

Article

Study on the Genetic Structure Based on Geographic Populations of the Endangered Tree Species: *Liriodendron chinense*

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Abstract: *Liriodendron chinense* (Hemsley) Sargent is a Class II protected plant in China as natural populations are on the verge of extinction. There is still a lack of systematic research on the genetic resources of its geographic populations. In this study, we used 20 pairs of SSR markers with high polymorphism to analyze a total of 808 *L. chinense* samples from 22 regions, and 63 *Liriodendron tulipifera* Linn samples from 2 regions were used as a comparison group. The results revealed a total of 78 alleles in *L. chinense*, and the average expected heterozygosity (H_e) was 0.558, showing a low level of genetic diversity. The degree of differentiation of *L. chinense* was high, with the differentiation coefficient (F_{st}) as high as 0.302, which is related to the low gene flow ($N_m = 0.578$). Based on the genetic structure, principal coordinate analysis (PCoA) and phylogenetic analysis of 24 *Liriodendron* spp. populations, *L. chinense* and *L. tulipifera* had obvious differentiation, while the differentiation between *L. chinense* geographic populations was very large and irregular. Inbreeding appears within the geographic populations, and the level of genetic diversity is very low. In order to protect the genetic diversity of *L. chinense*, in addition to protecting the existing population as much as possible, artificial cultivation should introduce materials from multiple populations.

Keywords: conservation biology; genetic diversity; genetic structure; *Liriodendron chinense*; phylogeography; species distribution pattern



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1. Introduction

The climate oscillations and topographical changes of the Late Tertiary and Quaternary have profound influences on the geographical distribution and genetic structure of species. During the glacial periods, species retreated to refugium where the conditions were stable and diverse, so that their genetic resources could be preserved [1]. After glacial periods, species migrate and expand from refugia to other suitable regions. Central China (25°47'~33°20' N, 103°30'~111°50' E) was an important glacial refugium for many Tertiary relict species due to the influence of the warm and humid climate in the Sichuan Basin and the Yunnan-Guizhou Plateau [2].

As a primitive group of angiosperms, Magnoliaceae plants are ideal natural resources for studying the origin, distribution and evolution of flowering plants [3,4]. The genus *Liriodendron* (Magnoliaceae), which is a relict species of the tertiary period, was widely distributed in the northern region of the Northern Hemisphere during the early to mid-Miocene but became extinct in Europe during the Pleistocene. There are only two sister species, *Liriodendron tulipifera* Linn and *Liriodendron chinense* (Hemsley) Sargent, of this genus [5], which are typical East Asian and North American intermittent distributions of “species pairs”. These trees are similar in shape and are famous for their rapid growth,

valuable wood, and unique leaf shape, so they have important economic and ornamental value [6,7]. *L. tulipifera* is widely distributed in broad-leaved forests in the eastern United States and southeastern Canada, while *L. chinense* is scattered in the mountains of southern China and northern Vietnam. Due to its biological characteristics, including a small population, low seed setting rate and low seed germination rate, as well as climate change and human interference, *L. chinense* has become an endangered species [8].

Because *L. chinense* is endangered, studies on the conservation biology of *L. chinense* have been carried out, which mainly focus on the fields of reproductive ecology [9–11], population distribution [1,12,13], genetic diversity and genetic differentiation [14–16] and other fields. Regarding the geographical distribution of *L. chinense*, some research suggests that the two modern distribution centers of *L. chinense* are the area between 108–110° east longitude and 25–31° north latitude, which is called the western subregion, and the area between 116–120° east longitude and 28–31° north latitude, called the eastern subregion [17]; other areas are scattered. There is also a study that interprets the distribution of *L. chinense* as “one belt and five islands”, referring to a northeast-southwest distribution belt and south-central (1 “island”) and eastern (4 “islands”) areas, for a total of 5 island areas [18]. Although *L. chinense* presents scattered and intermittent geographical distribution characteristics, research on the genetic structure of its geographic populations is still incomplete from the perspective of population genetics.

Different genetic markers have been used to explore the genetic diversity and genetic structure of this species [19–21], but the results are often different. The main reason for these differences is that the markers involved are mostly dominant markers, such as Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter-Simple Sequence Repeat (ISSR), etc., which may cause the loss of genetic information due to their inability to distinguish heterozygotes; although simple sequence repeats (SSR) that use codominance are also used as markers in research [22–25], due to the limited number of markers used and the lack of coverage of the geographic populations [14], it is difficult to provide comprehensive information on the characteristics of a given geographic population.

