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Altitudinal variation in *Ginkgo* leaf characters: Clues to paleoelevation reconstruction

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After detailed studies of leaf area, leaf shape index, petiole length, stomatal density, stomatal index, and carbon isotope discrimination of *Ginkgo biloba* L., growing in Northwest China, the change trends of these parameters with an altitude gradient and their differences between sun and shade leaves were assessed. The results show that leaf area, petiole length, and stomatal parameters have no obvious linear relationship with altitude, but the carbon isotope discrimination shows a negative correlation with altitude, which has a potential to be an applicable paleo-altimeter. The results also suggest that the differences in stomatal density and stomatal index between sun and shade leaves had more influence on paleoelevation reconstruction than that in other parameters. Based on the linear and nonlinear correlations between carbon isotope discrimination and altitude, the paleoelevation evolution during the Middle Jurassic of the Yaojie Basin, Lanzhou in Gansu Province was estimated. The results show that the paleoelevation of the Yaojie Basin increased at first, and then decreased from the Aalenian to the early Bajocian and then to the late Bajocian in the Middle Jurassic.

altitude, carbon isotope, *Ginkgo*, leaf area, leaf shape index, Middle Jurassic, paleoelevation, stomata

Paleoelevation is important for understanding biological evolution, topographic evolvement, climatic change, and tectonic history[1]. Certain plants need definite environment and climatic conditions, which are closely related to the altitude they lived at[2]. Atmospheric temperature and pressure are controlled by the elevation. The reconstruction of paleoelevation based on fossil floras has received increased interests from both paleobotanists and tectonicians[3−5]. So far, three kinds of methodologies have been used to estimate paleoelevation using fossil floras. The first is to infer paleoelevation based on the climate-dependent temperature-laps rate[4]. The second is to use leaf physiognomy to estimate enthalpy, and then together with gravitational acceleration to estimate elevation[3,6]. The third is a recently developed approach, which calculates paleoelevation based on altitudinal changes of stomatal frequency responding to CO_2 partial pressure[7]. Different climates in the same region may be due to the different elevations, and especially in the mountain region, there often has a three-dimensional climate responding to a vertical elevation. Thus, the elevation has a great influence on estimating paleoclimate. Because the survival and the growth of plants are controlled by environment and climate, plant morphological, anatomical characters and their carbon isotopic discrimination effects are potential indicators of elevation. Then the comparative analysis between fossil and extant species of certain plants helps to find some clues for estimating paleoelevation.

Using plants to infer paleoelevation has a relatively intricate problem. It needs to consider the potential change of extant plants. The floristic change of modern

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vegetation with elevation in temperate zone is similar to the law of latitudinal distribution change; from the lowest to the highest, they are broad-leaved deciduous forest zone, broad-leaved deciduous and conifer evergreen mixed forest, and alpine coniferous forest zone in order. This type of vegetation change is accompanied by the gradually reducing trend of the leaves' width. Investigation of leaves in a species shows that leaf length, width, and area decreased with the increase of elevation[8]. Hovenden et al.[9] investigated leaf morphological changes in *Nothofagus cunninghamii* with altitudes. *Ginkgo biloba* L., a kind of relict gymnosperm, has been considered to be a sensitive species to environmental changes since dinosaur age[10,11], but the relationship between its leaf characters and the elevation has not been well investigated. In addition, the variation rules in morphological, anatomical and carbon isotopic characters of *Ginkgo* leaves with altitude have a potential contribution for paleoelevation reconstruction. Therefore, this paper reports the investigation on the morphology, anatomy, and carbon isotope changes of extant *Ginkgo* leaf growing in Northwest China with an elevational increase. Moreover, we assess the potential for the characters including leaf area, leaf shape index, petiole length, stomatal density, stomatal index, and carbon isotope discrimination to be a paleo-altimeter. Finally, the paleoelevation changes of the Yaojie Basin located in Lanzhou of Gansu Province during the Middle Jurassic are preliminarily estimated.

