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Genetic diversity and genetic structure of the natural population in the critical production area of *Phoebe bournei*

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Abstract

Phoebe bournei, also known as "nanmu," is renowned for its exceptional material quality. However, the natural forest resources of *P. bournei* are increasingly scarce due to excessive logging and usage. This study utilized EST-SSR molecular marker technology to assess the genetic diversity and structure of nine natural populations of *P. bournei*, providing a theoretical foundation for the conservation and use of its germplasm resources. There was a notably high level of genetic diversity, with an average expected heterozygosity of 0.73. There were significant variances in genetic diversity among the populations. The Shunchang population in Fujian Province exhibited the highest genetic diversity (H_e = 0.83), which should be prioritized for conservation due to its extensive area and dispersed individual distribution. The population in Chongyi, Jiangxi Province, had the lowest genetic diversity (H_e = 0.60) due to inbreeding and its relatively small area. Genetic differentiation occurred primarily within populations (83.80%). On the other hand, high levels of differentiation existed between populations (F_{ST} = 0.1620). The genetic distance among populations was positively correlated with altitude, suggesting that altitude may impact the genetic differentiation of natural populations of *P. bournei*. Genetic structure and cluster analysis revealed that the nine natural populations of *P. bournei* were classified into two categories.

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Introduction

The genetic diversity of a species is determined by the sum of the total genetic differences among individuals. The emergence of new alleles in each generation is caused by DNA damage due to replication errors or mutagenesis. The presence and absence of genetic variations reflect the balance of genetic diversity, which is an essential basis for the sustainable survival and development of species in complex environments. Environmental adaptation during long-term species evolution shapes genetic diversity in populations[1]. In an ideal population, random mating between individuals, gene frequency, and genotype frequency remain consistent across generations[2]. However, real populations typically deviate from these assumptions. Factors such as population size[3], age structure[4], gender ratio[5], mating system, and seed dispersal mode[6] all affect reproductive and natural regeneration abilities, as well as the survival potential of endangered species, thereby influencing the maintenance of genetic diversity[7,8]. In addition, Genetic diversity is inversely proportional to population size, where small populations and great spatial isolation caused by habitat fragmentation result in severe genetic drift, inbreeding, and the accumulation of harmful mutations. Ultimately, these factors lead to the low genetic diversity of populations and even extinction[9].

Thus, evaluating genetic diversity is crucial for protecting and utilizing germplasm resources and genetic breeding. Such a procedure significantly reveals the genetic structure, evolutionary history, potential, and causes of species endangerment. Additionally, this evaluation provides predictive guidance for selecting parents, determining the degree of genetic variation in offspring, and assessing the level of heterosis.

Simple sequence repeats (SSRs) are widely distributed in the genomes of eukaryotes and are a more accurate way to study species diversity than using phenotype variations. SSR molecular marker technology has several characteristics, such as co-dominance, high resolution, and good repeatability, making it one of the most widely used molecular marker technologies in plant genetic diversity analysis[10]. However, the silver-staining technology of artificial band reading silver-staining technology during the experimental process may impact the accuracy of the identification of different allele genes. Capillary electrophoresis technology overcomes the disadvantages of traditional silver staining methods, allowing for automated processing and data collection of large-scale samples SSR analysis, which improves the reliability of the markers. Neophytou et al. (2020) used SSR molecular marker technology to discover a significant decrease in the genetic diversity of adult *Pseudotsuga menziesii* trees compared to natural regeneration seedlings[11]. Wu et al. (2020) found low genetic diversity within *Glyptostrobus pensilis* populations based on EST-SSRs[1]. Genetic differentiation among populations was substantial, suggesting that long-term geographical isolation may be the primary reason for this regional genetic pattern.

Phoebe bournei is a broad-leaved, evergreen tall tree belonging to the family Lauraceae of the genus Phoebe. It is one of the original plants of the commercial species of *Phoebe zhennan* and is a rare and endangered tree species at the national second-level protection. The wood of this species is tough, dense, and has beautiful patterns, making it a superior material for architecture, high-end furniture, and craft carving[12]. This species is also a rare and unique ornamental tree in China. *P. bournei* is named after the abbreviation of Fujian province, where its natural population is mainly distributed. However, it is also found in other provinces (autonomous regions), such as Zhejiang, Jiangxi, Hunan, Guangdong, Guangxi, and Guizhou. *P. bournei* is widely distributed and has an extensive range and considerable geographical distance between populations. Due to human activities, *P. bournei* habitat fragmentation has been severe for a long time, and the quantity of natural germplasm resources is scarce.

There are still fewer studies on the genetic diversity of natural populations of *P. bournei* based on SSR molecular markers on a large scale. The natural populations and sample sizes involved in previous studies are small, the distribution range is narrow, the geographical distance between populations is close, and there is a lack of in-depth systematic studies, which are not sufficient to comprehensively reveal the genetic diversity and genetic structure of natural populations of *P. bournei*[13]. Therefore, this study selected six natural populations of *P. bournei* in the Fujian province and three in other provinces as materials. The genetic diversity and structure of natural populations of *P. bournei* were systematically revealed based on EST-SSR molecular marker technology, which provides a theoretical basis for the protection of germplasm resources and the exploration and utilization of excellent germplasms of *P. bournei*.

