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GEOMETRIC MORPHOLOGIC VARIATION OF *ORMOSIA HENRYI* PRAIN LEAVES FROM DIFFERENT HUNAN PROVENANCES

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Abstract

This study was envisaged to explore the variation characteristics of *Ormosia henryi* Prain leaves from four different areas of Hunan provenance of China based on analyzing the geometric morphologic information of the leaf. Specifically, the leaf morphological indices were firstly calculated, and multiple comparison analysis, canonical analysis of the principal coordinates and fisher linear discriminant model were employed to decipher the morphological variability based on contour outlines. The results showed that the leaf morphological indices exhibited significant differences between the samples of different areas. Variability also exists among the *Ormosia henryi* of four areas of the provenance. The results provide a theoretical basis for the study of genetic diversity and genetic improvement of *Ormosia henryi*.

Introduction

Ormosia henryi Prain is a species of flowering plant in the family Fabaceae, native to southern China, Thailand and Vietnam. Plant populations of *O. henryi* have evolved in different forms during the process of long-term adaptation to the environment, and the genetic differences in the individual plants arise due to the combined effects of environmental factors and genetic background (Peterson *et al*. 2011, Li *et al*. 2018). Due to the combined effects of long-term reproductive isolation and surrounding environmental factors, the morphological characteristics and growth of different individuals and populations of the same tree species exhibit significant differences. As a result, the growth performance of species with different geographical provenances also varies in different regions (Li *et al*. 2020). Research of phenotypic variation of different provenances helps to develop an accurate understanding of the genetic variation of different provenances and the morphological variation of individuals in provenances, and provides a theoretical basis for breeding and germplasm innovation of excellent provenances (Schmidtling 1994, Matyas 1996). Leaves are one of the organs of plants that are most sensitive to environmental changes (Shipley and Almeida-Cortez 2003). Leaf traits are affected by both genetic and environmental factors (Zhang and Luo 2004) and are malleable to environmental changes (Vile *et al*. 2005). Changes in the environment increase the probability of the changes in the leaf traits. Therefore, phenotypic variation of plant leaves within and among the populations can better reflect the interaction between plants and their habitats than a genetic study, and the information about the plant survival, adaptability, adaptation range, and evolution can be obtained (Joesting *et al*. 2009, Rabara *et al*. 2014). Geometric morphometrics utilizes the relative positions of morphological points, boundary curves and surfaces rather than linear, areal or volumetric variables (Lawing and Polly 2010). Geometric morphometrics provide a more comprehensive quantification of the biomorphology than other methods, and is widely used in ecological and evolutionary studies (Heteren and Germonpre 2023, Díaz *et al*. 2023, Escobar-Suárez *et al*. 2023). This method is commonly used to reveal the phenotypic differences, identify convergent and parallel evolution, and study the macroevolutionary trends (Adams *et al*. 2007, Adams and Nistri 2010, Monteiro and Nogueira 2011).

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Ormosia henryi is a precious tree species in China and has great economic and ecological potential as a raw material for making high-grade furniture and handicrafts. Besides, it is an important tree species of the Chinese traditional medicinal crops with its roots, branches and leaves having medicinal activity (Ma and Zhang 2009, Zhang *et al*. 2012). As the wild resources of *O. henryi* are scarce, easy to be destroyed and scattered in villages or nature reserves, it has been listed in the second class of the state-protected tree species (Ma and Zhang 2009, Lu *et al*. 2019). Previous studies on *O. henryi* have focused its bioactive ingredients (Feng *et al*. 2012, Wu *et al*. 2020), medical usages (Lu *et al*. 2019), tissue culture (Wu *et al*. 2021) and genetic structure (Zhou *et al*. 2023). However, the leaf phenotypic variation and environmental adaptation of *O. henryi* from different provenances have not been studied.

In this study, the leaf samples of *O. henryi* were obtained from four different provenance forest areas in Hunan Province, China, and wavelet analysis and fourier analysis were employed to identify the different stocks of *O. henryi* with blade profile information. The study mainly focused the evaluation of the provenance differences based on the shape of blade contour shape parameters for *O. henryi* collected in central and northern Hunan Province. Simultaneously, the utility of blade contour shape was validated as an applicable technique in determination of the differences in plant provenance. The results can assist to generate a theoretical basis for the study of genetic diversity and genetic improvement of *O. henryi*.