1 Material and methods

1.1 Collection of extant *Ginkgo* and cuticle treatment

Based on the distributing region of the extant *Ginkgo* in Northwest China, we collected extant *Ginkgo* leaves in Lanzhou, Liangdang, Tianshui, Huixian of Gansu Province, and Xi'an of Shaanxi Province in the late September of 2003 (Figure 1). In order to eliminate the influence of the tree's young-age effect, all *Ginkgo* leaves were collected from the trees over 20 years old, and some of them are over 100 years (such as those 150 years old trees in Tianshui as indicated by the nameplate). Judged from the diameter, the age of the trees in other places is over 50 years old. We collected *Ginkgo* leaves from the trees in open space at about four to five meters above the ground, which can avoid the influence of organic humus on the ground that was decomposed and released more negative CO_2. Considering that *Ginkgo* trees have a dense canopy, we collected sun leaves and shade leaves from all sides of the trees. Sun leaves were sampled from the outermost branches of the canopy layer. Shade leaves were sampled from the innermost branches. About 80 leaves were collected from each sampling site.

The leaves from each sampling site were divided into three portions, which were used for morphological measurement, stomatal analysis, and carbon isotope determination, respectively. For chemical treatment, after

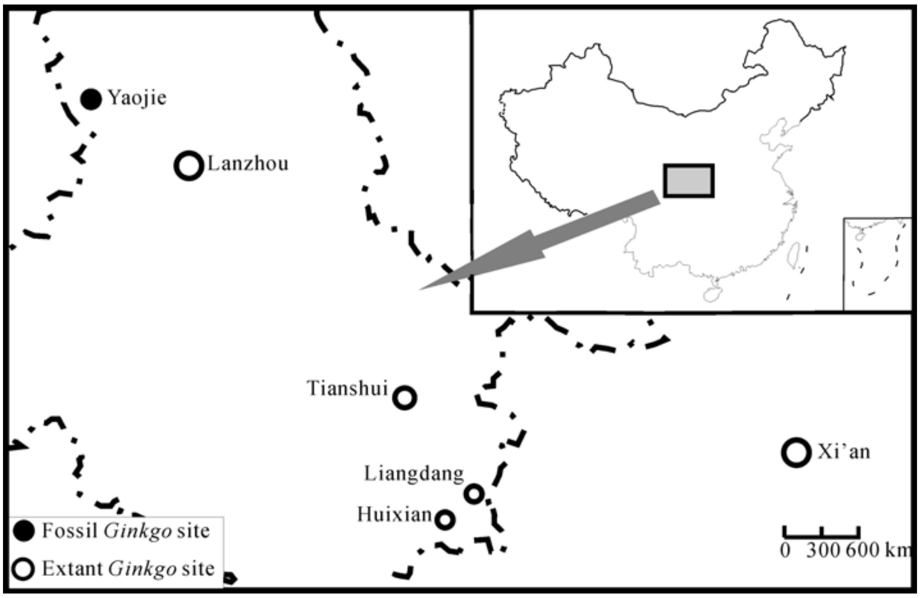

Figure 1 Collection localities of fossil and extant *Ginkgo* in Northwest China.

being cleaned, leaves were put into numbered beakers, added the mixture of hydrogen peroxide and 30% acetic acid (1∶1), then heated to 60℃. After about 12 hours, when the leaves became somewhat transparent, the leaves were taken out, cleaned and distracted into another beaker filled with distilled water, then the middle part of the lamina was taken with a scalpel, removed the cuticles from leaf surface with a forceps, then brushed mesophyll lightly. Finally the cuticles were dyed with red staining solution, mounted with glycerin glue and Canada balsam, and then labeled.

1.2 Collection of fossil *Ginkgo*, chemical treatment and carbon isotope measurement

Ginkgo cuticles (Figure 2) in this study were collected from the Yaojie Formation of the Yaojie Coalfield, Lanzhou, Gansu Province. The Yaojie Formation, 235.5 m in thickness, is underlain by the Lower Jurassic Tandonggou Formation and overlain by the Upper Jurassic Xiangtang Formation. In an ascend order, the Yaojie Formation is divided into five members, i.e., glutenite member (J_2y^1), coal-bearing member (J_2y^2), clay stone member (J_2y^3), oil shale member (J_2y^4), and sandy and muddy stone member (J_2y^5). The lower part (J_2y^1 and J_2y^2) of the Yaojie Formation belongs to the Aalenian (Jurassic) and the upper part (J_2y^3, J_2y^4 and J_2y^5) is the Bajocian (Jurassic), induced from assemblage of fossil plants and sporo-pollen[12].