Materials and methods

Plant materials

Nine natural populations of *P. bournei* were selected from its central distribution areas in China from November to December 2021, including six in Fujian Province and one each in Chongyi in Jiangxi Province, Taizhou in Guizhou Province, and Lianzhou in Guangdong Province (Fig. 1). Each population was required to collect at least 50 evenly distributed samples. Populations with less than 50 samples were collected entirely, resulting in the collection of 460 single plants. Details for each population are shown in Table 1. Five to ten fresh tender leaves were collected from each plant and placed in sealed bags, then stored in a refrigerator at −20 °C and transported to the laboratory for DNA extraction. The longitude, latitude, and elevation of the location of each plant were recorded using a GPS device, and the diameter at breast height was measured.

DNA extraction and microsatellite genotyping

A DNA extraction kit was used to extract the genomic DNA of *P. bournei*. After that, 1% agarose gel electrophoresis was used to verify the integrity of the DNA. Next, a NanoDrop spectrophotometer was employed to assess the concentration and purity of the DNA, aiding in successive PCR amplification.

According to the literature[14,15], 16 pairs of EST-SSR primers with clear bands and substantial polymorphisms were selected for PCR amplification (Table 2). The reaction system was 25 μl: 2 μl DNA template, 1 μl forward primer, 1 μl reverse primer, 12.5 μl PCR Master Mix, and 8.5 μl ultrapure water. The DNA PCR amplification was performed using the ABI Veriti amplification instrument, with the following reaction program: predenaturation at 94 °C for 5 min, denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, for 35 cycles. The final extension was performed at 72 °C for 10 min. PCR products were detected using a fully automated nucleic acid protein analyzer (Qsep-100), and Q-Analyser-for-100 software was used to identify gene fragments.

Data analysis

Null alleles for each locus were detected using Micro-Checker 2.2.3[16]. The parameters of genetic diversity, including the

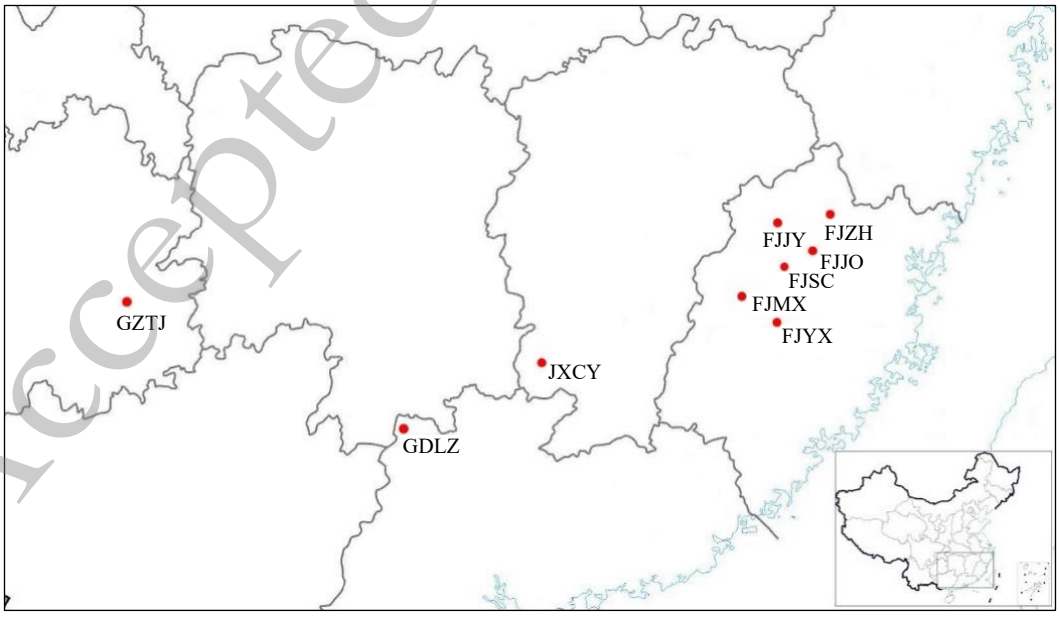

Fig. 1 Sampling location of the natural population of *P. bournei*

Table 1. Sample numbers and geographic information for experimental analysis of nine natural populations of *P. bournei*

Serial number	Population code	Location	Sample numbers	Longitude (E)	Latitude (N)	Altitude (m)
1	FJJO	Jian'ou, Fujian	28	118.37	27.08	148.16
2	FJJY	Jianyang, Fujian	50	117.84	27.39	197.82
3	FJMX	Mingxi, Fujian	56	117.27	26.41	442.37
4	FJSC	Shunchang, Fujian	69	117.94	26.87	434.72
5	FJYX	Youxi, Fujian	55	118.28	26.27	308.68
6	FJZH	Zhenghe, Fujian	50	118.62	27.44	299.60
7	GDLZ	Lianzhou, Guangdong	50	112.20	25.05	195.85
8	GZTJ	Taijiang, Guizhou	50	108.31	26.58	802.62
9	JXCY	Chongyi, Jiangxi	52	115.17	26.36	219.98