In this study, we collected 22 geographic populations from the main distribution areas of *L. chinense*. At the same time, two geographic populations of *L. tulipifera* were used as a comparison group, and 20 pairs of SSR markers were used to study geospatial genetic structure. This was the first time this research had been conducted using a large number of groups. We aimed to comprehensively study the status of genetic resources of *L. chinense* geographic populations from the perspective of population genetics, including the genetic diversity level and differentiation. Especially exploring the characteristics and reasons for the differentiation of the *L. chinense* geographic population, we hoped to provide a more reliable basis for formulating measures for the protection and utilization of *L. chinense*.

2. Materials and Methods

2.1. Plant Materials

From 2005 to 2006, a total of 22 *L. chinense* populations (Figure 1) were collected from 11 provinces (cities). Since most groups of *L. chinense* within its natural distribution area were small, there were very few large groups with more than 100 plants [18]. The larger geographic populations of *L. chinense* included those from Jinping, Yunnan (YJP), Liping, Guizhou (GLP), Monan, Guizhou (GMN) and Anji, Zhejiang (ZAJ) in this study. The remaining natural populations had less than 20 plants, of which 83% were small populations (less than 10 plants). For larger populations, it was necessary to select more than 15 plants (with an interval of more than 50 m apart). In groups of less than 10 plants, the seeds of all plants were mixed after collection. The collected seeds were sown in the germplasm resource nursery of Nanjing Forestry University (31°66′ N, 119°01′ E). Two *L. tulipifera* geographic populations (Louisiana, USA (LA), Missouri, USA (MO)) came from the provenance test forest of *L. tulipifera* at HuZhuaShan Forest Farm

(112°90' E, 31°08' N) in Jingshan County, Hubei Province, China. Both places have a subtropical monsoon climate and a yellow brown soil type.

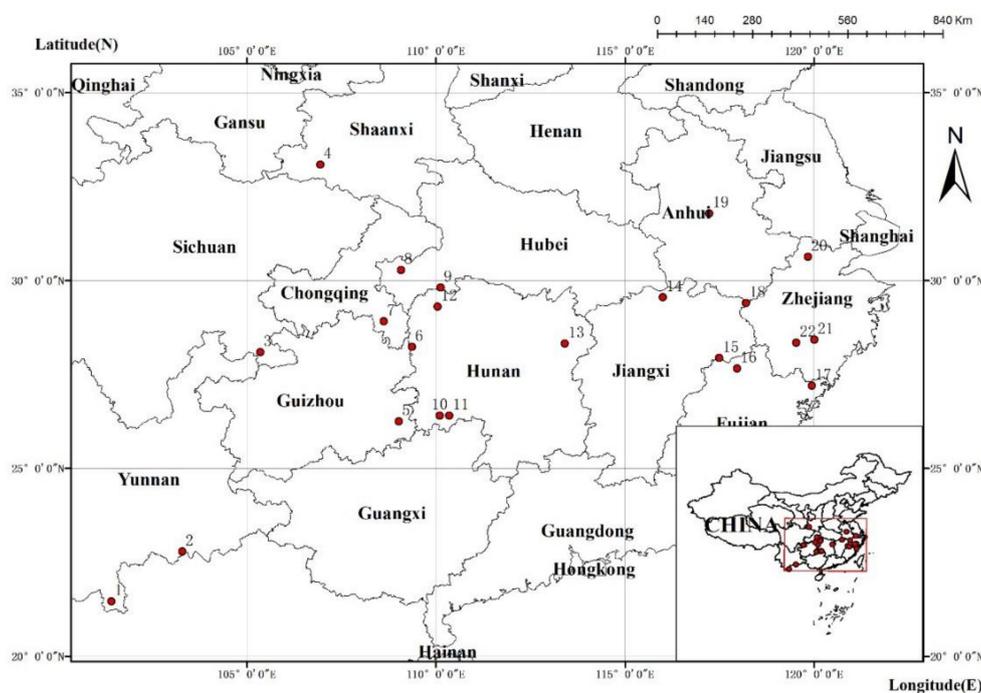


Figure 1. Locations of the 22 geographic populations of *L. chinense*. Numbers correspond to the population numbers in Table S1.

