Postprint: Genetic Structure of Magnolia sieboldii in Two Vegetation Zones of East China

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Abstract

Magnolia sieboldii is a valuable and rare ornamental tree species currently listed as a National Grade III protected plant. The vegetation types in its natural distribution range can be broadly classified into the temperate deciduous broad-leaved forest zone (north of the Yangtze River) and the warm temperate evergreen broad-leaved forest zone (south of the Yangtze River). East China represents one of the regions with a relatively high concentration of Magnolia sieboldii distribution and also constitutes the boundary zone between the two vegetation zones. The present study utilized microsatellite markers to analyze the genetic diversity and genetic structure of eight natural populations of Magnolia sieboldii within the two vegetation zones in East China. Results demonstrated that the genetic diversity of Magnolia sieboldii populations in East China was relatively low compared to closely related species (NA = 3.83, HO = 0.25, HE = 0.40). The genetic diversity of populations in the Dabie Mountains (deciduous broad-leaved forest zone) (mean HO = 0.18 and HE = 0.28) was significantly lower than that of populations in southern Anhui (evergreen broad-leaved forest zone) (mean HO = 0.33 and HE = 0.51), and significant genetic differentiation was detected between them. Population characteristics (such as population size, diameter at breast height, or number of clustered branches) within the two vegetation zones did not differ significantly, and no significant alterations in genetic diversity were detected between young and old subpopulations within populations. Therefore, we hypothesize that the relatively short population history experienced by Magnolia sieboldii populations in the Dabie Mountains (compared to those in southern Anhui) constitutes the primary cause of their lower genetic diversity. We recommend that Magnolia sieboldii populations in the two vegetation zones should be regarded as distinct evolutionary units for conservation, and in-situ conservation should continue to be the primary strategy at present.

Full Text

Preamble

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The Genetic Structure of *Oyama sieboldii* Within Two Vegetation Zones in Eastern China

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Abstract

Oyama sieboldii (K. Koch) N.H. Xia & C.Y. Wu is a rare and valuable ornamental tree species that has been listed as a national Grade III protected plant in China. The species is naturally distributed across two distinct forest vegetation zones: the temperate deciduous broad-leaved forest north of the Yangtze River and the warm temperate evergreen broad-leaved forest south of the Yangtze River. Eastern China represents one of the concentrated distribution areas of *O. sieboldii* and constitutes the boundary zone between these two vegetation types. This study analyzed the genetic diversity and structure of eight wild populations of *O. sieboldii* from Eastern China using microsatellite markers.

The results indicated that the genetic diversity of *O. sieboldii* in this region was relatively low (mean A = 3.83, H_O = 0.25, and H_E = 0.40) compared to its closely related species. No significant differences were observed in mean population size, diameter at breast height (DBH), or number of branches between populations from different vegetation zones. However, the genetic diversity of Dabie Mountain populations within the temperate deciduous broad-leaved forest (mean H_O = 0.18 and H_E = 0.28) was significantly lower than that of Southern Anhui populations within the temperate evergreen broad-leaved forest (mean H_O = 0.33 and H_E = 0.51), and their genetic characters had obviously differentiated from each other. The distinct population history might mainly explain the difference in genetic diversity between these two zones. The genetic diversity of young individuals was not significantly lower than that of old individuals within each population.

Therefore, we propose that the populations in the Dabie Mountain area and Southern Anhui area should be considered as two different evolutionary significant units for conservation, and an in-situ conservation strategy should be taken as the main protecting measure at present, given that the regeneration ability of wild populations was normal and the genetic diversity of young individuals did not significantly decrease.

Keywords: *Oyama sieboldii*; genetic diversity; genetic structure; vegetation zone; conservation strategy

Introduction

The Magnoliaceae family, as an important representative of extant primitive angiosperm groups, possesses high economic value for timber, medicinal, ornamental, and scientific research purposes. With fossil records indicating an origin in the Cretaceous Albian period, these ancient woody plants include many tall tree species that constitute important forest and timber resources. However, due to climate change, biological characteristics, and human disturbance including over-harvesting, more than half of Magnoliaceae taxa worldwide are now facing extinction, making the conservation and sustainable utilization of wild germplasm resources an urgent priority.

