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GEOMETRIC MORPHOLOGICAL VARIATION CHARACTERISTICS OF *LOROPETALUM CHINENSE* (R. BR.) OLIV. LEAVES AND THEIR RESPONSE TO INTRA-SPECIFIC COMPETITION

NAN DENG[1], JINGDI WANG[2*], QINGAN SONG[1] AND YUXIN TIAN[1]

Hunan Academy of Forestry, Changsha, Hunan, No.658 Shaoshan Road, Tianxin District, Changsha, 410004, China

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Abstract

This study aimed to explore the geometric morphological variation characteristics of the leaves of the understory plant *Loropetalum chinense* (R. Br) Oliv. under different competitive pressures. Specifically, the leaf morphological indices were calculated, and multiple comparison analysis and Fisher linear discriminant model were employed to decipher the morphological variability based on contour outlines. Results revealed that the leaf morphological indices exhibited significant differences among various sample plots. The lower stand density and diameter at breast height (DBH) facilitated more water and air exchange and led to a rounded leaf shape. The results provided a theoretical basis for the study of response of leaf morphological evolution to forest competition.

Introduction

Plant traits are considered the best explanation as to how and why species composition and biodiversity influence ecosystem functioning (Díaz *et al.* 2016, Chacón-Labella *et al.* 2023, Hagan *et al.* 2023). The functional traits are based on important aspects of plant physiology and morphology (Reich 2014, Carmona *et al.* 2021), the mechanisms governing competitive coexistence (Kraft *et al.* 2015, Klausmeier *et al.* 2020), and the processes that drive fluxes of matter and energy (Roscher *et al.* 2012, Furey and Tilman 2021). As leaves are the main organ of photosynthesis, they provide basic substances for plant growth and reproduction. The study of variation in leaf functional traits can thus help address many important ecological questions (Pérez-Harguindeguy *et al.* 2013). Leaf morphology is influenced by both genetic and environmental factors and varies between and within species, reflecting plant survival strategies (Gong *et al.* 2020). At the interspecific level, leaf morphology varies among species under the same environmental conditions in response to temperature, light, and other environmental conditions (Fisher 1954, Nicotra *et al.* 2008, Mocko *et al.* 2018, Gallaher *et al.* 2019). For example, the leaves of *Dactylis glomerata* and *Cibotium glaucum* become longer with increasing level of shade (Lohmann *et al.* 1983, Pritchard *et al.* 2000), and the leaves of *Alseuosmia pusilla* switch from left-sided elliptical shape to right-sided wedge shape with decreasing temperature (Yager *et al.* 2016). Geometric morphology is the shape obtained by removing all spatial positional information from an object and describing it by points and contours (Nery and Fiaschi 2019). The geometric morphological method digitizes the leaf. It helps quantify the degree of variability in leaf morphology (Gong *et al.* 2020) to elucidate the relationship between leaf morphology and environmental factors (Glennon *et al.* 2015).

[*]Author for correspondence: <197981235@qq.com>. [1]Hunan Cili Forest Ecosystem State Research Station, Cili, Hunan, Changsha, 410004, China. [2]Zhangjiajie Municipal Forestry Bureau, Zhangjiajie, 427000, Hunan Province, PR China.

The competition between trees is a widespread phenomenon in forest ecosystems and is one of the main factors influencing plant growth, morphology, and survival, as well as an essential driver of the direction of plant community evolution (Liu *et al.* 2020). Masson pine (*Pinus massoniana* Lamb.) is a major coniferous tree species widely distributed in the subtropical forests of South China (Quan and Ding 2017). In recent years, a large number of management activities were conducted in planted forests in the study area, improving competition within the stand to some extent. *Loropetalum chinense* (R. Br.) Oliv. is the most common shrub species in understory, which grows in a wide range of environments. This study aimed to explore the morphological changes of *L. chinense* leaves in response to different stand structures in pure Masson pine forest, and to examine the mechanisms of leaf morphology evolution on a small scale.