Methodology of chemical treatment, stomatal counting, and carbon isotope measurement of fossil and extant *Ginkgo* leaves followed the procedures in Sun et al.[11] and Xie et al.[13].

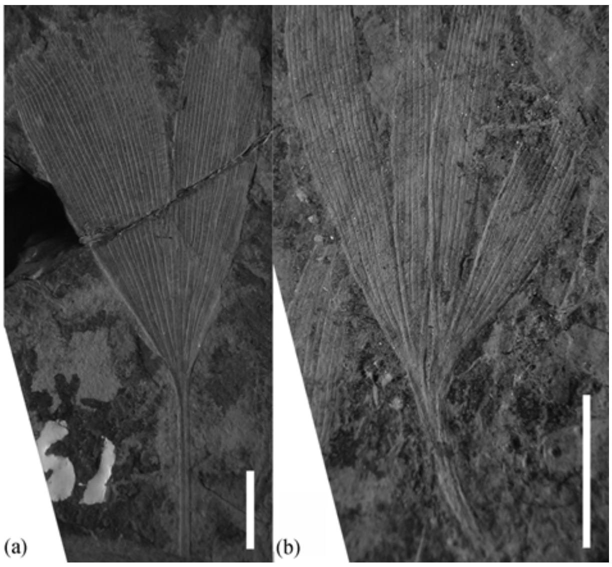

Figure 2 Fossil *Ginkgo* collected from the Yaojie Formation of Middle Jurassic. (a) *Ginkgo huttonii* (Sternb.) Heer, scale=1 cm; (b) *Ginkgo chilinense* Lee, scale=1 cm.

2 Analysis and discussion

2.1 Extant *Ginkgo* leaf morphology analysis

2.1.1 *Ginkgo* leaf area and leaf shape index

With the increase of elevation, *Ginkgo* leaf area (eight samples per collecting site) decreased, but leaf shape index (leaf length: leaf width) increased (Figure 3). However, the determination coefficient of linear regression between leaf area of *Ginkgo* and elevation is very low (R^2=0.1), which means a poorly fitted decreasing trend between *Ginkgo* leaf area and the elevation. Other factors (e.g., temperature, water, light) also affect the leaf size[14], and leaf area of *Ginkgo* did not show a good negative altitudinal trend. Similarly, the determination coefficient of leaf shape index with elevation is also very poor (R^2=0.4). Thus, these two morphological parameters are not good enough to contribute to the paleoelevation reconstruction. This result is consistent with the study of Kouwenberg et al.[15].

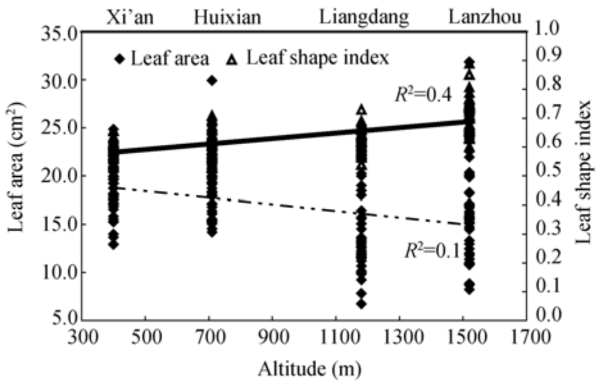

Figure 3 Linear relationship between leaf area and leaf shape index versus altitudes for *Ginkgo* growing in Northwest China (collected in 2003).