A total of 871 individuals were collected (Supplementary Materials Table S1), and samples of at least 30 plants were selected from each group (the sample sizes of the Mengla, Yunnan (YML), Hanzhong, Shaanxi (SHZ), Sangzhi, Hunan (HSZ), and Dabieshan, Anhui (ADB) groups were less than 30, so all were collected). The collected young leaves were quickly put into a Ziplock bag, preserved on dry ice, brought back to the laboratory, and stored at -80°C .

2.2. DNA Extraction

Total genomic DNA was extracted using the hexadecyl trimethyl ammonium bromide (CTAB) method [26]. The DNA concentrations were estimated with a NanoDrop-1000 spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA) and 1% agarose gel electrophoresis was used to check the quality of DNA. Qualified samples were stored in a -20°C freezer until use.

2.3. EST-SSR Primers and PCR Amplification

From the 162 pairs of primers designed by our laboratory based on the expressed sequence tags (EST) of *L. tulipifera* in the NCBI database (<https://www.ncbi.nlm.nih.gov/> (accessed on 21 June 2021)), 20 pairs of EST-SSR primers with high polymorphism were screened for this study. A list of the EST sequence data sources corresponding to 20 pairs of primers is found in Table S2. The primers were synthesized by Nanjing GenScript Biotechnology Co., Ltd. See Table S2 for details. Polymerase Chain Reaction (PCR) amplification for all EST-SSR markers was carried out using an ABI Veriti 96 PCR system (Thermo Fisher Scientific, MA, USA). The total volume of each single locus PCR was $10\ \mu\text{L}$, which consisted of 20 ng genomic DNA, 2.5 mM MgCl_2 , 0.2 mM dNTPs, $1\ \mu\text{L}$ $10\times$ buffer, $0.2\ \mu\text{M}$ each EST-SSR forward and reverse primer and 0.5 U Taq polymerase (Thermo Scientific). The PCR program involved an initial denaturation step of 4 min at 94°C , followed by 30 cycles at 94°C for 15 s, the appropriate annealing temperature

for 15 s, and 72 °C for 30 s, an extension cycle of 5 min at 72 °C, and final storage at 4 °C. Fragments resulting from PCR amplifications were separated by electrophoresis on 8% polyacrylamide denaturing gels and visualized with silver nitrate staining. According to the band sizes of the PCR products (representative examples were shown in Supplementary Materials Figures S1–S4), the samples were represented by capital letters A, B, C, etc.

2.4. Analysis of Data

The number of observed alleles (N_a), the number of efficient alleles (N_e), the observed heterozygosity (H_o), the expected heterozygosity (H_e), Nei's diversity index (H), the genetic differentiation index (F_{st}), gene flow (N_m), Shannon's information index (I) and inbreeding coefficient (F) [27] were determined using POPGENE version 1.32 software.

The polymorphism information content (PIC) and linkage disequilibrium (the construction of phylogenetic trees based on the unweight pair-group method with arithmetic means (UPGMA)) were calculated by using PowerMarker version 3.25 software [28]. Allelic richness (AR) was analyzed with FSTAT version 2.9.3 software [29]. Analysis of molecular variance (AMOVA) and principal coordinate analysis (PCoA) was carried out using GenAlEx version 6 software [30].

The population genetic structure was analyzed based on Bayesian clustering using STRUCTURE version 2.3.1 software [31]. For clustering from $K = 1$ to $K = 25$ (populations + 1), an admixture ancestry model and correlated allele frequency model were used to perform a Markov chain Monte Carlo simulation algorithm (MCMC) [32]. The length of the burn-in period at the start time was set as 100,000; the MCMC after the length of the burn-in period was set as 100,000, and for each K value, the simulation calculation was repeated 10 times. The method from Evanno [31] was used to determine the optimal K value and was implemented in STRUCTURE HARVESTER web version 0.6.94 [33]. The results were plotted using CLUMPP version 1.1.2 [34] and DISTRUCT version 1.1 [35]. NTSYS version 2.10 software [36] was used to perform Mantel test on the correlation between genetic distance and spatial geographic distance of 24 *Liriodendron* populations.

