of Biological Sciences and Don Sundquist Center of Excellence in Paleontology, Box 70703, East Tennessee State University, Johnson City, Tennessee 37614-1710, USA  
e-mail: liuc@mail.etsu.edu; zhouzk@mail.kib.ac.cn

### ABSTRACT

Leaf margin analysis (LMA) is a widely used method that applies present-day linear correlation between the proportion of woody dicotyledonous species with untoothed leaves (P) and mean annual temperature (MAT) to estimate paleotemperatures from fossil leaf floras. Previous works demonstrate that LMA shows regional constraints and to date, no equation has been modeled directly from Chinese forests. Fifty humid to mesic Chinese forests were chosen to understand the relationship between percentage of untoothed leaf species and MAT in China. Consistent with previous studies, the Chinese data indicate that P shows a strong linear correlation with MAT, but the actual relationship is a little different from those recognized from other regions. Among the several currently used LMA equations, the one resulting from North and Central American and Japanese data, rather than the widely used East Asian LMA equation, yields the closest values to the actual MATs of the Chinese samples (mean absolute error = 1.9 °C). A new equation derived from the Chinese forests is therefore developed, where  $MAT = 1.038 + 27.6 \times P$ . This study not only demonstrates the similarity of the relationship between P and MAT in the Northern Hemisphere, but also improves the reliability of LMA for paleoclimate reconstructions of Chinese paleofloras.

### INTRODUCTION

The morphology of leaves is largely affected by biological (Brown et al., 1991) and environmental factors, such as climate (Bailey and Sinnott, 1915; Baker-Brosh and Peet, 1997; Royer and Wilf, 2006; Royer et al., 2008), water availability (Kowalski and Dilcher, 2003; Greenwood, 2005a; Royer et al., 2009), and edaphic conditions (Greenwood et al., 2004). Even with the uncertainty of mechanisms of genome expression in response to environmental triggers, leaf physiognomy has long been considered to show a high correlation with climate and has been widely used in reconstructing terrestrial paleotemperatures (e.g., Davies-Vollum, 1997; Wilf et al., 1998; Kennedy et al., 2002; Liang et al., 2003; Martinetto et al., 2007; Xia et al., 2009). Previous works demonstrated that the proportion of woody dicotyledons with untoothed leaves (P) was strongly correlated to mean annual temperature (MAT) (Wolfe, 1979, 1993; Wing and Greenwood, 1993; Wilf, 1997; Greenwood et al., 2004; Miller et al., 2006; Adams et al., 2008). Therefore, untoothed leaf-margin percentage is suggested to be an effective proxy for quantitatively calculating MAT in terrestrial paleoclimate reconstructions (Wolfe, 1979; Wing and Greenwood, 1993; Wilf, 1997). Previous studies have suggested, however, that this relationship is inconsistent among different regions (Wing and Greenwood, 1993; Jacobs, 1999; Gregory-Wodzicki, 2000; Greenwood et al., 2004; Spicer et al., 2004; Traiser et al., 2005; Miller et

al., 2006; Adams et al., 2008) and have focused on the following seven regions:

1. East Asia: Wolfe (1979) compiled 34 humid to mesic floras from East Asia, including data from Wang (1961), and plotted untoothed margin percentage against MAT. Wing and Greenwood (1993) summarized the relationship in an equation based on these data.

2. North and Central America: Wilf (1997) studied vegetation in Central America. In combination with North and South American data sets, he pointed out that this LMA equation was similar to the one for East Asia (Wing and Greenwood, 1993). Miller et al. (2006) established a new equation based on 84 sites from both Central and North America and suggested a different method to predict the uncertainties of calculated MAT. Other authors have also studied the relationship between P and MAT in North America to investigate the physiological basis of the relationship and potential taphonomic errors associated with the data (e.g., Wiemann et al., 1998; Kowalski and Dilcher, 2003; Royer et al., 2005; Traiser et al., 2005; Adams et al., 2008).

3. South America: Gregory-Wodzicki (2000) studied 12 samples in Bolivia and suggested that the relationship between P and MAT in Bolivia was not significantly different from that in North America and Japan. Subsequently, Kowalski (2002) tested 15 leaf character–MAT models on thirty samples from tropical South America and concluded that models from tropical South American vegetation could better estimate MAT in this region. More recently, Aizen and Ezcurra (2008) studied temperate forests in southern South America and derived a new equation similar to the one from tropical South America (Kowalski, 2002).

