Genetic Structure in 21 Remnant Phoebe Sheareri Populations in Southern China: Implications for Genetic Resource Conservation

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Abstract

Background: *Phoebe sheareri* (Lauraceae) is a valuable and endemic tree species in China, with limited large natural communities remnant. Genetic diversity and differentiation analysis are essential to manage their conservation and utilization. To provide a conservation and utilization strategy of *P. sheareri* based on sound genetic diversity and differentiation data.

Results: We found medium level of genetic diversity and low inbreeding. Nei's gene diversity and Shannon's information index value showed medium genetic diversity in nature populations of *P. sheareri*, which was higher than other two *Phoebe* species. AMOVA showed the genetic differentiation among populations was significantly, and 21.2% of genetic variation was among populations. Bayesian clustering, obtained with STRUCTURE, grouped the populations into four genetic clusters, whereas UPGMA analysis distinguished three main groups approximately in line with the geographic area of occurrence.

Conclusion: Based on the study results, the establishment of gene conservation units must be considered in nature conserves in order to protect the genetic diversity of the species, and the proposal of sampling strategies for ex situ conservation and reforestation.

Background

Phoebe sheareri (Hemsl.) Gamble (Lauraceae) is a valuable tree species unique to China. Its hard wood, with smooth cut surfaces and beautiful texture is highly valued for quality furniture and engraving material. *P. sheareri* is an important original plant species of the commercial timber known as "golden nanmu", with the most beautiful wood vein and highest cold tolerance among *Pheobe* species. The habitat of this tree species usually was warm and humid environment, which is commonly found in broadleaf forests of mountain valleys below 1,000 m (Liu 1957). *P. sheareri* is naturally distributed in Zhejiang, Jiangxi, Guizhou, Fujian, Guangdong, Guangxi, Hunan and Hubei provinces in southern China, within the range 24.50–32.05°N, 106.23–121.45°E (Fig. 1). However, due to excessive harvesting, lack of protection, and weak natural regeneration capabilities (Chen et al. 2013), *P. sheareri* natural stocks are in decline, with limited natural communities remnant (Chen et al. 2014). This fragmentation of habitats will hinder the gene flow between two populations, increasing the possibility of the reduction of genetic diversity. With the aim to ensuring the genetic diversity from being decreasing, it is urgent to the proposal of an effective protection strategy for *P. sheareri.* The understanding of the genetic diversity and structure of *P. sheareri* natural population is the prerequisite to making conservation strategy.

Genetic diversity is an essential component of biodiversity, and is of great significance for understanding the origin of species, predicting adaptability, and estimating the distribution of genetic resources (Muriira et al. 2018). Genetic markers are the main tools for studying genetic diversity; these can include phenotypes, cytology, biochemistry, and molecular markers (Ge et al. 1988; Qin et al. 2016). The advantage of expressed sequence tags microsatellite (EST–SSR) markers is that their polymorphism is associated with the transcribed sequences, reflecting the variation in the expressed region of the genome. EST–SSR markers also have the advantages of good stability, high polymorphism, co-dominant inheritance, and easy operation. It has been widely applied to study genetic diversity in many plant species (Varshney et al. 2005). For example, Wang et al (2019) studied the genetic diversity and genetic structure of *Magnolia sinostellata* P.L.Chiu et Z.H.Chen by EST-SSR. They found that *M. sinostellata* has rich genetic diversity and obvious population genetic differentiation, and habitat destruction caused by human activities is the main threat to this species (Wang et al. 2019). Tanhuanpää et al (2019) using SSR markers found that most of the genetic diversity exists within population (83%), and revealed that geographical barriers separate European hazelnut (*Corylus avellana* L.) populations, and decrease gene flow (Tanhuanpää et al. 2019). Similar studies in *Phoebe zhennan* S. Lee (Shi et al. 2016), *Phoebe chekiangensis* C.B. Shang (Ding et al. 2015), *Prunus Armeniaca* L. (Hu et al. 2018), *Cinnamomum camphora* (L.) Presl. (Zhong et al. 2019), and

Fraxinus chinensis Roxb. (Wu et al. 2016) were conducted to understand their genetic diversity and genetic structure of natural populations.