Materials and Methods

Leaf samples of *O. henryi* plant were collected from four different areas in Hunan Province: Changning (CN), Shaoshan (SS), Zhuzhou (ZZ) and Changsha (CS). Samples were taken from two growth stages (e.g., 1 and 2 year), with 9 samples each. In order to avoid the obvious differences among the highly similar and closely related species, *O. glaberrima* (XY), a species of the same genus with similar leaves, was selected as a closely related similar species for analysis, with a total of 9 samples (Table 1).

Table 1. Information of leaf samples of *Ormosia spp.* of Hunan Province.

Sample name	Species	Area of Provenance	Sample numbers
CN	*Ormosia henryi*	Changning County	18
SS		Shaoshan City	18
ZZ		Zhuzhou City	18
CS		Changsha City	18
XY	*Ormosia glaberrima*		9

An HP scanner (resolution 600 dpi) was used to scan the leaf, with the same scale parameter. The outline of the blade in each scanned images was ensured to be clear enough, so that the edge of the blade could be seen. Image binaryzation analysis was carried out to obtain the contour information of the leaf (Fig. 1). By using the shapeR package for outline reconstruction based on Fourier/wavelet method, two data sets (Fourier/wavelet) were obtained for the following comparative analysis. Six morphological indices e.g., roundness, circularity, rectangularity, elipticity, aspectratio and form-factor was calculated in the study (Table 2).

Initially the preliminary analyses were carried out by comparing the leaf morphological characteristics of *O. henryi* and *O. glaberrima* collected in 5 provenances. They were analyzed using a univariate analysis of variance (ANOVA) test to assess the provenance and their interaction between *O. henryi* and *O. glaberrima*. A multiple comparison analysis (Tukey HSD) was used to assess the differences in coefficients between samples from 5 provenance. In addition, the reconstructed outline of leaf samples based on wavelet and Fourier method for different species, different leaf age and different provenance was plotted to analyze the variation in leaf shape caused by multiple factors.

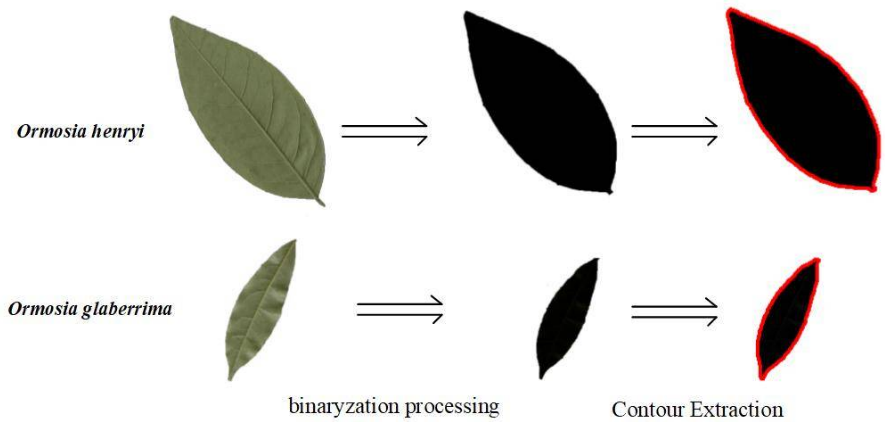

Fig. 1. Contour outlines extracted from *Ormosia henryi* and *Ormosia glaberrima* images. Red outline marks the shape of the leaf of two species.

Table 2. Leaf morphological indices calculated from the measurement data.

Morphological index	Formula*
Roundness	$4A/(\pi LL^2)$
Circularity	P/A^2
Rectangularity	$A/(LL*LW)$
Elipticity	$(LL-LW)/(LL+LW)$
Aspect ratio	LL/LW
Form-factor	$4\pi AP^2$

*A = Leaf area, LL = Leaf length (mm), P = Leaf perimeter (mm), LW = Leaf width (mm)

Canonical analysis of principal coordinates (CAP) was performed on the wavelet and Fourier transform data to explore the relationships between the leaf shape and the geographic provenance. Fisher Linear Discriminant Model was used to evaluate the classification effect of the leaf shape from 5 provenances.

Results and Discussion

There was a significant relationship between the four morphological indices and the species (Table 3). The growth stage had no significant correlation with any of the morphological indices. The statistical results provided a clear indication of the morphological differentiation between the *O. henryi* and *O. glaberrima*. The morphological differentiation among provenance was mainly focused on circularity.