4. Australia: Greenwood et al. (2004) used data from 113 rainforest sites in Australia and derived a different LMA equation that gave a lower estimate of MAT than any other equations when it came to the same percentage of entire-margined species.

5. Europe: Traiser et al. (2005) studied the distribution of 108 native woody dicotyledons in Europe with a new method that is quite different from direct sampling in the field: In their study, a species list is generated from floristic manuals and climate data are based on the global climate model (New et al., 1999), with a  $0.5^\circ \times 0.5^\circ$  latitude and longitude resolution. They concluded that leaf-base acuteness, rather than leaf margin, showed a higher correlation to MAT.

6. Africa: Jacobs (1999) studied 30 samples in equatorial Africa and concluded that the relationship between percentage of entire-margined species and MAT in equatorial Africa is weaker than the results derived from other regions.

7. New Zealand: Spicer et al. (2004) compared the spatial distribution pattern of P in different regions and found that the relationship between P and MAT in New Zealand was different from other regions and showed weak correlation.

In addition to these mainly regional studies, the widely used method, CLAMP (Climate Leaf Analysis Multivariate Program), has largely

\* Corresponding authors.

improved our understanding of the relationship between leaf characters and climate (Wolfe, 1993; Spicer et al., 2009). CLAMP consists of three data sets from North America and East Asia, including PHYSG3AR, PHYSG3BRC, and PHYSG3CRC. These data sets contain information on 31 leaf characters and 11 climate parameters from 173, 144, and 193 sample sites (Spicer, 2009).

Up until now, the East Asian LMA model was the only one that included data from China, although these data were derived from a single, out-of-date source (Wang, 1961). CLAMP does include some data from East Asia, but only from Japanese vegetation (Wolfe, 1993; Spicer, 2009). Therefore, little is known about the real relationship between leaf physiognomy and climate in Chinese forests. The East Asian LMA model, which has been the most widely used equation, was first developed from the data set of Wolfe (1979) by Wing and Greenwood (1993). The Chinese data utilized by Wolfe (1979), however, appeared largely questionable because these data represent incomplete compilations from Wang's personal collection (Wang, 1961). It is obvious that the vegetation types, their distribution patterns, and their floristic elements documented by Wang (1961) did not fully represent the diversity of vegetation in China (Wu, 1980). Furthermore, the climate data compiled by Wang (1961) only covered a short time frame and did not include observations after the first half of the 20<sup>th</sup> century. After 1949, numerous national botanical and vegetation surveys were comprehensively carried out and a great number of specimens were collected and added to Chinese herbaria. These activities have resulted in several important Chinese botanical references, such as the *Vegetation of China* (Wu, 1980) and the *Flora of China* in both Chinese and English versions (Wu, 1990, 2004). As a result, these references have helped us better understand the Chinese vegetation and floras. Additionally, many new weather stations have been established and their recordings have gradually become available to the public. It is now possible to use these data from China to elucidate the relationship between P and MAT. The main purpose of the present study is to fill the gap by providing data to explore the relationship between percentage of untoothed leaf species and MAT in China.

## MATERIAL AND METHODS

### Sampling

Two approaches are often used in developing LMA models: (1) sampling plant data in the field, and (2) compiling synthetic and chorological floras from publications. The sampling approach consists of collecting samples directly in the field and compiling climate data from nearby weather stations (Wolfe, 1979, 1993). This approach more precisely reflects the climate data of a local floral assemblage (Greenwood, 2005a), although it is hard to find undisturbed vegetation near climate stations (Spicer et al., 2009).

The second approach deals with extracting the spatial distribution of species from regional floras and calculating climate data from climatic models (Traiser et al., 2005; Adams et al., 2008). This approach yields a much higher number of samples at a larger spatial scale, but with less accurate climate data than the direct sampling strategy. Despite differences in data sources, both approaches conclude that the proportion of untoothed leaves strongly correlates with MAT (Greenwood, 2005b).