3. Results

3.1. Genetic Diversity of Different Geographic Populations

Twenty pairs of primers were used to detect 808 individuals from 22 *L. chinense* geographic populations. The results are shown in Table 1. A total of 78 alleles were amplified from *L. chinense*. The number of alleles per SSR locus varied from 2 to 5, with an average of 3.9 alleles per locus. The average N_e , H_e , and PIC were 2.582, 0.558, and 0.503, respectively, indicating that the 808 samples contained low levels of genetic diversity. Among the 20 pairs of SSR primers, the polymorphism level of LCEST-97 was the lowest, with the N_e , H_e , and PIC being 1.108, 0.017, and 0.017, respectively, and I had the lowest value of 0.05, while the N_e , H_e , and PIC at LCEST-61 were 3.772, 0.735, and 0.687, respectively, and I had the highest value of 1.411. The polymorphism level of this site was the highest. These 20 pairs of SSR primers could be effectively amplified in 63 individuals of *L. tulipifera*, but the polymorphism of the loci was different, and the polymorphism of the LCEST-119 locus was the highest (Table S3).

In general, the genetic diversity of the 22 geographic populations of *L. chinense* varied greatly. The H_e fluctuated from 0.177 to 0.539 (Table 2). In terms of the populations, the average genetic diversity of *L. chinense* (average $H_e = 0.399$) was lower than that of *L. tulipifera* (average $H_e = 0.454$). Among them, the Chengbu, Hunan (HCB) population had the highest genetic diversity, with the largest $N_e = 2.391$, $H = 0.532$, and $H_e = 0.539$. It may be that HCB is located in a refuge in central China and retains a high level of genetic diversity; second was HSZ, which had the largest AR = 2.985. The YML population had the lowest level of genetic diversity, and lowest detected N_e (1.333), H (0.170), and H_e (0.177). Second, the Zherong, Fujian (FZR) population had the lowest genetic diversity, with the

lowest Na = 1.550 and AR = 1.495. One possible reason for these findings might be that YML and FZR are small groups (the number of natural groups was less than 10).

Table 1. Polymorphism information for 20 microsatellite loci amplified in *L. chinense*.

Locus	Na	Ne	He	PIC	I	Fst	Nm
LCEST-41	4	2.899	0.655	0.602	1.202	0.273	0.666
LCEST-53	4	1.442	0.307	0.292	0.650	0.166	1.255
LCEST-57	3	1.830	0.454	0.371	0.719	0.267	0.687
LCEST-60	3	2.951	0.662	0.587	1.090	0.302	0.579
LCEST-61	5	3.772	0.735	0.687	1.411	0.165	1.262
LCEST-62	4	1.616	0.382	0.349	0.702	0.222	0.875
LCEST-68	5	2.775	0.640	0.576	1.183	0.256	0.727
LCEST-75	5	2.231	0.552	0.505	1.032	0.372	0.423
LCEST-80	4	3.519	0.716	0.662	1.306	0.298	0.588
LCEST-97	2	1.018	0.017	0.017	0.050	0.052	4.550
LCEST-103	4	1.169	0.145	0.138	0.318	0.125	1.756
LCEST-104	4	3.489	0.714	0.664	1.318	0.355	0.453
LCEST-107	4	2.946	0.661	0.591	1.146	0.430	0.332
LCEST-119	3	2.903	0.656	0.582	1.083	0.196	1.023
LCEST-131	4	3.019	0.669	0.616	1.232	0.259	0.715
LCEST-169	4	2.689	0.629	0.553	1.056	0.402	0.372
LCEST-181	4	2.910	0.657	0.584	1.113	0.362	0.442
LCEST-184	4	2.763	0.639	0.564	1.059	0.361	0.442
LCEST-186	5	3.398	0.706	0.649	1.290	0.220	0.889
LCEST-195	3	2.304	0.566	0.478	0.920	0.520	0.231
Mean	3.9	2.582	0.558	0.503	0.994	0.302	0.578

Na: Observed number of alleles, Ne: Effective number of alleles, He: Expected heterozygosity, PIC: Polymorphism information content, I: Shannon's information index, Fst: Genetic differentiation index, Nm: Gene flow.

Table 2. Mean values of genetic diversity for 20 microsatellite loci in 22 *L. chinense* geographic populations and 2 *L. tulipifera* geographic populations.