Table 2. Characteristics of sixteen SSR loci

Locus	Primer sequence (5'-3')	Length (bp)	Repeat motif	Annealing temperature (°C)	References
L1	F:TCGATTTGCAGAAGATAAGCC R:GGGGTAGAAAAGTGAAAGAGTTG	449	(ATT)14	63	Zhou et al., 2021
L2	F:AGAGGGCCTGTGCGTACGTTT R:ACATTTGAGTCGGTTCCGGTTCC	352	(TCT)12	63	Zhou et al., 2021
L3	F:GCTAGAGCTCAAAGGATCCC R:GGTGGTGATTGGACTGGTAGGAG	344	(GAA)12	63	Zhou et al., 2021
L5	F:GCCTGTGTTTGGAGTATGGA R:TTGAGTGGAGGAAGAAGTAGAAG	229	(AG)35	63	Zhou et al., 2021
L6	F:GAGAAGGGCATCAACACCAAC R:GCCTCTCCTAAGCTTTACCCA	259	(CT)31	63	Zhou et al., 2021
L8	F:GTGCTCTCTCTTGATTGTTCG R:CGGATAGGGTGATATTGTGTG	237	(CT)32	63	Zhou et al., 2021
L11	F:AAGTCCGATCTCGCAAAC R:CTCTTACCCTTCTTCCACC	283	(AG)34	63	Zhou et al., 2021
L13	F:CGTCTTCGTTTCGCTACT R:CCTTCTACTTCCCCAATCT	218	(GAA)10	63	Zhou et al., 2021
L14	F:TCTCGCCATCCTACTTCG R:GGTTTACGGTGACCTTCG	432	(TTC)10	63	Zhou et al., 2021
L15	F:AGGTTCGTCGGAGTTAGG R:TTGCGTCAATGTTGCTTC	333	(AG)33	63	Zhou et al., 2021
L17	F:AACAGGAGAAGGGAAGCAATGG R:GCCTTCAGCAATGGTGTCGG	375	(CTT)10	63	Zhou et al., 2021
L18	F:CAAGGGTGCCATGGTAGTGATAA R:AGCCTGACCCACGCACCTATAC	260	(GA)36	63	Zhou et al., 2021
L21	F:AGTAATACCAGCAGTACCAGTC R:CAGATAGCATCAGAAGCAGA	126	(AGA)11	63	Zhou et al., 2021
L23	F:AGGAATTGGAGCCGTTGGTTGT R:TACATTTGAGTCGGTTCCGGTTC	266	(TCT)12	60	Zhou et al., 2021
L24	F:GTCACAGCCCCCAAAGAATA R:GTTTCCCGCCATCACTCTTA	100	(AGG)5	60	Liu, 2019
L30	F:CCCCAAAATCACATTTCACC R:TCAACAGTTGCTTGGAATCG	218	(CCTTC)5	60	Liu, 2019

observed number of alleles (N_a), effective number of alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), Shannon's information index (I), and gene flow (N_m) for different loci and populations, were calculated using GenALEx 6.5 software[17]. A Mantel test was used to assess the genetic distance between populations concerning geographic distance and altitude difference. The correlation between genetic distance, geographic distance, and altitude difference was analyzed. Polymorphic information content (*PIC*) and fixation index (F) for different loci were calculated using Cervus software[18], in addition to performing a Hardy-Weinberg equilibrium test. Furthermore, HP-Rare software[19] was used to determine the allele richness (A_r) and private allele richness (PA_r) of the populations. FSTAT software[20] was applied to determine Nei's genetic diversity index (H), genetic differentiation coefficient (G_{st}), and inbreeding coefficient (F_{is}) of populations. Arlequin software[21] was utilized to examine the molecular variance (AMOVA) within and among populations.

Structure software[22] was used to analyze the genetic structure of the population. First, the number of clusters was set between 2–9, and 20,000 iterations of Markov chain Monte Carlo (MCMC) were run for 10 repetitions with a burn-in period of 10,000 iterations. Next, Structure Selector[23] online (https://lmme.ac.cn/StructureSelector/) was used to calculate the ΔK value, which will determine the optimal grouping number, K. The sampling analysis using CLUMPP software[24] was repeated to generate a population genetic structure diagram using Distruct software[25]. Additionally, individual evolutionary trees were built using genetic distance and the neighbor-joining method with MEGA 11 software[26].