Mountainous regions cover ~66% of the 9.6 million km<sup>2</sup> of China, mainly in the western part. Compared to lower altitude regions in eastern China, the mountainous areas show a complicated relationship between climate parameters and plant distribution which limits the use of the chorological method for western China. For this reason, we adopted the sampling method in the present study. Fifty humid to mesic floras spread from tropical to cold temperate climates in China were selected as sample sites (Fig. 1, and see Supplementary Data 1<sup>1</sup>). No

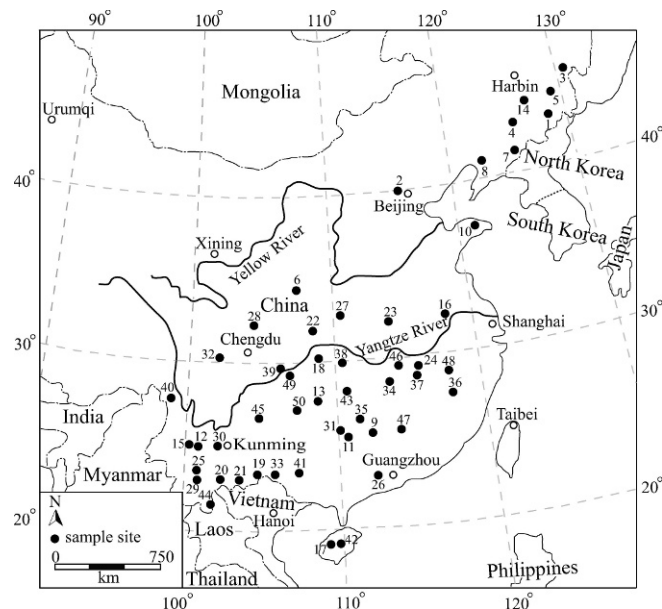


FIGURE 1—Distribution of 50 sample sites. Numbers of sample sites on the map correspond to Supplementary Data 1<sup>1</sup>.

sample was collected in the western dry regions (e.g., Qinghai and Xinjiang) due to the rareness of mesic broadleaved forests there (Wu, 1980). All of these sites were chosen from natural forests with low levels of human activities and elevation <2400 m to avoid the disproportion of untoothed leaves found in alpine zones (Velázquez-Rosas et al., 2002). Moreover, only sites ≤30 km away from the nearest weather station were selected. The area of each site was usually 1–3 hectares. Field work was carried out during the summer and fall of three consecutive years from 2006 to 2008. MAT and mean annual precipitation (MAP) of the sample sites ranged from 2.1–22.4 °C and 469.7–2458.5 mm, respectively.

Samples were collected randomly at each site. Both sun and shade mature leaves of all woody dicotyledons within the site were included. The average sample size for most sites is >20 species; however, five sites in northeastern China, where plant diversities are low due to the cold temperate climate, contain <20 species of woody dicotyledons. One monocot genus, *Smilax*, was included in samples for its foliar similarity to woody dicot leaves (Wilf, 1997). Species that are parasitic or not indigenous were excluded in sample species. All voucher specimens are deposited in the Herbarium of the Kunming Institute of Botany, Academia Sinica (KUN).

### Climatic Data

Climatic data of sample sites were retrieved from the following two sources: (1) China Meteorological Data Sharing Service System (CMDSSS) available online (<http://cdc.cma.gov.cn/>, access open only to registered users); and (2) for data unavailable in CMDSSS, we consulted non-public data from the library of Yunnan Provincial Meteorological Bureau. All climate data used in this study are from pre-1991 and include 27 years of records on average. Ranges of records were largely based on the time of establishment of each weather station. When the altitude difference between a given sample site and the nearest weather station is >100 m, which includes 35 sites, a calibration in MAT calculation was made to correct the elevation effect on climate (Wang, 2006; D.R. Greenwood, personal communication, 2009). We chose 5–11 climate stations within 1 degree of latitude from the sample site to set up a single linear regression. Climate stations are from

<sup>1</sup> [www.paleo.ku.edu/palaios](http://www.paleo.ku.edu/palaios)

**TABLE 1**—LMA models derived from data sets of different regions; numbers correspond to data sets in Figure 2. P = proportion of woody dicotyledonous species with untoothed leaves; n = number of samples; SE = standard error.