Species	Population Code	Na	Ne	H	Ho	He	AR	F
<i>L. chinense</i> (Hemsl.) Sarg.	YML	1.600	1.333	0.170	0.100	0.177	1.555	0.434
	YJP	2.150	1.362	0.226	0.125	0.229	1.900	0.453
	SXY	2.600	1.910	0.372	0.320	0.377	2.286	0.150
	SHZ	2.650	1.862	0.402	0.222	0.425	2.650	0.477
	GLP	3.050	2.046	0.429	0.280	0.434	2.668	0.354
	GMN	2.550	1.745	0.332	0.223	0.336	2.207	0.337
	CYY	3.150	2.178	0.480	0.310	0.486	2.849	0.362
	HLC	2.800	2.025	0.426	0.471	0.431	2.497	-0.093
	HHF	2.800	1.908	0.409	0.217	0.413	2.542	0.476
	HSN	2.750	2.066	0.430	0.183	0.437	2.617	0.581
	HCB	3.150	2.391	0.532	0.384	0.539	2.884	0.288
	HSZ	3.250	2.251	0.508	0.295	0.521	2.985	0.433
	HLY	2.550	1.768	0.343	0.290	0.347	2.213	0.164
	JLS	2.700	1.870	0.375	0.223	0.379	2.365	0.412
	JWY	2.550	1.893	0.399	0.241	0.404	2.377	0.403
	FWY	2.300	1.702	0.345	0.278	0.350	2.064	0.206
	FZR	1.550	1.362	0.194	0.179	0.196	1.495	0.090
	AHS	3.250	2.165	0.471	0.294	0.478	2.938	0.386
	ADB	2.950	2.067	0.472	0.213	0.484	2.766	0.561
	ZAJ	3.200	2.141	0.481	0.285	0.487	2.796	0.415
ZLS	2.700	1.907	0.407	0.330	0.412	2.456	0.198	
ZSY	3.300	1.987	0.437	0.301	0.442	2.890	0.318	
mean		2.707	1.906	0.393	0.262	0.399	2.455	0.337
<i>L. tulipifera</i> L.	LA	3.250	2.279	0.473	0.363	0.481	2.914	0.245
	MO	3.000	2.203	0.420	0.358	0.427	2.744	0.161
	mean		3.125	2.241	0.446	0.360	0.454	2.829

Ho: observed heterozygosity, H: Nei's diversity index, AR: allelic richness, F: inbreeding coefficient.

The average F of the 22 *L. chinense* geographic populations was 0.337. Except for the Lichuan, Hubei (HLC) group ($F = -0.093$), the rest of the *L. chinense* populations showed $F > 0$, indicating that most of the populations were heterozygous. However, the average H_o (0.262) of the 22 *L. chinense* geographic populations was less than H_e (0.399), which indicated that mating within the *L. chinense* geographic population was imbalanced and inbreeding occurred.

3.2. Genetic Differentiation among Geographic Populations

The average F_{st} of *L. chinense* was 0.302 (Table 1). The results of AMOVA (Table S4) showed significant genetic differences among geographic populations, which was inferred to result from distribution in island-like populations, and most populations were small. According to Fang [17], the geographic populations of *L. chinense* were divided into western subregion and eastern subregion; the Nei standard genetic distance between the regions was not large, much smaller than the genetic distance between the two geographic populations of *L. tulipifera* (Table S5). The results showed that the genetic differentiation of *L. chinense* and *L. tulipifera* was obvious.

3.3. Genetic Structure of Geospatial

To further study the genetic structure of *L. chinense* geographic populations, STRUCTURE software was used to analyze the common ancestral relationship of the *L. chinense* geographic populations based on a Bayesian clustering model. The research results show that when the ΔK value reached the maximum value (Figure S5), the corresponding best K value was 21, which could be divided into 21 groups according to genetic information (Figure S6). The two geographic populations of *L. tulipifera* (LA, MO) had the same origin, while the diversification among the geographic populations of *L. chinense* was very large. Only Wuyishan, Jiangxi (JWY) and Wuyishan, Fujian (FWY), ADB and Lishui, Zhejiang (ZLS) had relatively close origins, while the other geographic populations had no consistent genetic information.

When $K = 4$, ΔK was the second largest value, and its structure map could divide the 24 groups into 4 groups (Figure 2). The *L. tulipifera* population (LA, MO) was still a single group, while the *L. chinense* geographic populations could be divided into 3 groups, but the rule between and within groups could not be found, indicating random differentiation.