However, the genetic diversity and structure of natural residual *P. sheareri* populations were unclear, which hinders the proposal of protection strategy in *P. sheareri.* In the present study, with the aim to proposing a conservation strategy for this endangered species *P. sheareri*, 32 pairs of polymorphic EST-SSR primers were applied to analyze 21 natural populations involving in 428 individuals. Firstly, the genetic diversity and structure of 21 populations were evaluated. Secondly, the genetic differentiation within and between populations was also evaluated and combined with the geographic distance and climatic index to reveal the potential force driving the present structure. Lastly, the conservation strategy for this valuable tree species has been proposed.

Methods

Field study and experimental materials

Following an *in situ* survey of the natural distribution of *P. sheareri*, 21 natural populations with representative habitat conditions were selected for sampling, from which we collected a total of 428 samples. Sampling trees were separated by a minimum distance of 30 m. The 21 sampled populations included one population in Jiangsu province (ZJS), 12 in Zhejiang province (XH, TMS, THY, CH, LHT, ZJB, LJ, CA, NH, SC, SY, and QY), and two populations from each of the Jiangxi, Hunan, Guizhou and Guangxi provinces (populations WY, LC; ZJ, XN; FJS, XS; and ZY, LS, respectively), representing the major natural *P. sheareri* habitats of China (Table 1, Fig. 1). We collected 15 pieces of young leaves from each tree; sampled leaves were quickly placed in ziplock bags, stored in a cooler on ice, and brought to the laboratory, where they were frozen at − 40 °C until DNA extraction. The Global Positioning System (GPS) was used to record the latitude, and the longitude. The main climatic factors of each sampling site were obtained through the China Meteorological Science Data Sharing Center platform (Table 1).

Table 1
Locations and ecological characteristics of the sampled *P. sheareri* populations

Number	Populations	Sample size	Location	Longitude (E)	Latitude (N)	Annual mean temperature (°C)	Annual Precipitation (mm)
1	ZJS	21	Zijinshan,Jiangsu	118°52'11'	32°03'05'	19.6	1530.1
2	XH	19	Xihu,Zhejiang	120°05'36'	30°13'44'	17.8	1454.5
3	TMS	22	Tianmushan,Zhejiang	119°26'19'	30°19'49'	14.5	1400.0
4	THY	20	Taihuyuan,Zhejiang	119°33'29'	30°23'18'	15.9	1425.5
5	CH	27	Changhua,Zhejiang	119°07'29'	30°07'48'	15.5	1450.5
6	LHT	15	Lvhetang,Zhejiang	119°07'47'	29°26'38'	17.1	1550.0
7	ZJB	19	Zhujiabu,Zhejiang	119°12'02'	29°26'56'	17.4	1600.5
8	LJ	17	Longjiang,Zhejiang	119°40'23'	29°36'09'	15.8	1650.0
9	CA	16	Chunan,Zhejiang	119°07'56'	29°28'02'	17.0	1430.0
10	NH	17	Ninghai,Zhejiang	121°24'01'	29°26'34'	16.1	1300.0
11	SC	27	Suichang,Zhejiang	119°27'50'	28°37'35'	16.8	1510.0
12	SY	10	Songyang,Zhejiang	119°24'04'	28°23'43'	17.7	1700.0
13	QY	26	Qingyuan,Zhejiang	119°23'20'	27°40'44'	17.4	1760.0
14	WY	14	Wuyuan,Jiangxi	117°27'03'	29°10'37'	16.8	1821.0
15	LC	30	Lichuan,Jiangxi	116°55'27'	27°02'35'	17.9	1749.5
16	ZJ	20	Zhijiang,Hunan	109°32'27'	27°31'19'	16.4	1294.6
17	XN	12	Xinning,Hunan	110°43'40'	26°16'34'	16.2	1680.5
18	FJS	28	Fanjingshan,Guizhou	108°43'31'	27°53'32'	13.9	1850.5
19	XS	21	Xishui,Guizhou	106°23'12'	28°29'24'	13.1	1109.9
20	ZY	24	Ziyuan,Guangxi	110°32'27'	25°51'20'	16.4	1761.1