2.1.2 *Ginkgo* petiole length

Petiole is an important tissue in plants that transports water and nutrition. Plants absorb water from soil through roots, then transport it into lamina through the leaf petiole and finally into leaf cells for photosynthesis. Thus, the length of leaf petiole may relate to physiological response of plants to water conditions[16]. The present study shows that while the altitude increased, the *Ginkgo* leaf petiole length (eight samples per collecting site) also slightly increased (Figure 4). Although this trend needs further testing, if this is a universal law, one reasonable explanation is that with the increase of elevation, the content of soil moisture decreases, plants water stress increase, then the increase of the leaf petiole length can reduce water loss through the leaves' stomata[16]. However, the correlation between petiole length and altitude is poor (R^2<0.1), and thus using this relation to estimate paleoelevation is not credible.

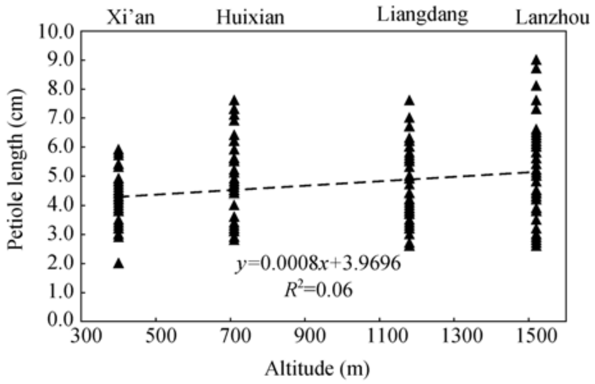

Figure 4 Linear relationship between leaf petiole lengths versus altitudes for *Ginkgo* growing in Northwest China (collected in 2003).

2.2 Relation between stomatal parameter and altitude

It is well known that with the increase of altitude, atmospheric CO_2 partial pressure declines[17,18], and stomatal parameters (stomatal index and stomatal density) are expected to increase[7,19]. Indeed this phenomenon appears in some species, such as *Picea crassifolia*[19], *Quercus kelloggii*[7]. However, from the *Ginkgo biloba* sampled from Northwest China in 2003 (Figure 5) (eight samples per collecting site), with the altitude increase, stomatal density and stomatal index have a slight downward trend, regression coefficient is less than 0.1, and no significant linear correlation with the altitude exists. This may be related to the following factors. Firstly, *Ginkgo* trees has become cultivated plants for a long time, most of our samples were collected from non-wild environment. Secondly, *Ginkgo* trees are mostly inclined to live at lower altitudes where water is sufficient[20], and high-altitude condition may be a limited factor for the growth of *Ginkgo biloba*, as well as the altitudinal trends observed from leaf shape index and petiole length. Lastly, different climatic conditions in sampling region may also bias the results. Thus, different from the observations of some previous studies[18,21], our results show the increased trend of stomatal parameters with attitudinal increase does not always occur under any circumstance. Using stomatal parameters as a barometer, we should collect wild samples on the same mountain, which will avoid the collection bias from the different growing environments.

2.3 Carbon isotope discrimination in response to elevation

Ginkgo biloba is a woody plant, which uses C_3 way to photosynthesis, so its leaves' carbon isotope discrimination follows the C_3 carbon isotope discrimination model[22]. This model is a multiple-variable function whose factors include carbon isotopic composition of atmospheric CO_2, climatic parameters, geographic elements, and microenvironment. In certain circumstances, however, one or more factors may play a key role. Therefore, while using carbon isotopic composition of