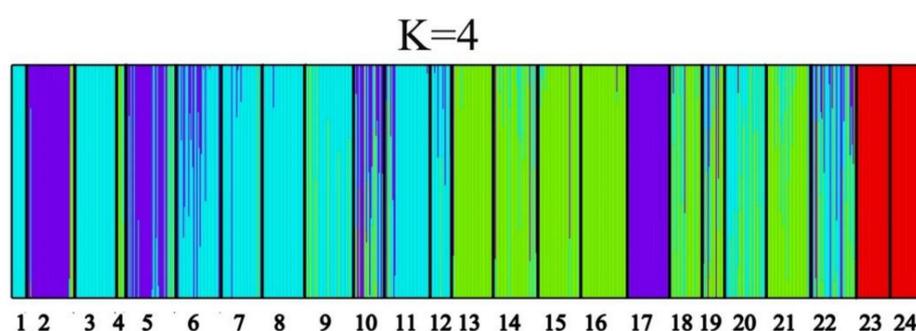


Figure 2. Population genetic structure based on the Bayesian clustering model among 871 samples at $K = 4$. Numbers correspond to the population numbers in Table S1.

Similarly, the results of the phylogenetic tree (Figure S7) based on the UPGMA method and PCoA (Figure S8) further illustrated the irregularity of the differentiation and geographical distribution among the geographic populations. Although, the structure and differentiation analysis (STRUCTURE (Figure 2) and UPGMA (Figure S7)) showed that no. 6–7 and no. 10–12 were divided into the same group (the western group), no. 14–16 and no. 19–22 were put into the same group (the eastern group). The results provided some evidence of geographic differentiation between an eastern and a western group of *L. chinense*.

3.4. Mantel Test

A Mantel test was used on 24 *Liriodendron* groups to detect the correlation between geographic distance and genetic distance. The results showed that the spatial geographic distance after standardized logarithmic processing had an extremely significant correlation with the Nei standard genetic distance between 24 *Liriodendron* populations (Figure 3, $r = 0.680$, $p < 0.01$).

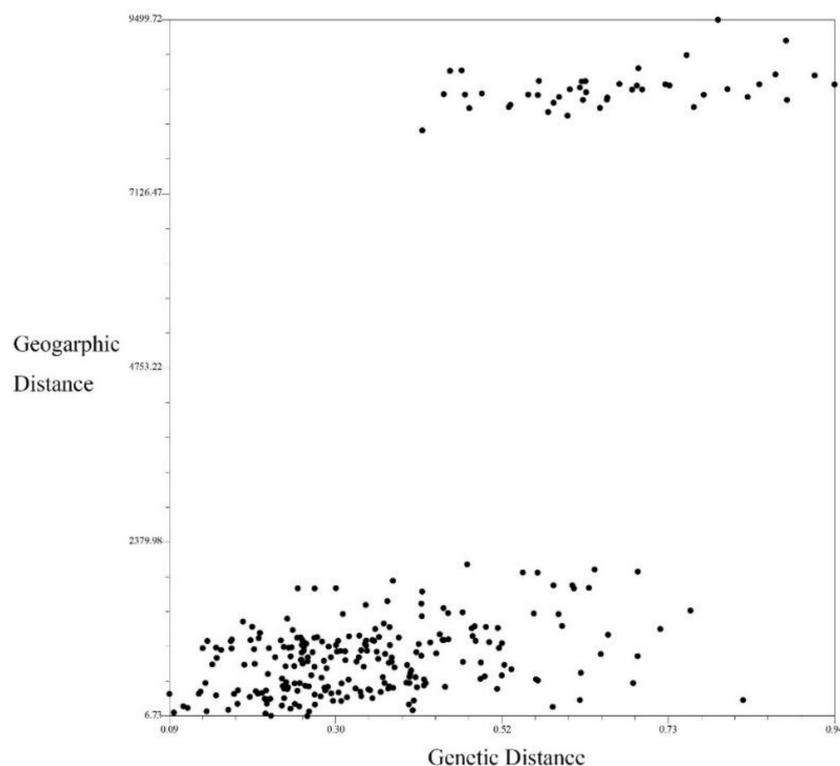


Figure 3. Spatial genetic structure of 24 *Liriodendron* populations.