Number	Populations	Sample size	Location	Longitude (E)	Latitude (N)	Annual mean temperature (°C)	Annual Precipitation (mm)
21	LS	23	Longsheng,Guangxi	109°55'14'	25°37'52'	18.1	1705.5

Population Age-class Determination

We established one or two 20 m × 20 m plot in each sampling area, based on the population size. The height and basal diameter of each plant was measured. In each of the 400 m^2 plots, 2 m × 2 m subplots to investigate species composition and height and canopy coverage of the shrub layer were established. We also established one 1 m × 1 m plot at the center of each subplot to measure species composition, height, and canopy coverage of the herbaceous layer (Fang et al. 2009).

P. sheareri is a protected species, wood cores or destructive sampling was forbidden to determine the age of individuals. Therefore, we analyzed population age structure according to diameter class, combing with the sapling height, and basal diameter (D) was used as a standard to categorize the diameter classes (Liu et al. 2015). Recording to Qu et al (1952), the first diameter class (I) was defined as $D \leq 2.5$ cm and $H \leq 0.33$ m; the second diameter class (II) was defined as $D \leq 2.5$ cm and $H > 0.33$ cm; the third diameter class (III) was defined as $2.5 \text{ cm} < D \leq 7.5$ cm; the fourth diameter class (IV) was defined as $7.5 \text{ cm} < D \leq 22.5$ cm; and the fifth diameter class (V) was defined as $D > 22.5$ cm. Then, the plotted diagrams of population diameter-class structure were plotted (Qu et al. 1952).

Dna Extraction

Approximately 100 mg of young leaves per sample were used for genomic DNA extraction using the CTAB Plant Genomic DNA Rapid Extraction Kit (Aidelai Biotechnology Company, Beijing, China). DNA quality and concentration were determined using Nanodrop 2000 system, and DNA integrity was evaluated using 1% agarose gel electrophoresis. DNA was diluted to a final concentration of 25 ng/μL and stored at − 20 °C prior to EST-SSR amplification.

Est–ssr Primer Screening

A total of 6958 SSR loci were detected in *P. sheareri* transcriptome data using MISA (http://pgrc.ipk-gatersleben.de/misa/misa.html), and batch-developed EST-SSR primers were produced using Primer 3.0, as described previously (Lu et al. 2018). Ninety-four pairs were randomly selected and synthesized by the Nanjing Kingsray Biotechnology Co. Primers, then were selected using 10 different *P. sheareri* DNA sources, and polymorphisms were used in this study (Table S1).

Pcr Amplification And Product Detection

PCR was performed in 10 μL reaction volumes containing 1 μL genomic DNA (25 ng/μL), 0.5 μL each of forward and reverse primers (10 μmol/L), 5 μL Taq premix (0.25 U Taq, TaKaRa), and 3 μL of ddH_2O. The reaction mixture was initially denatured at 94 °C for 4 min, followed by 32 cycles of amplification at 94 °C for 30 s, 50–54 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 5 min on a Bio-Rad thermocycler (Bio-Rad Laboratories, Hercules, CA, USA). The PCR amplification products were electrophoresed on 8% non-denaturing polyacrylamide gel for 90 min. The bands were visualized by silver staining (Bassam et al. 1991; Charters et al. 1996), observed on a visible light box, photographed, and read band length.