Oyama sieboldii (K. Koch) N.H. Xia & C.Y. Wu, also known as small-flowered magnolia, is a deciduous small tree and typical Tertiary relict plant. It is scattered across Northeast and East China, with small distributions in Japan and Korea, and is recognized as a rare and valuable ornamental tree species endemic to East Asia. The plant' s extracts can be used in cosmetics, fragrances, and pharmaceuticals, giving it high economic utilization value. However, its distribution area is gradually shrinking due to climate change, human logging, and other factors, leading to sharp declines in population and individual numbers. Consequently, it has been listed as a national Grade III protected plant in China' s Rare and Endangered Plant Species Red List (1984).

Previous research on *O. sieboldii* has primarily focused on population community structure, seed germination characteristics, reproductive strategies, and rapid propagation techniques. However, studies on population genetic diversity and genetic structure remain unreported. Vegetation, defined as the totality of plant communities in a region, represents the most important feature of habitat landscapes and provides the necessary material foundation and environment for the survival of plants and microorganisms. It significantly influences the genetic characteristics of regional flora and fauna.

The vegetation in *O. sieboldii*' s current wild distribution area can be broadly classified into two types: the temperate deciduous broad-leaved forest zone north of the Yangtze River and the warm temperate evergreen broad-leaved forest zone south of the Yangtze River. Whether the genetic diversity and genetic structure of *O. sieboldii* populations differ between these two vegetation zones, and what factors might cause such differences, are important questions that can guide genetic management and effective conservation strategies for this rare and endangered plant.

This study investigated eight natural populations of *O. sieboldii* along both sides of the boundary between these two vegetation zones—specifically, in the Dabie Mountains (representing the temperate deciduous broad-leaved forest) and the Southern Anhui mountainous region (including Jiangxi Sanqingshan, representing the warm temperate evergreen broad-leaved forest). We surveyed population characteristics including population size, mean DBH, and number of branches, and analyzed their genetic diversity and structure using highly polymorphic microsatellite primers to provide guidance for scientific conservation measures.

1. Sample Collection and Population Characteristics Investigation

Based on extensive herbarium records and field surveys, we identified and sampled eight natural populations of *O. sieboldii* in Eastern China. Three populations were located in the Dabie Mountain region north of the Yangtze River (temperate deciduous broad-leaved forest zone), and five were located in the Southern Anhui mountainous region south of the Yangtze River (warm temperate evergreen broad-leaved forest zone), including the Jiangxi Sanqingshan population. Young leaves were collected from each sampled plant, dried with silica gel, and stored for DNA extraction. For each sampled individual, we counted the number of above-ground branches and measured the DBH of the thickest branch, while also estimating and recording population size.

Total genomic DNA was extracted using a modified CTAB method and detected via electrophoresis in 1% agarose gel. From related species including *Houpoea obovata* and *Yulania stellata*, we screened seven microsatellite primers that showed stable amplification and polymorphism in *O. sieboldii*. The 5' ends of forward primers were fluorescently labeled with FAM, HEX, or TAMRA.

PCR amplification was performed in a 15 L reaction volume containing 5 ng DNA, 1.5 L buffer, 2 mmol/L MgCl , 0.2 mmol/L dNTPs, 0.4 U Taq polymerase, and 0.4 mol/L primers. Amplification conditions were: initial denaturation at 95°C for 5 min; followed by 30 cycles of 95°C for 30 s, annealing at 50–58°C for 30 s (Table 2), and extension at 72°C for 30 s; with a final extension at 72°C for 15 min. PCR products were analyzed automatically on an ABI 3730XL sequencer and genotyped using GeneScan 3.7 software (Applied Biosystems, USA).