4. Discussion

4.1. Genetic Diversity of Different Geographic Populations

Genetic diversity can be used to measure the abundance of population variation and the ability of populations to adapt to the environment. The study of genetic diversity will provide a basis for the formulation of strategies for the protection and utilization of genetic resources, which is a prerequisite for the survival and development of populations. The degree of heterozygosity is expected to be proportional to the degree of locus variation, which can reflect the stability and abundance of population alleles. Regarding the study of the genetic diversity of the *L. chinense* populations, Li et al. [21] and Yang et al. [12] used dominant RAPD and AFLP markers to demonstrate that the genetic diversity levels of *L. chinense* were $H_e = 0.257$ and $H_e = 0.174$, respectively. Li et al. [37] used ISSR markers to verify a lower level of genetic diversity of *L. chinense* (the average Nei's gene richness was 0.258). However, Li et al. [14] used 14 pairs of SSR markers to reveal that the genetic diversity of the 12 populations of *L. chinense* was high (average $H_e = 0.612$), which was different from the results obtained with the detection of 20 pairs of SSR polymorphic primers in this study (average $H_e = 0.399$). It may be that the progeny populations of the natural populations were used in this study, and not all the mother trees were fruit-bearing, so a lower genetic diversity was detected. Interpretation of the results of polyacrylamide gel electrophoresis could also lead to divergence of results. At the same time, the number of geographic populations involved in this study was very large, basically covering the entire distribution area of *L. chinense*, and these *L. chinense* geographic populations were

mostly small groups (the number of natural populations was less than 10), which was also one of the reasons for the low level of genetic diversity.

At the species level, the genetic diversity of *L. chinense* was also low ($H_e = 0.558$). Compared with the results of other plant genetic diversity studies based on SSR markers, *Quercus variabilis* Bl. ($H_e = 0.707$ [38]), *Ginkgo biloba* L. ($H_e = 0.808$ [39]), *Quercus wutaishansea* Mary ($H_e = 0.754$ [40]) and other plants had higher genetic diversity. This was related to the biological characteristics and current status of the distribution of *L. chinense*. Its scattered and intermittent distribution form and population size were very small. These geographic populations have been damaged to different degrees, resulting in lower genetic diversity. These findings also reflected the current endangered status of *L. chinense* and explained the importance of protecting its genetic diversity.

4.2. Genetic Differentiation among Geographic Populations

Natural selection is the most important evolutionary force leading to population differentiation, and gene flow is an important factor against selection. Wright believes that when $Nm > 1$, gene flow can prevent genetic differentiation between populations caused by genetic drift. Compared with other forest trees, the population differentiation level of *L. chinense* ($F_{st} = 0.302$) was much higher than that of *Quercus variabilis* Bl. ($F_{st} = 0.046$) [41], *Castanopsis fargesii* Franch. ($F_{st} = 0.031$) [42], *Pinus massoniana* Lamb. ($F_{st} = 0.072$) [43] and other tree species. The differentiation results obtained in this study were similar to those of Li et al. [21] and Li et al. [36] ($F_{st} = 0.3154$ and $G_{st} = 0.291$, respectively) but higher than those of Li et al. [14] ($F_{st} = 0.1956$), who also used SSR markers, which may be related to the large number of groups in this study and many of them being small populations. *L. chinense* is an ancient relic plant, and its biological characteristics lead to low natural fecundity [44], mostly occurring in small groups [45] on high mountains at an altitude of 600 m to 1500 m. The mountain topography is complex, and the geographical distance between populations is large, so it is difficult to have gene exchange among populations. There was a certain degree of inbreeding, leading to a high degree of genetic differentiation.

The mantel test showed that the spatial geographic distance had an extremely significant correlation with genetic distance between 24 *Liriodendron* populations. There is a certain geographical gradual change in the genetic variation of *L. chinense* populations.

The genetic relationship between *L. chinense* and *L. tulipifera* could be traced back to the Miocene [17]. At that time, the genus *Liriodendron* had a diversified morphology and free mating between species. Since the late Miocene, the unified distribution area began splitting and disintegrating, resulting in geographical isolation, and differentiation of the two species from their respective ancestors; the differentiation time of *L. chinense* was earlier than that of *L. tulipifera*, and the two have a sister relationship. In terms of genetic relationships, the results of this study indicated that there was obvious genetic differentiation between *L. chinense* and *L. tulipifera*, which was consistent with the research results of Zhong et al. [15] and Chen et al. [16].

4.3. Genetic Structure of Geospatial

The distribution of genetic variation at the small scale within populations and the large-scale spatial distribution among populations is one of the important characteristics of the population genetic structure. Whether it was the eastern-western subregion or the distribution pattern of "one belt and five islands", *L. chinense* was clustered and distributed in the large-scale spatial, which was largely affected by the climate fluctuations since the Tertiary. However, the accumulation of *L. chinense* in the mountains of central and eastern China was more closely related to the Quaternary and interglacial periods. Most of the habitats of *L. chinense* were high-altitude. Since the Tertiary, it had been relatively stable in geology and had not experienced the southward invasion of the Quaternary ice sheet.