Results

Different locus polymorphisms

The null allele frequency of each locus was less than 0.25 and the average was 0.07 (Table 3). Therefore, the null alleles had no significant effect on the estimation of population differentiation. A total of 150 alleles were detected in 460 samples using 16 loci, resulting in an average of 9.38 alleles per locus. The locus with the highest number of alleles was L15, with 55. Additionally, an average of 5.13 effective alleles per locus was observed, resulting in 82.14 effective alleles, and no ineffective alleles were found. Shannon's information index ranged from 0.93 to 2.26, averaging 1.67, suggesting high genetic diversity among the nine natural Fujian Nanmu populations at different loci. The average polymorphic information content was 0.86, all greater than 0.5, indicating high polymorphism at all 16 loci, effectively reflecting the genetic diversity of natural populations of *P. bournei*. The observed heterozygosity varied greatly across different loci, ranging from 0.32 to 0.99, with an average of 0.55, while the expected heterozygosity ranged from 0.50 to 0.84, with an average of 0.73. Except for loci L5 and L21, the expected heterozygosity at the other loci was higher than the observed heterozygosity, indicating a higher frequency of homozygotes in most loci. Furthermore, the average gene flow among all loci was 1.51, all greater than 1, indicating sufficient gene flow between populations. Four loci, namely L8, L13, L23, and L24 deviated from the Hardy-Weinberg equilibrium ($P < 0.05$), while the remaining loci were in equilibrium.

Genetic diversity of different populations

The overall level of genetic diversity in the nine natural populations of *P. bournei* was relatively high (Table 4). Still, there were significant differences in genetic diversity among the different populations. The Fujian Shunchang (FJSC) population had the highest level of genetic diversity ($N_a = 13.06$, $N_e = 7.48$, $H_o = 0.52$, $H_e = 0.83$). On the other hand, the Jiangxi Chongyi (JXCY) population had the lowest genetic diversity level ($N_a = 7.50$, $N_e = 3.69$, $H_o = 0.31$, $H_e = 0.60$), with the highest inbreeding coefficient ($F_{is} = 0.50$). The observed heterozygosity of each population was generally lower than the expected heterozygosity, and the inbreeding coefficient was greater than zero, with an average of 0.39, indicating the phenomenon of heterozygote deficiency and inbreeding in the population.

Genetic differentiation of populations

The results of the AMOVA analysis (Table 5) show that the genetic differentiation coefficient ($F_{ST} = 0.1620$) between popu-

Table 3. Genetic diversity parameters of *P. bournei* at different loci

Locus	N_a	N_e	I	H_o	H_e	PIC	G_{st}	N_m	F	$Null$	HW
L1	9	4.36	1.65	0.41	0.76	0.91	0.149	1.26	0.46	0.15	NS
L2	7	4.80	1.74	0.46	0.78	0.84	0.088	2.25	0.42	0.00	NS
L3	7	4.64	1.67	0.45	0.76	0.86	0.113	1.76	0.45	0.11	NS
L5	8	6.37	2.05	0.99	0.83	0.94	0.106	1.96	−0.20	0.00	NS
L6	17	9.27	2.26	0.57	0.84	0.95	0.109	1.84	0.37	0.00	NS
L8	13	3.93	1.45	0.34	0.67	0.82	0.175	1.09	0.55	0.01	**
L11	9	7.08	2.02	0.68	0.81	0.94	0.134	1.49	0.16	0.10	NS
L13	7	2.69	1.00	0.35	0.51	0.70	0.291	0.58	0.31	0.07	**
L14	11	6.32	1.88	0.47	0.80	0.90	0.108	1.84	0.45	0.09	NS
L15	15	6.90	2.03	0.46	0.81	0.96	0.139	1.38	0.43	0.08	NS
L17	7	4.67	1.63	0.63	0.76	0.84	0.096	1.99	0.17	0.12	NS
L18	9	6.13	1.94	0.32	0.81	0.94	0.129	1.51	0.61	0.21	NS
L21	8	3.83	1.38	0.81	0.67	0.80	0.179	1.10	−0.18	0.13	NS
L23	9	5.69	1.83	0.70	0.80	0.88	0.095	2.15	0.16	0.00	NS
L24	6	2.48	0.93	0.45	0.50	0.74	0.350	0.45	0.15	0.00	**
L30	8	2.98	1.18	0.64	0.64	0.70	0.133	1.52	0.01	0.00	*
Total	150	82.14									
Over all	9.38	5.13	1.67	0.55	0.73	0.86	0.15	1.51	0.27	0.07	

N_a number of alleles, N_e effective number of alleles, I Shannon's information index, H_o observed heterozygosity, H_e expected heterozygosity, *PIC* Polymorphic information content, G_{st} genetic differentiation coefficient, N_m gene flow, F fixation index, *Null* null allele frequency, *HW* Hardy-Weinberg equilibrium, NS no significant, * $P < 0.05$ Deviation from Hardy-Weinberg equilibrium.