Materials and Methods

The present study was conducted at Hunan Cili Forest Ecosystem State Research Station, located in Cili, Hunan Province, China. A total of three pure Masson pine sample plots (20 × 50 m^2), without management (M0) and with different management models (M1 and M2), were selected as the study sites. The management intensities of M1 was higher than M2. The soil of the forest is mainly yellow-red and yellow, and belonged to the humid subtropical climate zone. A terrestrial laser scanning radar (SLAM100, Shenzhen Feima Robotics Co. Ltd.) was used to acquire the spatial data of the forest. Lida360 software was used to process the point cloud data (Fig. 1A), where the point cloud preprocessing included de-noising, Octree algorithm (Octree) thinning, ground point classification, improved progressive triangular irregular network (TIN) densification, ground point filtering, and normalization. The stand density, tree height, DBH, crown diameters (S–N and E–W), crown area, and crown volume (m^3) were extracted after the processing.

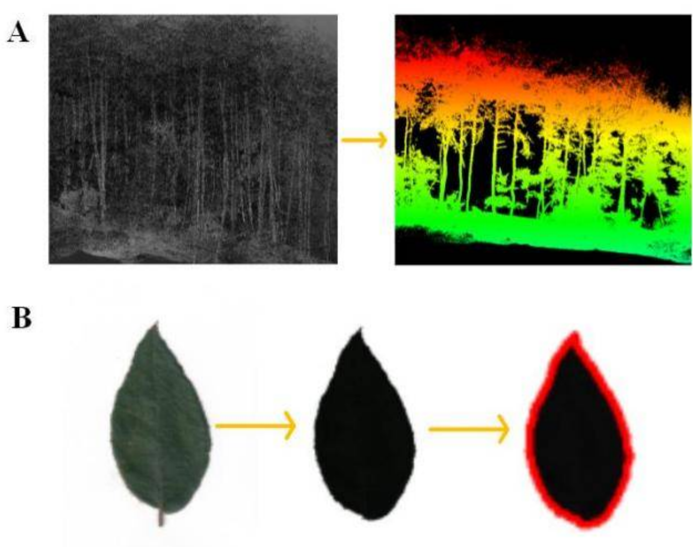

Fig. 1. Diagram of extracting stand parameters (A) and contour outlines (B).

Nine *L. chinense* plants were selected from each sample plot. The leaf samples were collected from plant's canopy, and 149 leaves were collected from each plot. All sampled leaves were scanned (HP scanner, resolution 600 dpi). The outline of the leaves in each scanned image was clear enough to ensure that the edge of the leaves was seen. Image binarization analysis was carried out to obtain the contour information of the leaves (Fig. 1B). The shapeR package was used for outline reconstruction based on Fourier/wavelet method, obtaining two data sets (Fourier/wavelet) for a comparative analysis. Subsequently, the following six morphological indices were calculated and analysed: roundness, circularity, rectangularity, ellipticity, aspect ratio, and form factor (Table 1).

Table 1. Leaf morphological indices calculated from the measurement data.

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

A, Leaf area; LL, leaf length (mm); LW, leaf width (mm); P, leaf perimeter (mm).

Preliminary analyses were first carried out comparing the morphological characteristics of leaves collected from the three sample plots. They were analyzed using a univariate analysis of variance test. A multiple comparison analysis (Tukey honestly significant difference) was used to assess the differences in coefficients among different leaf samples. In addition, we reconstructed an outline of leaf samples based on the wavelet and Fourier method for different sample plots. Different plots and positions were plotted to analyze the variation in leaf shapes caused by multiple factors. The Fisher linear discriminant model was used to evaluate the classification effect of leaf shape sampled from three different plots.

Results and Discussion

According to the analysis of forest stand structure (Table 2), the M0 sample plot had the maximum values for tree height and DBH and the M1 sample plot had the maximum values for crown area and crown volume. The crown diameter of each plot was similar. The M1 sample plot had the lowest stand density compared with others. The area, length, width, perimeter, roundness, and circularity of leaves were significantly different among the three sample plots (Table 3). This result indicated a morphological differentiation among various forest structures.