Data Analyses

The DNA fragments amplified by the SSR primers were recorded as alleles based on the comparison with external standards, and a bp data matrix was obtained. Use DateTrans1.0 software to convert the data into the required form for subsequent analysis (Gai et al. 2011). Number of observed alleles per locus (*Na*), number of effective alleles (*Ne*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), *Nei's* gene diversity (*Nei's*), Shannon's information index (*I*), inbreeding coefficient at the population level (*Fis*), inbreeding coefficient at total populations (*Fit*), inter-population differentiation coefficient (*Fst*) and gene flow (*Nm*), estimation of allele frequency were calculated by PopGene 32 software(Yeh et al. 2000). The polymorphic information index (*PIC*) was calculated by using the PIC_CALC software. Analysis of molecular variance (AMOVA) to estimate the distribution of genetic variation among and within populations, estimating F-tatistics to test the actual proportion of genotypes in the population, Hardy-Weinberg equilibrium testing was analyzed in GenAlEx 6.5 (Peakall et al. 2012). *Nm* was estimated by $Nm = 0.25 (1-Fst)/Fst$ (Slatkin et al. 1989).

The Bayesian clustering analysis was conducted to evaluate the genetic structure of *P. sheareri* populations using STRUCTURE version 2.3 (Daniel et al. 2003). This method estimates the number of genetic clusters (*K*) in the data and estimates the ancestry of individuals in these clusters (Daniel et al. 2003). The admixture model with correlated allele frequencies was applied to Bayesian analysis. Models were tested for *K*-values (testing from $K = 1$ to $K = 10$), and the model was run with 10 independent stimulations for each *K*, and a burn-in period of 100,000 iterations and 100,000 Markov chain Monte Carlo (MCMC) repetitions (Daniel et al. 2003). The most likely *K* value was determined using Structure Harvester based on both the log likelihood and the maximum ΔK (Earl et al. 2012).

Due to the mutation rate of the microsatellite sequence and the effective content of the population, the accuracy of the phylogenetic tree by UPGMA (Unweighted Pair Group Method with Arithmetic means) is slightly higher than that of Bayesian analysis (Takezaki et al. 1996; Nei et al. 2000). The UPGMA tree was constructed based on *Nei's* genetic distance using PopGene 32(Yeh et al. 2000).

Geographic distances between populations were estimated with Earth Explorer 6.5, the Mantel test between genetic distance and geographic distance, annual precipitation difference, annual mean temperature difference was detected by XLSTAT software (XLSTAT 2017).

Results

The population characteristics of P. sheareri

The diameter-class of 21 populations showed varied distribution patterns (Fig.S1). The class I seedlings were predominantly in the Population LS; the class II saplings had proportional advantage in the Population ZJS, CH, and WY; the class III young trees were predominantly in the Population THY, ZJB, QY, and LC; the class IV middle-aged trees had dominant position in the Population TMS, CA, LJ, XH, NH, SC, SY, ZJ, XN, FJS, XS, and ZY, especially this class tree accounting for above 50% in Population LJ and ZY; while the class V big-aged trees were predominantly in the Population LHT. However, none class V tree was observed in nine populations such as ZJS, THY, and ZJB (Fig.S1).

Polymorphisms Of The Est-ssr Markers

A total of 428 samples from 21 *P. sheareri* populations were amplified using 32 pairs of EST-SSR primers, and 105 alleles were detected. The value of *Na* ranged from 2 to 5 (mean = 3.219), and *Ne* ranged from 1.179 to 3.728 (mean = 2.159). *Ho* and *He* was 0.603 (0.318–0.884) and 0.493 (0.267–0.848), respectively. The average *Nei's* was 0.507, with a minimum at ZN-35 (0.152) and a maximum at site ZN-77 (0.732). *I* averaged 0.832, with a minimum at ZN-35 (0.320) and maximum at

ZN-77 (1.427). In addition, the *PIC* values for all of the loci ranged from 0.144 (ZN-35) to 0.710 (ZN-72), with an average of 0.428, showing medium informative scores (0.25 < *PIC* < 0.5) (Table S2).