Table 3. Leaf morphological indices of different leaf samples using ANOVA tests.

Morphological indices	Parameters	Sum of square	Mean of square	*P*
Roundness	species	3645474	5.399	0.02*
	Growth stage	1617003	2.395	0.13
	Provenance	1452573	2.151	0.10
	Growth stage×provenance	2466821	3.653	0.02*
Circularity	Species	0.025342	8.631	0.005*
	Growth stage	0.00606	2.064	0.16
	Provenance	0.015992	5.447	0.002*
	Growth stage×provenance	0.029925	10.192	1.26E-05*
Rectangularity	Species	0.06946	16.418	0.0001*
	Growth stage	0.00678	1.603	0.21
	Provenance	0.00905	2.14	0.10
	Growth stage×provenance	0.00541	1.279	0.29
Elipticity	Species	0.12084	1.347	0.25
	Growth stage	0.18484	2.061	0.16
	Provenance	0.16855	1.88	0.14
	Growth stage×provenance	0.16896	1.884	0.14
Aspectratio	Species	0.0719	0.122	0.73
	Growth stage	0.9779	1.661	0.20
	Provenance	0.47	0.798	0.50
	Growth stage×provenance	1.2378	2.103	0.11
Formfactor	Species	0.21649	101.926	3.69E-15*
	Growth stage	0.00838	3.947	0.05
	Provenance	0.00373	1.754	0.16
	Growth stage×provenance	0.00082	0.386	0.76

* indicates significant differences at the level of $P = 0.05$.

The boxplot of the leaf morphological indices between the geographic provenance and multiple comparative analysis is showed in Fig. 2. The rectangularity and form-factor of XY was significantly different in all *O. henryi* samples ($P < 0.05$), the roundness and circularity of only XY was significantly different from CS, the circularity of ZZ was significantly different from CS. The remaining morphological indices exhibited no significance among *O. henryi* samples. The samples from CS provenance presented a smaller circularity than others.

The results of CAP analysis were showed in Fig. 3. The first two canonical axes explained 27.61% of the variation based on the wavelet analysis (CAP1: 25.42%; CAP2: 2.19%), demonstrating a clear difference between the XY and other four *O. henryi* samples (Fig. 3A), and there was samller difference between the four *O. henryi* provenances. The first two canonical axes only explained 1.14% of the variation based on the wavelet analysis (CAP1: 1.01%; CAP2: 0.13%) (, Fig. 3B), and XY showed a clear difference with *O. henryi* samples.

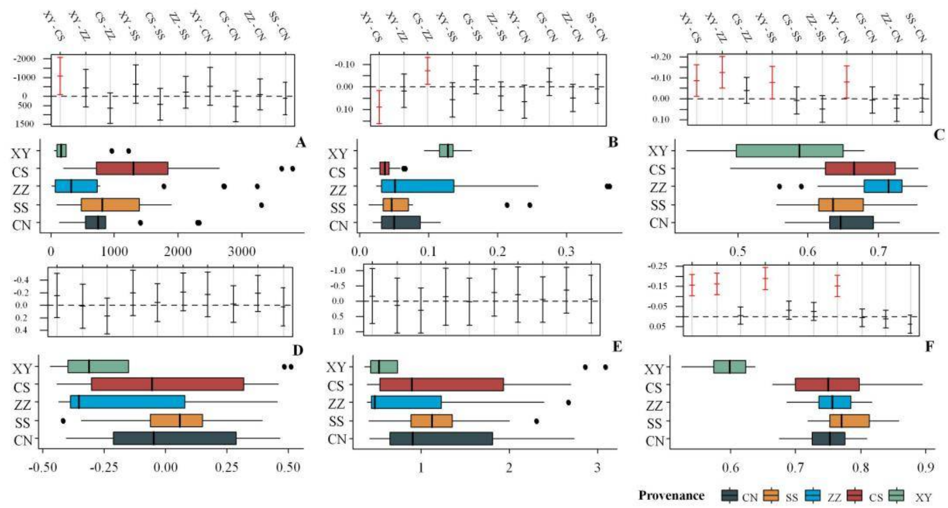

Fig. 2. Boxplot and multiple comparison analysis (Tukey HSD) of 6 morphological indices. A-F: roundness, circularity, rectangularity, elipticity, aspect ratio and form-factor. Tukey HSD analysis was performed at 95% family-wise confidence level.