[Figure 1: see original paper] The locations of the 8 sampled populations of *O. sieboldii*

The locations and information of the studied populations of *O. sieboldii*

The characterizations of the used SSR loci and their primers

3. Data Analysis

GenePop 4.0 software was used to test for linkage disequilibrium and deviation from Hardy-Weinberg equilibrium. Micro-Checker 2.2.3 software was employed to detect null alleles at each microsatellite locus. The Bottleneck program was used to test whether populations had experienced recent bottleneck effects. GenAlEx 6 software calculated population genetic parameters including observed heterozygosity (H__O), expected heterozygosity (H__E), and inbreeding coefficient (F__IS). GENETIX software computed genetic differentiation coefficients (G__ST) between populations. TFPGA software performed Mantel tests to examine correlations between geographic distance and genetic differentiation.

Since DBH and branch number may correlate positively with plant age, we divided individuals within each population into young and old subpopulations using the median values of DBH and branch number as thresholds. Genetic parameters for both subpopulations were calculated separately using the aforementioned software to detect trends in population genetic characteristics. Statistical analyses were conducted in SPSS 11.0, with one-way ANOVA testing for significant differences in population characteristics (branch number, DBH, and population size). Wilcoxon signed-rank test and Mann-Whitney U test were used to examine significant differences in genetic diversity parameters between vegetation zones and between young and old subpopulations.

For genetic structure analysis, Arlequin 3.5 software performed Analysis of Molecular Variance (AMOVA) within and among populations. Structure 2.2 software conducted Bayesian clustering analysis with K values set from 1 to 8, using an admixture model with a burn-in of 100,000 and run-length of 1,000,000. Evanno' s method was used to calculate the optimal K value. Additionally, GenAlEx software performed individual-based Principal Coordinate Analysis (PCoA) that does not rely on genetic equilibrium assumptions. The 2MOD software was used to detect and calculate the relative contributions of gene flow and drift-isolation models.

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2. Results

2.1 Population Characteristics

Through extensive herbarium research and field investigation, we identified eight natural populations of *O. sieboldii* along the Yangtze River, which forms the boundary between temperate deciduous broad-leaved forest and warm temperate evergreen broad-leaved forest vegetation zones. Three populations were located in the Dabie Mountains north of the Yangtze River (temperate deciduous broad-leaved forest zone, hereafter referred to as Dabie Mountain populations), while five were located south of the Yangtze River (warm temperate evergreen broad-leaved forest zone, hereafter referred to as Southern Anhui populations), including the Jiangxi Sanqingshan population.

Population sizes varied considerably among the eight populations, with the smallest being the Baimajian (BMJ) population and the largest being the Huangshan (HS) population. The mean DBH values were 4.37 cm for Dabie Mountain populations and 4.89 cm for Southern Anhui populations, but the difference was not significant (F , = 1.993, $P > 0.05$). Mean branch numbers ranged from 3.73 to 6.36 across populations, also showing no significant differences between the two vegetation zones (F , = 9.652, $P = 0.057$).

2.2 Genetic Diversity

From related species including *Houpoea obovata* and *Yulania stellata*, we selected seven microsatellite primers that amplified stably and showed high polymorphism in *O. sieboldii*. A total of 69 alleles were detected across all loci, with an average of 9.86 alleles per locus. Micro-Checker detected only a few loci in individual populations that might have null alleles or linkage, while GenePop found no loci with null alleles in more than two populations or linked to other loci, confirming these primers were suitable for genetic diversity and structure analysis in *O. sieboldii*.

The genetic diversity parameters, bottleneck effects, and 2MOD analysis results for studied populations of *O. sieboldii*

The mean genetic diversity parameters for the eight *O. sieboldii* populations were A = 3.83, H_O = 0.25, and H_E = 0.40. The Dabie Mountain populations showed significantly lower genetic diversity (mean A = 3.11, H_O = 0.18, H_E = 0.28) compared to the Southern Anhui populations (mean A = 5.36, H_O = 0.33, H_E = 0.51). The BMJ population exhibited the lowest genetic diversity parameters among all populations.