The population genetic structure is inseparable from the biological evolution process. Based on the STRUCTURE software, cluster analysis of all individuals was based on the Bayesian model, in which the determination of the best K value is key. Evanno et al. [31]

and others believed that it was difficult to obtain the correct K value using $\ln P(D)$, and the highest ΔK value was more reliable. In this study, when the ΔK value was the highest, the K value was 21. At this time, in addition to the two geographic populations of *L. tulipifera*, the *L. chinense* geographic populations were divided into 20 groups based on genetic composition. Except for the two geographic populations (JWY and FWY) located in different locations on WuYi Mountain, the geographical distance was very close, and there was no obvious differentiation; the population genetic information of ADB and ZLS was also similar, and these populations might have been introduced from the same place. The rest of the groups had different origins, and they were even more divided.

When the value of ΔK was the second highest, *L. chinense* populations were divided into three groups, and it was difficult to find the regularity of the relationship among *L. chinense* geographic populations. Li et al. [46] and Luo et al. [47] used RAPD markers to cluster *L. chinense* populations, but also found it difficult to classify *L. chinense* geographic populations by region. Although the markers used were different, they also proved that the genetic differentiation among *L. chinense* geographic populations was extremely large, and there was no rule to follow. The “island-like” distribution of the existing *L. chinense* populations resulted in mostly small populations, so the degree of inbreeding within the populations was high and the genetic isolation among the geographic populations resulted in random differentiation among the populations.

4.4. Conservation of *L. chinense* Genetic Resources

The discontinuous distribution pattern, population structure decline and specific requirements of the habitat reflect that *L. chinense* is an endangered species. The genetic diversity of the *L. chinense* geographic populations was low, and the degree of differentiation was high. There is little gene exchange between the populations due to geographical isolation, and the genetic basis is gradually narrowing. From the perspective of protection, the genetic diversity of this species must be maintained first, and the existing geographic populations should be protected if possible; second, the genetic diversity of populations should be improved. By promoting gene exchange among the populations, it is possible to introduce *L. chinense* from different geographic populations into suitable habitats and increase the genetic diversity of the artificial populations.

5. Conclusions

In this article, a large number of populations of *L. chinense* were used to study genetic diversity and genetic differentiation for the first time. The results found that the level of genetic diversity of *L. chinense* was relatively low, and a certain degree of inbreeding occurred within the population. *L. chinense* was clustered and distributed in the large-scale spatial area, which was largely affected by the climate fluctuations since the Tertiary. The distribution status of the existing *L. chinense* populations resulted in mostly small populations. Geographical isolation and genetic isolation among the geographic populations resulted in random differentiation among the populations, and it is difficult to have gene exchange among populations. This article puts forward the protection strategy of *L. chinense* from two aspects: on-site protection and introduction protection.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/f12070917/s1>, Table S1. Geographical distribution of 22 *L. chinense* geographic populations and two *L. tulipifera* geographic populations, Table S2. Repeat motif, primer sequence, fragment size, T_m and data sources information for 20 EST-SSR loci, Table S3. Polymorphism information for 20 microsatellite loci amplified in *L. tulipifera*, Table S4. Analysis of molecular variance (AMOVA) in *L. chinense*, Table S5. Pairwise genetic distances between three regions of *Liriodendron*, Figure S1. LCEST-41 SSR amplification products of Chengbu, Hunan (HCB) population, Figure S2. LCEST-41 SSR amplification products of Lichuan, Hubei (HLC) population, Figure S3. LCEST-186 SSR amplification products of Chengbu, Hunan (HCB) population, Figure S4. LCEST-186 SSR amplification products of Lichuan, Hubei (HLC) population, Figure S5. Relationships between the number of clusters (K) and the corresponding ΔK statistics calculated according to ΔK (Evanno et al. 2005) based on

STRUCTURE analysis, Figure S6. Population genetic structure based on the Bayesian clustering model among 871 samples at $K = 21$. Numbers correspond to the population numbers in Table 1, Figure S7. UPGMA tree based on Nei's genetic distances. Numbers correspond to the population numbers in Table 1, Figure S8. Score plot generated using PCoA.

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