Transfer function	Region	r <sup>2</sup>	n	SE	Authors
(1) MAT = 1.141 + 30.6 × P	East Asia	0.98	34	0.8	(Wolfe, 1979; Wing and Greenwood, 1993)
(2) MAT = 2.240 + 28.6 × P	North, Central, and South America	0.94	9	2.0	(Wilf, 1997)
(3) MAT = -0.266 + 29.1 × P	North and Central America and Japan	0.76	106	3.4	(Wilf, 1997)
(4) MAT = -0.059 + 31.6 × P	South America	0.89	14	1.6	(Gregory-Wodzicki, 2000)
(5) MAT = -2.120 + 27.0 × P	Australia	0.63	74	—	(Greenwood et al., 2004)
(6) MAT = 0.512 + 31.4 × P	Europe	0.60	1835	1.7	(Traiser et al., 2005)
(7) MAT = 1.320 + 29.0 × P	North and Central America	0.91	84	—	(Miller et al., 2006)

different elevations and this method shows the high correlation between MAT and elevation ( $r^2 > 0.9$ ); therefore, this method works well to predict MATs (see Supplementary Data 2<sup>1</sup>):

$$\text{MAT}_{\text{sam.}} = a_s - b_{\text{lr}}/100 \times H$$

MAT<sub>sam.</sub> is the calculated MAT of a sample site;  $a_s$  is the constant in this equation;  $b_{\text{lr}}$  is the lapse rate (°C/100 m), which represents the decrease rate of temperature per 100 meters of elevation; and H is the elevation of the sample site (Wang, 2006; D.R. Greenwood, personal communication, 2009).

#### Data Analysis

A tooth is defined as a projection from the leaf margin that always includes vasculature; the length of the marginal sinus should be less than one quarter of the distance from margin to midvein (Ellis et al., 2009). A species with only untoothed margins is scored 1; if both untoothed and toothed margins occur within one species, a score of 0.5 is assigned, and a score of 0 is assigned to species with toothed margins (Wolfe, 1993). The percentage of untoothed leaf species in each sample was recorded as the sum of scores of all species divided by the total number of scored species. We also scored 30 other leaf physiognomic characters (see Supplementary Data 3<sup>1</sup>) according to the leaf morphological definitions of CLAMP (Spicer, 2009), in order to evaluate relationships between each leaf character and MAT, and to compare the estimated value from CLAMP with the actual MAT for every sample.

Relationships among 31 leaf physiognomic characters, including the percentage of species with untoothed margins and the MAT were analyzed statistically by using Single Linear Regression (SLR). Furthermore, we applied seven published LMA models (Table 1) to the Chinese data to evaluate the accuracy of these models for climate estimation. Two CLAMP data sets were used (Spicer, 2009); one is PHYSG3ARC, which includes 173 modern floras, and the other is PHYSG3BRC, which includes 144 floras, excluding alpine sites. Estimated MATs were compared with the actual MATs by using a paired-samples t-test. We compared the predicted values of all models above using the mean absolute error, which is calculated by the mean absolute difference between estimated and observed values.

## RESULTS

### Correlations among Leaf Characters and MAT in China

The proportion of untoothed leaf margin is the parameter that shows the strongest correlation with MAT ( $r^2 = 0.79$ , see Supplementary Data 4<sup>1</sup>), which is consistent with previous studies (Wolfe, 1979; Wing and Greenwood, 1993; Gregory-Wodzicki, 2000; Kowalski, 2002; Greenwood et al., 2004; Greenwood, 2005b; Traiser et al., 2005; Adams et al., 2008). Ratios of leaf length to width show a relatively lower correlation to MAT than untoothed leaf-margin percentage (e.g., L:W = 1–2:1,  $r^2 = 0.58$ ; L:W = 3–4:1,  $r^2 = 0.46$ ). Weak correlations are found between

leaf sizes and MAT. Characters such as leaf apex, leaf base, and leaf shape also show weak correlations to MAT (e.g., apex attenuation, cordate base, and ovate shape, with  $r^2 = 0.25$ ,  $r^2 = 0.40$ , and  $r^2 = 0.23$ , respectively).