Table 4. Genetic diversity parameters of different natural populations of *P. bournei*

Populaton	N_a	N_e	I	H_o	H_e	A_R	PA_R	F_{is}	H
FJSC	13.06	7.48	2.10	0.52	0.83	11.17	1.64	0.38	0.84
FJYX	10.13	6.68	1.93	0.44	0.81	9.21	0.80	0.46	0.82
GZTJ	13.06	6.73	2.01	0.41	0.80	11.35	1.29	0.49	0.81
FJJO	8.81	5.33	1.74	0.44	0.76	8.81	0.90	0.45	0.78
FJZH	8.56	4.83	1.65	0.52	0.76	7.53	1.10	0.32	0.77
FJMX	7.00	4.06	1.47	0.47	0.69	6.36	0.48	0.33	0.70
FJJY	8.06	4.24	1.55	0.64	0.71	7.27	0.74	0.11	0.72
GDLZ	4.69	3.16	1.21	0.38	0.65	4.43	0.14	0.43	0.66
JXCY	7.50	3.69	1.33	0.31	0.60	6.60	0.57	0.50	0.61
Mean	8.99	5.13	1.67	0.46	0.73	8.08	0.85	0.39	0.75

A_r allele richness, PA_r private allele richness, F_{is} inbreeding coefficient, H Nei's genetic diversity index.

Table 5. Analysis of AMOVA molecular variance of natural populations of *P. bournei*

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	*P*	F_{ST}
Among populations	8	984.88	1.15	16.20	<0.01	0.1620
Within populations	911	5425.84	5.96	83.80		
Total	919	6410.72	7.11			

lations is greater than 0.15, indicating a high level of genetic differentiation among populations. Moreover, the intrapopulation variation (83.80%) is greater than the interpopulation variation (16.20%), suggesting that the genetic variation of *P. bournei* primarily exists within populations.

The Mantel test results indicated a significant positive correlation (R_2 = 0.18, p = 0.06) between genetic distance and altitude difference (Fig. 2b) among populations. However, there was no significant correlation with geographical distance (Fig. 2a), suggesting that altitude differences primarily influence the genetic differentiation of natural populations of *P. bournei*.

Genetic distance and genetic consistency between populations

According to Fig. 3, the range of Nei's genetic distance and genetic consistency among populations was 0.47–1.48 and 0.23–0.63, respectively. These findings indicated some genetic

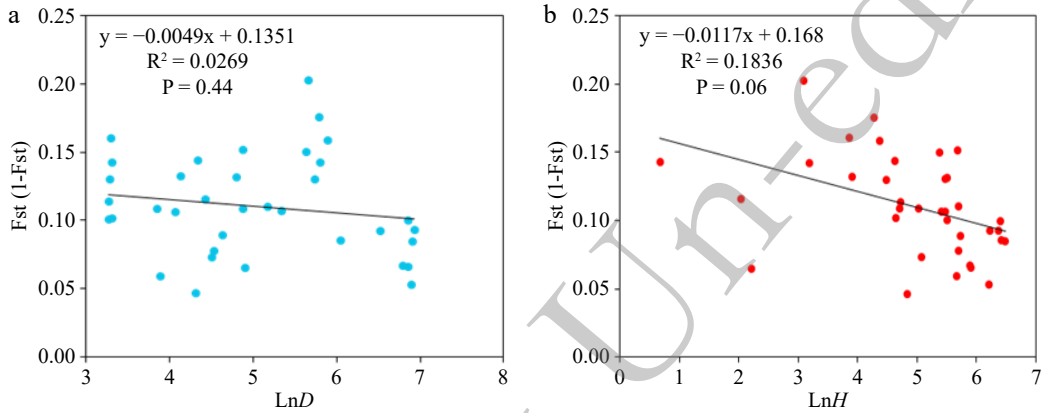

Fig. 2 Mantel test of genetic differentiation between populations and geographical distance (A) and altitude difference (B)

Population	JXCY	GZTJ	GDLZ	FJJO	FJJY	FJMX	FJSC	FJYX	FJZH
JXCY		0.63	0.53	0.32	0.29	0.62	0.35	0.46	0.38
GZTJ	0.47		0.58	0.42	0.43	0.63	0.46	0.58	0.37
GDLZ	0.64	0.55		0.25	0.40	0.57	0.30	0.41	0.52
FJJO	1.14	0.86	1.37		0.28	0.24	0.55	0.47	0.32
FJJY	1.24	0.83	0.93	1.27		0.41	0.33	0.33	0.23
FJMX	0.48	0.47	0.56	1.44	0.88		0.32	0.52	0.44
FJSC	1.06	0.77	1.22	0.60	1.12	1.15		0.58	0.41
FJYX	0.79	0.55	0.88	0.76	1.11	0.66	0.55		0.52
FJZH	0.98	0.99	0.66	1.14	1.48	0.82	0.90	0.64	

Fig. 3 Genetic distance and genetic consistency between populations. Note: Below the diagonal was genetic distance, above the diagonal was genetic consistency.

differentiation among populations, which was consistent with the results of AMOVA. Despite their proximity, Fujian Zhenghe and Fujian Jianyang exhibited the largest genetic distance (1.48) and the smallest genetic consistency (0.23), suggesting a relatively distant relationship. In contrast, Guizhou Taijiang and Jiangxi Chongyi had the smallest genetic distance (0.47) and the largest genetic consistency (0.63), implying a relatively close relationship.