Genetic diversity parameters among P. sheareri **populations**

Genetic diversity parameters among the 21 *P. sheareri* populations are shown in Table 2. The average percentage of polymorphic loci (*PPL*) was 95.83%. The average *Na* was 2.131, and the average *Ne* was 1.701. The average *Ho* across all populations was 0.609, with minimum and maximum values of 0.532 and 0.684 at the ZJS and XS sites, respectively. The average *He* was 0.614, with a minimum and maximum of 0.579 and 0.689 at FJS and SY, respectively. *Nei's* ranged from 0.295 at SY to 0.413 at FJS, with the average of 0.376. The average *I* among all populations was 0.576, ranking from high to low as FJS > ZJ > THY > XH > SC > CH > LHT > ZJS > TMS > WY > ZJB > LS > LC > CA > QY > XS > ZY > NH > XN > LJ > SY.

Table 2
Genetic diversity among the 21 sampled *P. sheareri* populations

Populations	*PPL*	*Na*	*Ne*	*Ho*	*He*	*Nei's*	*I*
ZJS	96.88	2.091	1.762	0.532	0.591	0.399	0.596
XH	96.88	2.242	1.773	0.579	0.583	0.406	0.622
TMS	100.00	2.242	1.717	0.601	0.616	0.375	0.590
THY	100.00	2.273	1.779	0.544	0.592	0.398	0.624
CH	100.00	2.182	1.742	0.610	0.594	0.398	0.607
LHT	93.75	2.152	1.770	0.579	0.593	0.394	0.601
ZJB	96.88	2.182	1.682	0.576	0.620	0.370	0.576
LJ	90.63	2.030	1.595	0.633	0.654	0.336	0.511
CA	100.00	2.121	1.674	0.584	0.610	0.378	0.573
NH	93.75	2.030	1.589	0.635	0.646	0.344	0.523
SC	96.88	2.152	1.806	0.617	0.582	0.410	0.619
SY	84.38	1.909	1.531	0.670	0.689	0.295	0.451
QY	96.88	2.030	1.698	0.614	0.610	0.382	0.572
WY	96.88	2.061	1.709	0.599	0.603	0.383	0.579
LC	96.88	2.030	1.705	0.629	0.605	0.388	0.574
ZJ	96.88	2.212	1.793	0.582	0.580	0.409	0.633
XN	93.75	2.030	1.591	0.657	0.649	0.336	0.519
FJS	100.00	2.394	1.800	0.595	0.579	0.413	0.649
XS	93.75	2.182	1.663	0.684	0.636	0.355	0.558
ZY	96.88	2.091	1.626	0.633	0.644	0.348	0.537
LS	90.63	2.121	1.719	0.635	0.619	0.373	0.575
Mean	95.83	2.131	1.701	0.609	0.614	0.376	0.576

PPL = The percentage of polymorphic; *Na* = Observed number of alleles; *Ne* = Effective number of alleles; *Ho* = Observed heterozygosity; *He* = Expected heterozygosity; *Nei's* = Nei's gene diversity; *I* = Shannon's information index.

The genetic structure among P. sheareri populations

Using a ΔK value to determine a reasonable K, the ΔK value reached a maximum when $K = 4$ (Fig. 2a). The 21 natural populations of *P. sheareri* could then be divided into four distinct groups. The first category included ZJS, XH, TMS, THY, CH, LHT, ZJB, LJ, and CA; the second category comprised NH, SC, WY, and LC; the third category included SY, QY, and XN; and the fourth was composed of ZJ, FJS, XS, ZY, and LS (Fig. 2b).