A new P and MAT transfer function (Fig. 2), based on the Chinese data set, was developed:

$$\text{MAT} = 1.038 + 27.6 \times P$$

$$\text{with } r^2 = 0.79, F = 180, P < 10^{-17}.$$

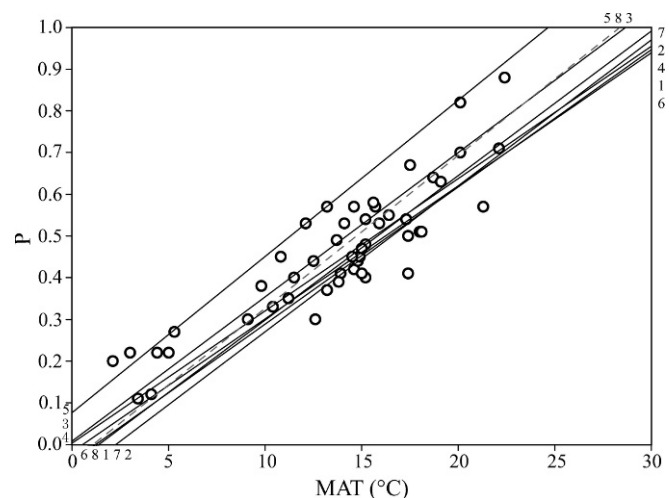
When applying an LMA equation to an investigated flora, standard error (SE) of the equation and the extra binomial error can be estimated as:

$$\text{SE} = b \times \sqrt{[1 + \phi(n-1)p(1-p)] \times \frac{p(1-p)}{n}} \quad (\text{Miller et al., 2006})$$

Where b is the slope in the equation;  $\phi$  is the overdispersion factor, which we follow from Miller et al. (2006) (for their study,  $\phi = 0.052$ ); p is the percentage of woody dicotyledons with untoothed leaves; and n is the total number of woody dicotyledons scored in a given flora.

### Previous LMA Models to Test Chinese Samples

We calculated MATs of 50 Chinese sites using the new Chinese LMA equation, seven previous LMA models (Fig. 2), and CLAMP to test the reliability of each model (see Supplementary Data 5<sup>1</sup>). Generally speaking, these models yielded estimated values that are not very different from the actual observed MATs, but the mean absolute



**FIGURE 2**—The Chinese leaf margin analysis (LMA) equation and seven other LMA data sets with best-fit linear equations. Numbers of equations correspond to the following regional data sets: (1) East Asia; (2) North, Central, and South America; (3) North and Central America and Japan; (4) South America; (5) Australia; (6) Europe; (7) North and Central America; and (8) China.



standard errors of models range from 1.9–3.5 °C. Among all tested models, the Chinese LMA model and the North and Central American and Japanese LMA model (Wilf, 1997) appear to have the closest predicted values, with the mean absolute SE for both being 1.9 °C. The most widely used East Asian LMA model (Wolfe, 1979; Wing and Greenwood, 1993), however, yields a higher value (mean absolute SE = 2.2 °C). A similar result is obtained from the North, Central, and South American LMA model (mean absolute SE = 2.3 °C) and the European LMA model (mean absolute SE = 2.1 °C). Two other LMA models, including the North and Central American LMA and the South American LMA (with mean absolute SE = 2.0 °C), had a relatively low mean absolute standard error. The Australian LMA model underestimates MATs in most samples and the mean absolute SE is the highest among all tested models (3.5 °C). The errors of estimated values from PHYSG3ARC and PHYSG3BRC are relatively higher than most other models (mean absolute SE = 3.1 °C and 2.9 °C, respectively).

According to the slope equality test (Sokal and Rohlf, 1995), only the North and Central American and Japanese LMA equation is not statistically different from the Chinese LMA equation in slope ( $p > 0.01$ ).