Genetic structure of populations

The results of the structure analysis indicated that the genetic differentiation of each population was most effectively reflected when K = 2 (Fig. 4). The maximum value of ΔK suggested that the genetic composition of all individuals could be classified into two clusters (Fig. 5). The first group (represented by the blue section) comprised Fujian Mingxi (100%), Guangdong Lianzhou (100%), Fujian Jianyang (98%), Jiangxi Chongyi (92.31%), and Guizhou Taijiang (54%). The second group (represented by the orange section) included Fujian Shunchang (100%), Fujian Jianou (100%), Fujian Youxi (81.82%), and Fujian Zhenghe (62%). In conclusion, the population Fujian Mingxi, Guangdong Lianzhou, Fujian Shunchang and Fujian Jianou exhibited lower gene penetration, while other populations underwent varying degrees of gene exchange.

After analyzing the Q values for all individuals (Fig. 5), 421 individuals were discovered to have Q values greater than 0.6, accounting for 91.52% of the total. This indicated that the genetic composition of the individuals in these populations was relatively uniform, with only a few individuals exhibiting mixed genetic ancestry. On the other hand, the remaining 49 individuals had Q values less than 0.6, accounting for 8.48%. This suggests that the genetic composition of these individuals was more mixed.

We used the neighbor-joining method to construct a population clustering tree according to the genetic distance between individuals (Fig. 6). Based on this analysis, individuals from the tested populations were classified into two groups, consistent with the results obtained from the structure analysis. Group Ⅰ consisted 278 individuals, primarily sourced from individuals belonging to five distinct populations: Mingxi in Fujian, Jianyang in Fujian, Chongyi in Jiangxi, Lianzhou in Guangdong, and Taijiang in Guizhou. Furthermore, it included 10 individuals samples from Youxi in Fujian, 8 individuals from Shunchang in Fujian, 2 individuals from Jianou in Fujian, and 1 individuals from Zhenghe in Fujian. Group II contained 182 individuals, mainly from individuals of four populations including Zhenghe, Youxi, Jianou, and Shunchang in Fujian, as well as 1 individual from Taijiang, Guizhou.

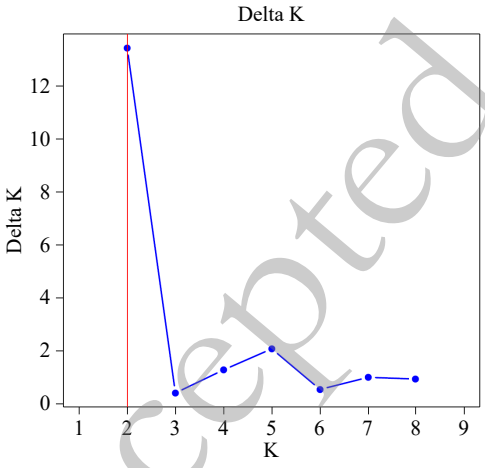

Fig. 4 The relationship between the optimal group number K and the inferred value ΔK

Discussion

The use of polymorphic primers is a requirement when performing genetic diversity analyses. The level of polymorphism of the primers can be reflected in the polymorphism information content (PIC). If the PIC value is greater than 0.5, it indicates that the primers have a high contribution rate and adequate polymorphism and can fully portray the genetic diversity of populations[27]. Here, 16 primer pairs were used to examine the genetic diversity of 460 samples drawn from nine natural populations of *P. bournei*. The average PIC value obtained was 0.86. These results suggest that the SSR primers used in this study had an adequate polymorphism and were suitable for analyzing genetic diversity. They provided accurate and effective information on the genetic diversity of *P. bournei* populations in their natural habitats.

Genetic diversity within and between species is crucial for evolution and conservation[2]. Furthermore, a high level of genetic diversity within a species leads to great adaptability to environmental changes and improved vitality[28]. The current study investigated the genetic diversity of the natural population of *P. bournei*, which demonstrated the presence of 150 alleles, an expected heterozygosity range of 0.50 to 0.84, and an average value of 0.73. In particular, although the Fujian Jianou population had a small population size (28), it had a high level of expected heterozygosity (H_e = 0.76).

Furthermore, the genetic diversity of our studied species was found to be roughly equivalent to that of the relic species *Ginkgo biloba* (H_e = 0.808)[29], lower than the endangered species *Semiliquidambar cathayensis* (H_e = 0.816)[30] and the widely-distributed *P. menziesii* (H_e = 0.90)[11], significantly higher than that of the relic species *G. pensilis* (H_e = 0.272)[1]. Therefore, despite human disturbance, the natural population of *P. bournei* has maintained a high level of genetic diversity, indicating its potential for genetic evolution[31]. This may be attributed to the longer lifespan and shorter fragmentation duration of *P. bournei*, as the genetic diversity changes resulting from fragmentation have not yet been manifested[32].