Correlation analysis between genetic diversity parameters and population characteristics revealed only a significant positive correlation between expected heterozygosity (H_E) and population size in Southern Anhui populations ($r = 0.982$, $P = 0.018$); other correlations were not significant. The inbreeding coefficient (F_IS) ranged from 0.06 to 0.50 across populations, significantly deviating from Hardy-Weinberg equilibrium. The 2MOD analysis indicated that the gene flow and drift models were roughly equivalent across all sampled populations, with Dabie Mountain populations tending more toward the gene flow model (0.52) while Southern Anhui populations showed stronger drift effects (0.66). No significant bottleneck effects were detected in any population.

The mean genetic diversity index and statistical analysis results of Dabie Mountain and Southern Anhui populations

When populations were divided into young and old subgroups based on DBH or branch number, the genetic diversity of young subpopulations was not significantly lower than that of old subpopulations within each population.

[Figure 2: see original paper] The comparison of genetic diversity between young and old sub-populations within populations of *O. sieboldii*

2.3 Genetic Structure

The genetic differentiation coefficient (G_ST) among *O. sieboldii* populations in Eastern China ranged from 0.108 to 0.546, with an overall mean of 0.315. The maximum G_ST value occurred between certain Dabie Mountain and Southern Anhui populations, while the minimum was found within the same vegetation zone. The difference between these values was not statistically significant (F , = 1.882, P = 0.089).

The mean G_ST values were 0.269 for Dabie Mountain populations and 0.198 for Southern Anhui populations. Overall, genetic differentiation showed a positive correlation with geographic distance (Mantel test: r = 0.436, P = 0.020), though this correlation was not significant within each vegetation zone (P > 0.05). The overall mean gene flow was N_m = 0.340, with Dabie Mountain populations showing higher gene flow (N_m = 0.632) than Southern Anhui populations (N_m = 0.352).

Pairwise G_ST values (below the diagonal) and geographical distances (above the diagonal) among the 8 sampled populations of *O. sieboldii*

AMOVA revealed that the main genetic variation existed among populations, accounting for 54.16% of total variation. Moreover, *O. sieboldii* populations in different vegetation zones had diverged significantly, with differences between zones accounting for 24.91% of total genetic variation (P < 0.001).

[Figure 3: see original paper] Principal coordinates analysis (PCoA) for 8 sampled populations of *O. sieboldii*

[Figure 4: see original paper] STRUCTURE analysis results of 8 sampled populations in *O. sieboldii*

Both STRUCTURE and PCoA analyses supported these results, showing that populations from different vegetation zones clustered separately, with each zone forming a distinct group.

Hierarchical analysis of molecular variance (AMOVA) within/among surveyed populations of *O. sieboldii*

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3. Discussion

Magnoliaceae represents an important ancient lineage of extant angiosperms that originated in the Cretaceous Albian period according to fossil records. While most Magnoliaceae species exhibit relatively high genetic diversity due to their ancient woody nature and outcrossing breeding systems (e.g., Japanese *Houpoea obovata*: H_O = 0.666, H_E = 0.719; *Magnolia tomentosa*: H_O = 0.650, H_E = 0.675), the *O. sieboldii* populations in Eastern China showed notably low genetic diversity (H_O = 0.25, H_E = 0.40). This is significantly lower than its Japanese subspecies *O. sieboldii* subsp. *japonica* (H_O = 0.88,

$H_E = 0.87$) and other related species such as *Yulania stellata* ($H_O = 0.360$, $H_E = 0.427$).

Conversely, genetic differentiation among *O. sieboldii* populations was substantially higher ($G_{ST} = 0.315$) than in related species, with 54.16% of total genetic variation existing among populations—significantly exceeding the mean values for perennial plants (0.19) and outcrossing species (0.22). This pattern of low within-population genetic diversity coupled with high among-population differentiation likely results from several factors.