## DISCUSSION

Based on this new data of 50 samples from humid to mesic forests in China, this study confirms that P appears mostly correlated to MAT among leaf characters ( $r^2 = 0.79$ ), which is consistent with previous studies (Wolfe, 1979; Wing and Greenwood, 1993; Wilf, 1997; Gregory-Wodzicki, 2000; Greenwood et al., 2004; Miller et al., 2006; Adams et al., 2008). The Chinese LMA equation shows a similar slope to other LMA models (Fig. 2). Furthermore, when applying other LMA equations to the 50 Chinese samples, all the models could approximately estimate MATs, indicating a similar trend in the relationship between P and MAT among different LMA equations. The similarity of LMA equations might be due to the same physical mechanism of teeth of leaves in woody dicotyledonous leaves that are in response to their surrounding climates (Baker-Brosh and Peet, 1997; Royer and Wilf, 2006).

In all tested LMA equations, the Chinese LMA is closest to the North and Central American and Japanese LMA. When applied to 50 Chinese samples, both of the two LMA equations yielded the best fit values amongst all tested equations (mean absolute error = 1.9 °C and  $P > 0.05$ ). According to the slope equality test, these two LMA equations share an equal slope ( $p > 0.01$ ), showing that there is no statistical difference in the relationship between P and MAT in East Asia and North and Central America.

The similarity in the spatial distribution of untoothed woody species in East Asia and North and Central America may be due to plant migrations between North America and Asia in the geological past, such as the migration across the Bering land bridge in the Eocene (Flora of North America Editorial Committee, 1993). On the other hand, both East Asia and North and Central America presently occupy latitudes similar to those in the Eocene, whereas other regions in the Northern Hemisphere, such as Europe and Africa, are now spread across a wider latitudinal range and experience markedly different climates.

Although the Chinese LMA is somewhat similar to the other LMA equations, there are still differences. Firstly, the Chinese LMA is statistically different from the most widely used East Asian LMA (Wolfe, 1979; Wing and Greenwood, 1993). We sampled leaf data from 50 Chinese forests, whereas the East Asian LMA was derived from the vegetation record of Wang (Wolfe, 1979). In Wang's (1961) flora book, he provides lists of woody species from a wide range of forests throughout China; however, a more recent work from Wu (1980) demonstrates that Wang's (1961) work is far from complete. Although this book presents the flora of woody species in a forest (Wang, 1961), it

does not appear to provide all information on the woody species in a flora (Wu, 1980). Therefore, the East Asian LMA only roughly reflects the relationship between P and MAT for the Chinese vegetation.

Secondly, the Chinese LMA is significantly different from the Australian LMA. As shown above, when applying models with the 50 Chinese samples, the Australian LMA yields the highest mean absolute error. The main causes of error may be due to the unrelated biogeographic history and different types of climates as Australia drifted northward into middle and low latitudes during the Cenozoic (Pubellier and Monnier, 2003). This drift resulted in the mass extinction of the cold-adaptive flora in the Pleistocene and the continent today lacks cold temperatures (Greenwood et al., 2004). For other warmer regions, such as equatorial Africa, the relationship between P and MAT again is totally different from milder regions such as East Asia (Jacobs, 1999). The morphology of leaf margins in warmer regions might be more affected by water availability than by temperature. Therefore, the biogeographic history and climate conditions in a region should be taken into account when regional constraints are concerned.

Beside the Australian LMA, both of the CLAMP data sets, PHYSG3ARC and PHYSG3BRC, also yielded larger mean absolute error than any other tested LMA equations (3.1 °C and 2.9 °C, respectively). The CLAMP data sets are mainly compiled from North American and Japanese vegetation with basically no Chinese samples included. With >9.6 million square km<sup>2</sup>, China is the only country in the Northern Hemisphere which includes a complete spectrum of forests from tropical in the south to cold-temperate zones in the north (Axelrod et al., 1996). Hence, the Chinese data from these diverse and continuous forests are a very important region to deduce the relationship between P and MAT. It would be valuable to integrate the Chinese data with CLAMP data sets so that CLAMP might become more robust for quantitative paleoclimatic reconstructions of Chinese paleofloras.

## CONCLUSIONS

A new LMA equation based on Chinese vegetation was developed based on new data from 50 Chinese leaf floras. This new equation, rather than the widely used East Asian LMA equation, is more appropriate to be employed for paleoclimate reconstructions of Chinese paleofloras. Further work will be needed to compare data from China and other regions and incorporate the Chinese data into other data sets, such as CLAMP, to improve the accuracy of quantitative paleoclimate reconstructions.

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