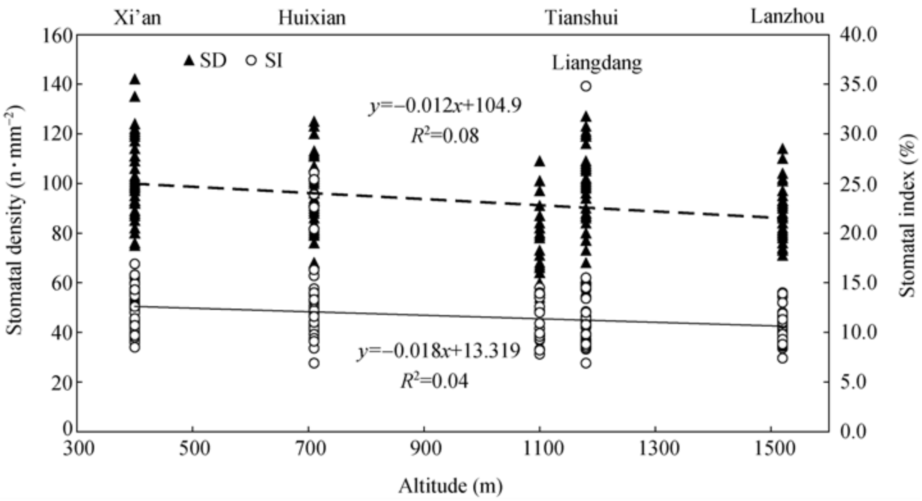

Figure 5 Linear relationships of stomatal density and stomatal index versus altitudes for *Ginkgo* growing in Northwest China (collected in 2003).

plant fossils to explain paleoenvironment, we must carefully consider all the factors. It is possible to make a reasonable and conclusive explanation of the paleoenvironmental condition when the key factors are taken into account. Carbon isotopic compositions of *Ginkgo* leaves on different altitude in Northwest China have been measured (eight samples per collecting site) in the present study (Table 1).

Based on carbon isotopic composition of *Ginkgo* leaves, we calculated the value of carbon isotope discrimination according to the formula of Farquhar et al.[23]. Taking the industrial pollution of Lanzhou City into account, we used −11‰ as atmospheric CO_2 carbon isotopic composition of Lanzhou[24], and the other locations (Liangdang, Tianshui, Huixian, Xi'an) adopted global background value −8.0‰ (http://cdiac.esd.ornl.gov/). Beerling et al.[25] got the value of carbon isotopic composition of atmospheric CO_2 in the Middle Jurassic from marine carbonate. Additionally, the difference of carbon isotopic composition between whole leaf and cuticles has been revised as 1.5‰[26]. From the result (Figure 6), we can see with the elevation increasing, *Ginkgo* leaf carbon isotope discrimination shows a decreasing trend, which is consistent with the previous research results[27,28]. Whether using linear regression or binomial regression method, *Ginkgo* leaf carbon isotope discrimination and elevation show a very strong correlation (R^2=0.8). Sun et al.[11] has pointed out that although the *Ginkgo* trees have evolved for millions of years, their carbon isotope discrimination has maintained a relative stability. We believe that *Ginkgo* has kept an approximate constant in the carbon absorption during its long geological history, similar to the conditions in its morphology[29]. The changes of carbon isotope discrimination across an altitudinal gradient provide an opportunity for estimates of paleoelevation. We applied the regression equation in Figure 6 to the reconstruction of paleoelevation of the Yaojie Basin during the Middle Jurassic.

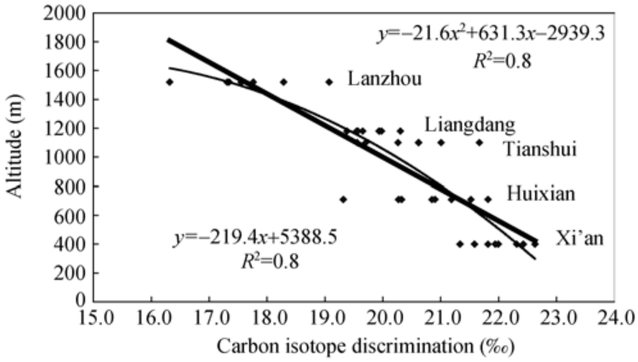

Figure 6 Relationship between altitude and leaf carbon isotope discrimination in *Ginkgo biloba* L. growing in Northwest China (collected in 2003).

2.4 The difference between sun and shade leaves

Some trees like *Ginkgo biloba* L. with dense crown canopy have potential differences between sun and shade leaves in the morphology, anatomy, and carbon isotopic composition. This introduces bias into paleoenvironment or paleoelevation reconstruction[10,11]. Our investigation (Figure 7) shows that leaf area, leaf shape index, petiole length, and leaf carbon isotope discrimination of *Ginkgo* have no significant differences (P>0.1) between sun and shade leaves, but shade leaves of *Ginkgo* show a lower stomatal density (P<0.001) and stomatal index (P<0.01) significantly compared to sun leaves. So, if applying the above parameters to paleoenvironment reconstruction, stomatal density and stomatal index have more influence than other parameters on the estimates due to the differences between sun and shade leaves. However, in practice it is much difficult to distinguish sun and shade leaves from a mixed fossil leaf assemblage, so a total regression together with sun and shade leaves in this study was expected to get a more accurate estimates. Nevertheless, if fossil sun and shade leaves could be discriminated with sufficient specimens, then the separate investigation between sun and shade morphotypes would be possible.