First, *O. sieboldii* inhabits highly specific, fragmented island-like habitats, typically limited to shady slopes and valleys above 1000 m elevation in Eastern China. These habitats are usually small, isolated, and characterized by high humidity. Such fragmented distributions restrict gene flow between populations. Second, despite being protogynous, *O. sieboldii* retains a self-compatible breeding system, as demonstrated by artificial pollination experiments showing that self-pollination can produce viable seeds. This adaptation helps ensure reproductive success in environments with limited pollinator availability. Indeed, studies have documented low natural seed set rates that can be significantly improved through artificial pollination. The combination of small, isolated habitats, self-compatibility, and pollinator limitation results in low gene flow ($N_m = 0.340$) that is insufficient to counteract genetic drift, leading to reduced genetic diversity within populations and increased differentiation among them.

Different vegetation types can significantly influence the genetic diversity of resident species. Our results demonstrate clear differences in genetic diversity between *O. sieboldii* populations in the two vegetation zones, with Dabie Mountain populations showing significantly lower diversity than Southern Anhui populations. While larger populations and stronger gene flow generally maintain higher genetic diversity, we found no significant differences in population size, DBH, or branch numbers between vegetation zones. Paradoxically, gene flow was stronger among Dabie Mountain populations, likely due to their closer geographic proximity. Additionally, the genetic diversity of young subpopulations was not significantly lower than that of old subpopulations, suggesting that population size, gene flow, and human activity are not the primary causes of lower genetic diversity in the Dabie Mountains.

Field surveys revealed that although *O. sieboldii* populations occur in the evergreen broad-leaved forest zone south of the Yangtze River, they are actually restricted to microhabitats characterized by deciduous broad-leaved forest communities at elevations above 1000 m. This indicates that *O. sieboldii* is fundamentally a deciduous broad-leaved forest species whose distribution closely tracks this vegetation type. During the Last Glacial Maximum, deciduous broad-leaved forests retreated southward to approximately 22°–30°N, meaning that *O. sieboldii* populations likely survived only south of this latitude. Post-glacial warming then allowed northward recolonization. Consequently, populations at higher latitudes have shorter evolutionary histories, more pronounced founder effects, and consequently lower genetic diversity. This historical demography likely explains the reduced genetic diversity in Dabie Mountain populations and suggests that populations in North China may have even lower diversity—a hypothesis requiring further verification.

The primary goal of species conservation is to preserve genetic diversity and evolutionary potential. Greater within-species genetic diversity confers larger evolutionary potential and better adaptation to environmental changes. Genetic diversity and structure analyses provide crucial information for assessing conservation value and formulating protection strategies for rare and endangered species.

Our genetic structure analysis of *O. sieboldii* in Eastern China reveals that populations in the two vegetation zones have diverged into distinct evolutionary units that cluster separately. Although Dabie Mountain populations have lower genetic diversity and likely originated from northward post-glacial expansion from low-latitude populations, they have become genetically distinct from Southern Anhui populations due to habitat selection and genetic drift. Their unique genetic characteristics are not encompassed within Southern Anhui populations. Therefore, we recommend that Dabie Mountain and Southern Anhui populations be treated as independent evolutionary significant units for conservation.

Despite relatively low genetic diversity and small gene flow compared to related species, the fact that young subpopulations show no significant decline in genetic diversity suggests that natural populations maintain stable age structure with adequate regeneration capacity. Field observations confirm the presence of numerous seedlings. The 2MOD analysis indicates that populations are currently in a gene flow-drift equilibrium state. Consequently, we recommend prioritizing in-situ conservation, with particular attention to protecting suitable habitats. Where conditions permit, artificial pollination within the same vegetation zone could be implemented to increase natural seed set and enhance natural regeneration capacity.

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