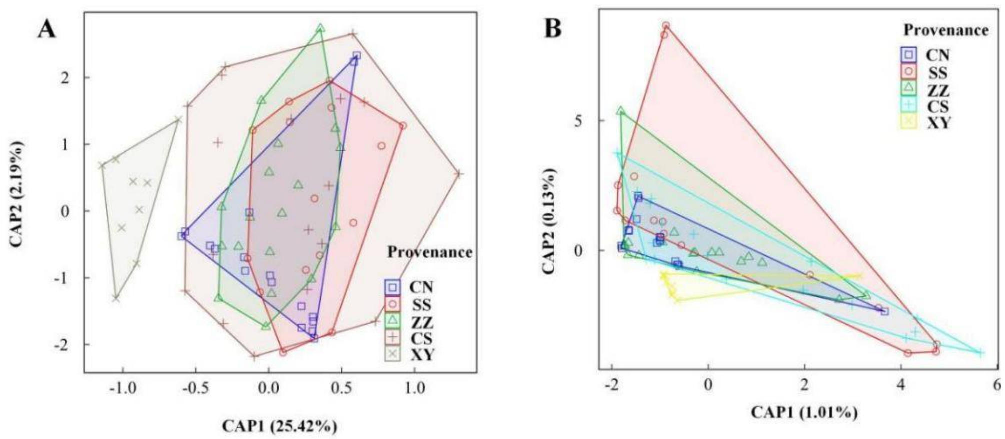

Fig. 3. Canonical analysis of principal coordinates of the Wavelet (A) and Fourier (B) coefficients for five provenances. CAP1 and CAP2 are the first and second discriminant axis, respectively.

To further determine whether the grouping is appropriate, the fisher linear discriminant model analysis was employed (Table 4). In the discriminant analysis based on wavlet and fourier analysis, LD1 and LD2 accounted for the largest proportion of variance. Among the four linear discriminant axis, the samples were best divided, when LD1 and LD2 were used (Figs. 4A and B). The four different provenances and XY were delineated based on wavelet, but an overlap was observed between CN and ZZ, and SS and CS (Figs. 4C and D).

Discriminant analysis showed that the wavelet analysis could effectively separate different provenances with a higher accuracy (98.7%) than that of the fourier analysis (83.12%) (Table 4). In particular, the wavelet analysis yielded the highest discrimination rate (100%) of CN, ZZ, CS and XY. It can be seen that the discriminant model exhibited a good discriminant effect.

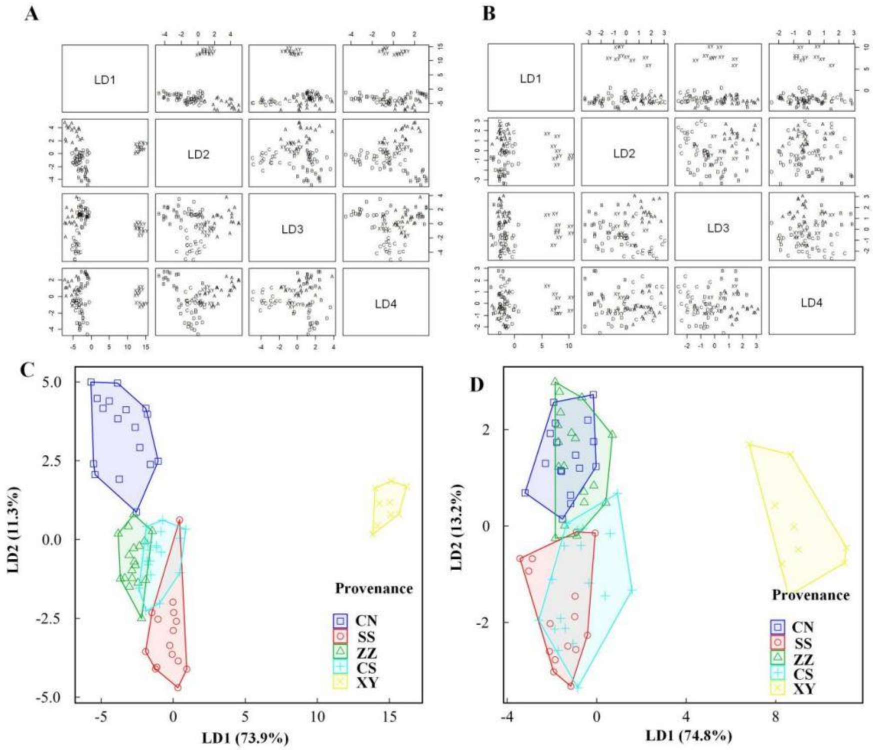

Fig. 4. Discriminant scatter plot of the Wavelet (A and C) and Fourier (B and D) coefficients for five provenances using Fisher Linear Discriminant model.