Table 1 Carbon isotopic composition (‰) of *Ginkgo biloba* leaves from five localities in Northwest China

Locality	Sun leaf				Shade leaf				Mean
	East	South	West	North	East	South	West	North	
Lanzhou	−27.9	−28.1	−27.8	−26.9	−28.1	−29.5	−28.8	−28.3	−28.2
Liangdang	−27.4	−27.1	−27.4	−27.0	−26.9	−27.7	−27.0	−27.0	−27.2
Tianshui	−28.0	no date	no date	−27.7	−29.0	−27.2	−27.0	−28.4	−27.9
Huixian	−27.7	−28.3	−28.3	−27.8	−29.2	−26.8	−28.6	−28.9	−28.2
Xi'an	−28.7	−29.7	−29.2	−29.0	−30.0	−29.8	−29.3	−29.4	−29.4

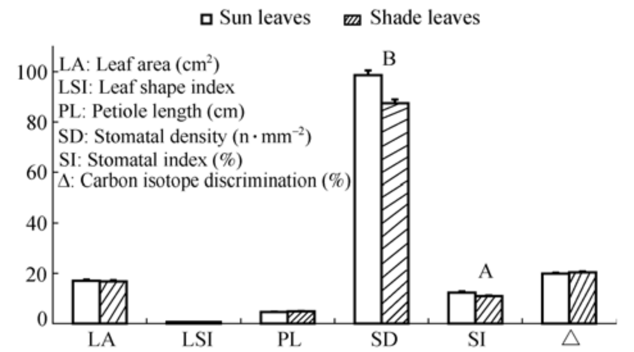

Figure 7 Morphological, anatomical parameters and carbon isotope discrimination for sun and shade leaves of *Ginkgo* from Northwest China. Means are compared using the independent-samples variance two-tailed *t*-test. A, $P<0.01$; B, $P<0.001$.

2.5 Estimation of paleoelevation of the Yaojie Basin during the Middle Jurassic

Two regression models (linear and polynomial) got similar estimates (Table 2). From the Aalenian to the early Bajocian then to the late Bajocian in the Middle Jurassic, the estimates of paleoelevation based on linear regression changed from 839 m to 1206 m, then declined to 977 m. Polynomial regression estimates showed a similar change trend, which increased from 863 m to 1246 m, then decreased at 1022 m, but the paleoelevation estimates are slightly higher than that from linear regression. This is mainly due to the differences in the regression methodologies. Further work is to find other evidences that can confirm this result.

Table 2 Paleoelevation estimates based on carbon isotope discrimination

Horizon	$\delta^{13}C_p$ (‰)	Paleoelevation from linear (m)	Paleoelevation from polynomial (m)
J_2y^5	−26.7	977	1022
J_2y^4	−25.7	1206	1246
J_2y^2	−27.4	839	863

3 Conclusion

Investigation of *Ginkgo biloba* L. leaf characters across an altitudinal gradient in five locations in Northwest China shows that with the altitudinal increase, leaf shape index and petiole length slightly increase but with no linear coefficient relationships. Meanwhile, leaf area, stomatal density, stomatal index, and carbon isotope discrimination have a decreased trend. The investigation of the differences of the above parameters (leaf area, leaf shape index, petiole length, stomatal density, stomatal index, and carbon isotope discrimination) between sun and shade leaves demonstrates that stomatal density and stomatal index have more influence on the paleoenvironmental reconstruction than others do. Regression analysis suggests a negative correlation between carbon isotope discrimination and elevation. The application of this correlation to the reconstruction of paleoelevation changes based on fossil *Ginkgo* cuticles from the Yaojie Basin shows that the paleoelevation of the Basin decreased after a considerable rise from Aalenian to Bajocian in the Middle Jurassic.

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