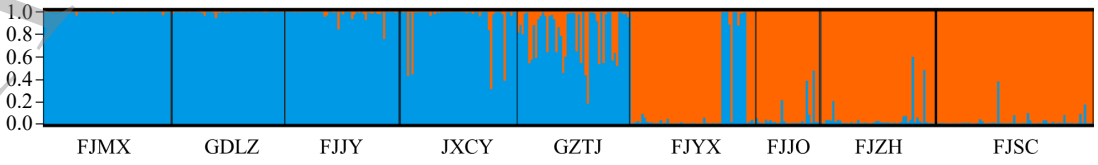

Fig. 5 Population genetic structure based on STRUCTURE analysis

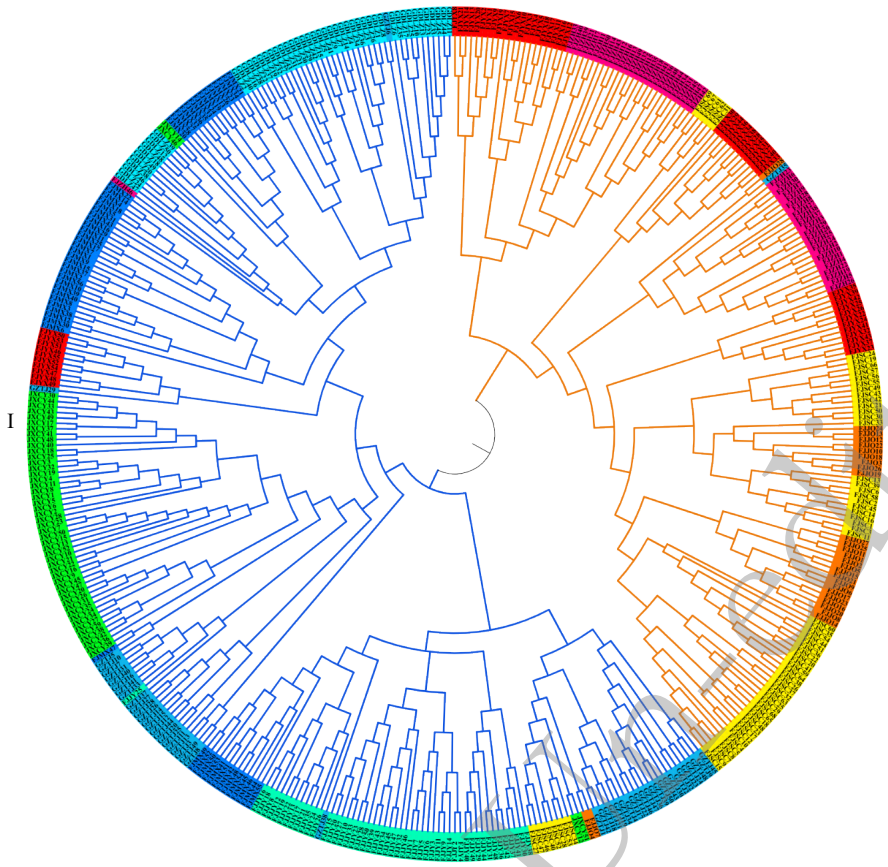

Fig. 6 Cluster analysis based on genetic distance among individuals

Genetic diversity parameters can reveal the genetic structure and differentiation within a species. This study compared genetic parameters from natural populations of *P. bournei* from different geographical regions and found differences in genetic diversity among the different populations of the species. The population from Shunchang, Fujian, had the highest genetic diversity, while the population from Chongyi, Jiangxi, had the lowest. The Shunchang population had a relatively large area with a high population density and large distances between individuals, growing in a scattered manner conducive to insect pollination and cross-pollination. Additionally, the Shunchang population was located at a high altitude (430 m), was relatively less affected by human activity, and had a lower rate of habitat fragmentation, maintaining its original population size and reducing the possibility of genetic drift, which maintained a high level of genetic diversity. Similarly, the population located at the highest altitude (800 m) in Taijiang, Guizhou, also had a high level of genetic diversity.

In contrast, the Chongyi population had a small area with short pollination distances between individuals, leading to a greater likelihood of inbreeding and inbreeding depression. Besides, *P. bournei* exhibited strong understory regeneration ability, and this population also displayed a parent-child coexistence phenomenon, with prominent kinship relationships among individuals. Furthermore, the Chongyi population was located at a low altitude, close to human settlements, and was heavily affected by human disturbances, resulting in small population sizes. The population at the lowest altitude in Lianzhou, Guangzhou, had a similar situation, with less genetic diversity. After conducting the sampling survey, it was discovered that although Zhenghe, Fujian, has the largest population area of the nine populations studied, the individuals within the population are highly concentrated, resulting in a high probability of inbreeding. This, in turn, has led to moderate genetic diversity within the population. Therefore, conservation and utilization strategies for *P. bournei* germplasm resources should prioritize populations with high genetic diversity, such as those in Shunchang, Fujian, which is well-preserved and has a large population size.

Perennial interspecific hybrids with a broad distribution and strong adaptability are believed to exhibit higher levels of population genetic differentiation[2]. Therefore, the population genetic differentiation coefficient is essential for evaluating the population genetic structure. A differentiation coefficient of 0.05–0.15 between populations is usually considered a moderate differentiation level. This study found that the natural population of *P. bournei* had a genetic differentiation coefficient of 0.1620, which was at a high level. It was higher than that of endangered species *Ormosia henryi* (G_{st} = 0.0918)[33] and *G. biloba* (G_{st} = 0.093)[29] but lower than that of *G. pensilis* (G_{st} = 0.452)[1], and similar to that of *Taxus mairei* (G_{st} = 0.140)[34].