Table 2. Nine spatial indices of each sample plot.

Sample plot	P1	P2	P3	P4	P5	P6	P7	P8
M0	8.08	18.49	3.32	3.75	3.56	12.63	49.32	162
M1	8.01	16.21	3.42	3.87	3.69	13.34	72.22	119
M2	6.62	17.29	3.42	3.71	3.84	12.00	45.56	120

P1–P8 represent tree height, DBH (m), crown diameter (m), crown diameters (S–N and E–W), crown area (m^2), crown volume (m^3), and stand density (per hm^2), respectively.

Table 3. Analysis of variance tests for the morphological indices of different leaf samples.

Leaf morphological indices	Sum of square	Mean of square	*P*
Area	140.2	70.1	7.17E-05*
Length	3.051	1.526	0.000359*
Width	8.03	4.013	0.00137*
Perimeter	52.7	26.365	0.000218*
Roundness	58575	29288	0.000132*
Circularity	0.1094	0.055	0.000506*
Rectangularity	0.00213	0.001	0.182
Ellipticity	0.0034	0.002	0.619
Aspect ratio	0.0062	0.003	0.591
Form factor	0.0032	0.002	0.6

[#]Indicates significant differences at the level of $P = 0.05$.

The boxplot of leaf morphological indices among the different plots and multiple comparative analysis are shown in Fig. 2. The area, length, perimeter, and roundness of leaves from the M1 sample plot were significantly higher than those of leaves from the other plots. The circularity of leaves from the M1 sample plot was significantly lower than that of leaves from the other plots. The width of leaves from the M1 sample plot was significantly higher than that of leaves from the M0 sample plot.

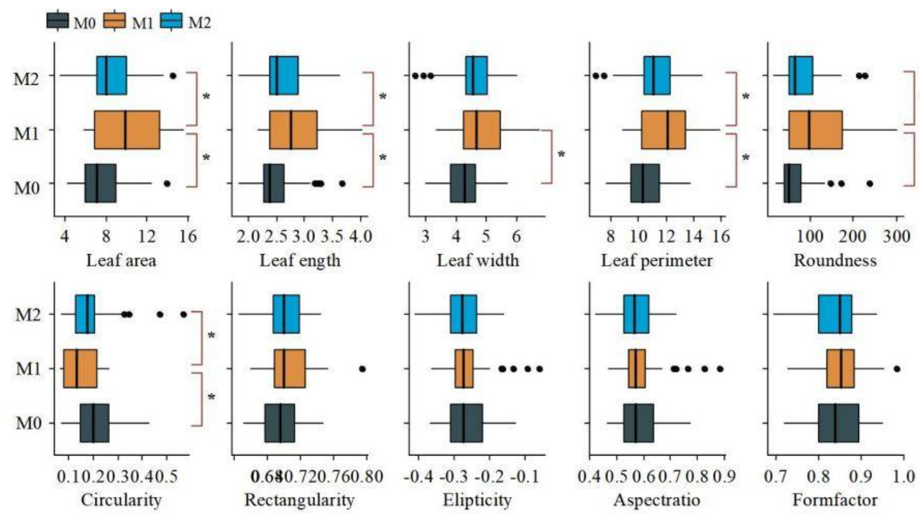

Fig. 2. Boxplot of 10 morphological indices. Asterisks in parentheses indicate significant differences.

The Fisher linear discriminant model analysis was performed to further determine whether the grouping was appropriate. In the discriminant analysis based on wavelet and Fourier analyses, LD1 and LD2 accounted for the largest proportion of variance. The samples were best divided when LD1 and LD2 were used among the four linear discriminant axes (Figs. 3A and 3B). The plots were delineated based on wavelet and Fourier analyses, but overlapping was observed among the three plots.