Table 4. Discriminant analysis and accuracy of different reconstruction methods.

Reconstruction method		CN	SS	ZZ	CS	XY	Accuracy (%)
Wavelet (98.7%)	CN	17	0	0	0	0	100.00
	SS	0	14	0	1	0	93.33
	ZZ	0	0	18	0	0	100.00
	CS	0	0	0	18	0	100.00
	XY	0	0	0	0	9	100.00
Fourier (83.12%)	CN	16	0	1	0	0	94.12
	SS	2	10	0	3	0	66.67
	ZZ	1	0	15	2	0	83.33
	CS	0	2	2	14	0	77.78

The reconstructed average leaf shapes based on wavelet and fourier analysis are shown in Fig. 5. According to the results of wavelet analysis, the differences were mainly at the leaf tip and petiole (about 0° and 180°) (, Figs. 5A-C), which was further confirmed by examining the variability of the mean wavelet coefficients and the proportion of variation between the between the two groups, summarized by the ICC (intra-class correlation) (, Figs. 5D-E). When compared to the results of the wavelet analysis, the differences were mainly concentrated on the sides of leaves based on fourier data (about 90° and 270°), especially between the different species and ages. Leaf shape can affect the photosynthetic area of a leaf, and plants can also influence the water-air exchange by changing the perimeter-area ratio of the leaf (Boyce 2009). In hot and humid environments, a near-circularity leaf shape can promote the water and air exchange with the outside environment (Hirokazu 2005). From to the results of the fourier analysis, it was observed that leaf of *O. microphylla* tended to be more rounded than *O. henryi*. This data indicates that *O. henryi* was distributed in more humid and hot environment. Similarly, the habitat of CN provenance presented the best hydrothermal conditions, followed by SS, CS and ZZ. The difference between the 2nd growth stages also indicated an allometry of the *O. henryi* leaves, which can be explained by water limitation, leaf temperature regulation, and biomechanical limitation (Nicotra *et al*. 2011).

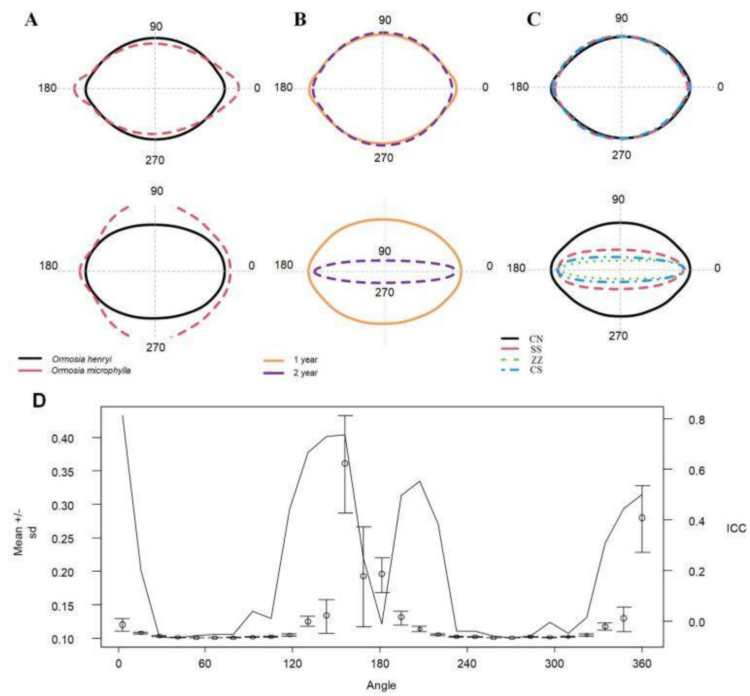

Fig. 5. Mean leaf shape based on Wavelet (up) and Fourier (down) reconstruction. A: Between *Ormosia henryi* and *Ormosia microphylla*, B: Between1st and 2nd year, C: Between different provenances, D: Mean and standard deviation (dots and whiskers) of the Wavelet coefficients for all combined leaf and the proportion of variance within groups (species and provenance) for the intra-class correlation (ICC, black solid line)

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