P. bournei is a woody perennial plant with many suitable habitats. The species has undergone long-term evolutionary sedimentation, resulting in rich genetic variation. However, due to overharvesting and human disturbances over a prolonged period, the area and number of natural populations have declined sharply, accelerating the process of habitat fragmentation. The reduction in population size may increase inbreed-

ing and lead to population decline. However, since habitat fragmentation in natural populations of *P. bournei* is relatively recent, the degree of reduction in genetic differentiation levels is still relatively small. This study also found that in nine populations, about 64.78% of the individuals had a relatively single genetic composition. The observed heterozygosity was lower than expected, indicating a lack of sufficient heterozygotes, and the natural population of *P. bournei* had to inbreed. As an allogamous plant, habitat fragmentation restricts pollen dispersal, reducing the probability of random mating and making inbreeding more likely in the population. Therefore, a reasonable evaluation of the genetic structure of the natural population of *P. bournei* should also consider its genetic mating characteristics. Further research into the genetic mating system of *P. bournei* is needed to evaluate its genetic diversity more accurately and to provide a scientific and comprehensive theoretical basis for formulating appropriate protection strategies.

Molecular variance analysis revealed that genetic variation in *P. bournei* primarily occurred within populations, similar to outcrossing plants such as *T. mairei* and *G. biloba*. *P. bournei* reproduces through cross-pollination, and its pollen can travel long distances, promoting strong gene flow and genetic exchange between populations, leading to a gradual convergence of gene frequencies. Consequently, conservation efforts should prioritize genetic diversity within populations. In addition, altitude differences cause water, heat, light, and soil variations, leading to plant adaptation and genetic differentiation among populations[35,36]. This study found that genetic distance among *P. bournei* populations strongly correlates with altitude differences but is not significantly related to geographic distance, consistent with previous research on the woody plant *Amygdalus mira* in the tibet plateau in China[37]. This result suggests that natural selection in different environments at varying altitudes leads to significant genetic differences among populations. However, populations at the same altitude exhibit similar genetic variations.

The population genetic structure results of *P. bournei* indicated that there may be two gene sources in the tested materials. There was varying degrees of gene penetration between populations, and some individuals within the populations had complex genetic relationships. Although gene penetration between populations was not strictly clustered according to geography, it was still affected by certain geographical isolation. Despite Taijiang in Guizhou, situated on the Yunnan-Guizhou Plateau, and Lianzhou in Guangdong and Chongyi in Jiangxi, located in the Nanling Mountains, are situated far away from Jianyang and Mingxi in Fujian, which are near the Wuyi Mountains, these five natural populations all reside in high-altitude mountainous environments and may be subject to similar pressures of natural selection. In the long-term evolution process, there may be gene exchange between them, so they have relatively similar genetic structures. Fujian Zhenghe, Fujian Jianou, Fujian Shunchang, and Fujian Youxi are four populations situated in the low hilly regions of central and northern Fujian, sharing similar growth environments. Due to their proximity to each other, there may be seed exchange between these populations. The pronounced genetic difference between these two groups could potentially stem from geographical isolation, which is a result of the significant environmental differences between mountain ranges, plateaus, and hills.

Conclusions

Although *P. bournei* has been heavily harvested, their relatively high genetic diversity suggests that there is still immense potential for genetic evolution within their natural populations. Genetic diversity varied noticeably among different populations, with the Shunchang population in Fujian boasting the largest geographical area, scattered distribution of individuals, and the highest level of genetic diversity. Therefore, this population deserves priority protection. Despite the low genetic diversity in the Lianzhou, Guangdong, and Chongyi, Jiangxi populations, the individuals in these groups still contained many alleles. Therefore, it is crucial to prevent the loss of genetic diversity due to human activities or natural disasters. Genetic variation in *P. bournei* existed mainly within populations, and genetic differentiation between populations was moderate. The nine natural populations of *P. bournei* could be divided into two categories, with varying degrees of gene infiltration between populations. Group Ⅰ included populations in Fujian Mingxi, Jianyang Fujian, Jiangxi Chongyi, Lianzhou Guangdong and Guizhou Taijiang. Group II consisted populations in Zhenghe, Youxi, Jianou, and Shunchang from Fujian.

Author Contributions

The authors confirm contribution to the paper as follows: study conception and design: conducting the experiments: Wang Y; data curation: Wang Y; writing - original draft: Wang Y; resources collection: Fan H, Pan X, Tang X; writing–review & editing: Zhou Z; project administration: Fan H, Zhou Z; designed and supervised the project: Fan H, Zhou Z; All authors reviewed the results and approved the final version of the manuscript.

Data archiving statement

In this manuscript, there is no raw sequences such as nucleic acid sequences, and protein sequences. Nevertheless, all the data for calculation are in possession of the authors and available for the reviewers or for submission in any database if it is necessary.

Data availability

The data are available upon request from the corresponding author.

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Conflict of interest

The authors declare that they have no conflict of interest.

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