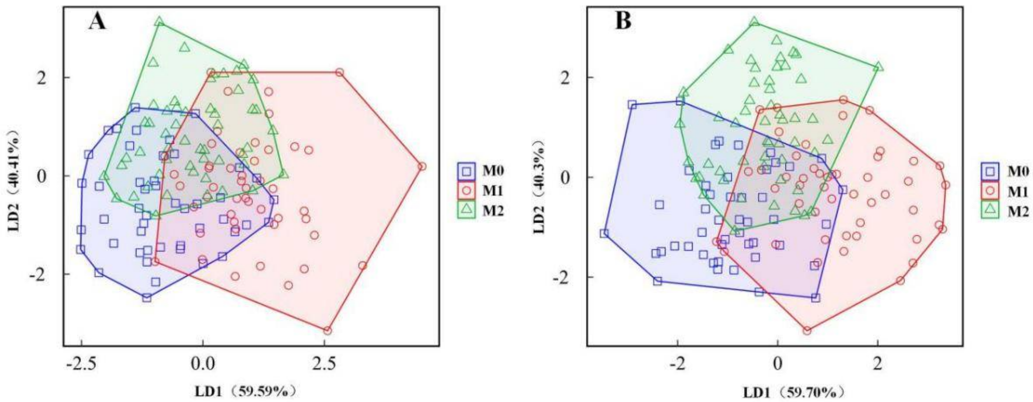

Fig. 3. Discriminant scatter plot of the wavelet (A) and Fourier (B) coefficients for the three sample plots using Fisher linear discriminant model.

The discriminant analysis showed that wavelet analysis could effectively separate different provenances with greater accuracy (70.27%) compared with Fourier analysis (66.22%) (Table 4). In particular, the wavelet analysis yielded the highest discrimination rate for the M0 sample plot (70.21%), followed by the M1 sample plot (71.43%), and the M2 sample plot (69.23%). The discrimination rate yielded by Fourier was 69.23%, 69.39%, and 67.31% for the M0, M1, and M2 sample plots, respectively. The discriminant model revealed a good discriminant effect.

Table 4. Discriminant analysis results and accuracy measures of different reconstruction methods considered in the present study.

Reconstruction method		M0	M1	M2	Accuracy (%)
Wavelet (70.27%)	M0	33	5	9	70.21
	M1	5	35	9	71.43
	M2	10	6	36	69.23
Fourier (66.22%)	M0	29	9	9	61.70
	M1	6	34	9	69.39
	M2	9	8	35	67.31

The reconstructed average leaf shapes based on wavelet and Fourier analyses are shown in Fig. 4. The reconstructed average leaf shapes were used for comparison among leaf samples from different plots. The wavelet analysis showed that the differences were mainly at the leaf tip and petiole (180°) (Fig. 4A). However, the differences were mainly concentrated on the sides of leaves according to Fourier analysis (about 90° and 270°). The leaf shape can affect the photosynthetic area of a leaf, and the plants can also influence the water–air exchange by changing the perimeter–area ratio (Boyce 2009). In hot and humid environments, a near-circularity leaf shape facilitates more water and air exchange with the external environment (Hirokazu 2005). According to the Fourier analysis, the leaves from the M1 sample plot were more rounded than those from other plots, explaining the lower stand density and DBH, which facilitated more water and air exchange. The shorter tree height and higher DBH of the M2 sample plot indicated that the trees were compressed within the forest, leading to narrower leaves.

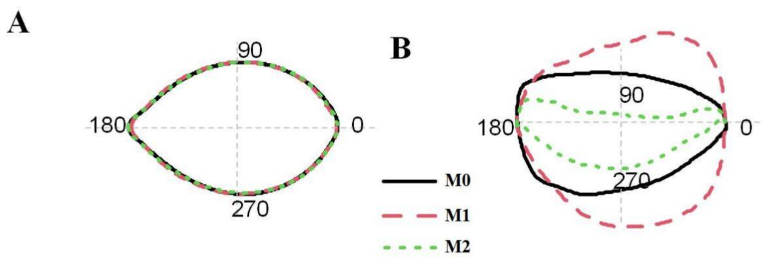

Fig. 4. Mean leaf shape based on Wavelet (A) and Fourier (B) reconstruction.

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