Fst and Nm among P. sheareri populations

The AMOVA indicated larger genetic variation within populations than among them (Table 3). Nevertheless, genetic differentiation among populations was extremely significant ($P < 0.001$). *Fst* analysis indicated that 21.2% of genetic

variation was among population (Table 3). Similar results were obtained when calculating by PopGene software (*Fst* = 0.227, Table S3). The inbreeding coefficient (*Fis*) was predicted to be low, only 0.036. The average *Nm* calculating by all SSR loci was 1.322, and the *Nm* derived from above formula involving in *Fst* was 0.927, suggesting that there is relatively low gene flow among *P. sheareri* populations.

Table 3
AMOVA results for within and among population variations in *P. sheareri*

Source of variation	df	Sum of square	Mean square	Variance component	Percentage of variance(%)
Among populations	20	1623.640	81.182	1.819	21%
Among individuals within populations	407	2962.198	7.278	0.535	6%
Within individuals	428	2657.000	6.208	6.208	73%
Total	855	7242.838		8.562	100%
Fst	0.212***				
Fis	0.079***				
Fit	0.275***				
Nm	0.927				
***: P < 0.001					

Genetic Distance Among Populations And Cluster Analysis

Nei's genetic distance and *Nei's* genetic identity among the 21 natural populations of *P. sheareri* were shown in Table S4, and they ranged from 0.077 to 0.492 and from 0.612 to 0.926, respectively. TMS and THY had the largest genetic identity and the smallest genetic distance, indicating that their kinship was closer than that of other populations. Genetic identity was smallest between the SY and LS populations and their genetic distance was the largest, indicating higher genetic differentiation between two populations.

Based on the *Nei's* genetic distance between populations, UPGMA cluster analysis was performed on the 21 populations (Fig. 3). All populations were divided into three categories. The first major category included ZJS, XH, TMS, THY, CA, CH, LHT, ZJB, LJ, SC, LC, WY, QY, and NH, which located in the eastern part of the distribution. The first category could be further clustered into four sub-categories: 1) ZJS and XH; 2) TMS, THY, CA, CH, LHT, ZJB, and LJ; 3) SC, LC, WY, and QY; and 4) NH, which located in the eastern part of the distribution. The second category comprised ZJ, ZY, XN, FJS, LS, and XS, which located in the southwestern part of the distribution. The population SY was in the third category, locating in the southern part of Zhejiang province.

Mantel test between genetic distance and geographical distance and climate difference

Mantel test analysis showed a significant correlation between geographic and genetic distance among the *P. sheareri* populations (r = 0.624; P < 0.0001; Fig. 4). It indicated that the geographic distance observed among populations were key factors influencing genetic differentiation. The Mantel test showed that the genetic distance was insignificantly positively correlated with temperature difference (r = 0.114, P = 0.098, Supplementary Fig.S2a), but significantly correlated with the precipitation difference (r = 0.204, P = 0.003, Supplementary Fig.S2b). Furthermore, the mantel test showed that the genetic distance was significantly positive correlated with geographic distance among western populations (r = 0.525, P <

0.0001), whereas insignificantly with precipitation difference (r = -0.027, P = 0.784, Supplementary Fig.S3). In the case of eastern populations, neither precipitation difference nor geographic distance was significantly with genetic distance (Supplementary Fig.S3).

Discussion

Genetic diversity in P. sheareri

Higher genetic diversity might reflect better adaptation to the varied environment (Gadissa et al. 2018). The genetic diversity of larger and older population is higher compared to smaller and newly established populations due to higher levels of accumulation and maintenance of genetic variation (Rampersad et al. 2013). The information of markers based on *PIC* were generally defined as low (*PIC* < 0.25), medium (0.25 < *PIC* < 0.5), or high (*PIC* > 0.5) (Botstein et al. 1980). In the present study, the average *PIC* was 0.428, indicating moderate polymorphism (Nagy et al. 2012). The primers used in this study were suitable genetic diversity markers for *P. sheareri* populations. The average observed heterozygosity (*Ho* = 0.609) and the average expected heterozygosity (*He* = 0.614) showed the intermediate level of genetic variation within *P. sheareri* population, which was higher than those tree species such as *Dalbergia odorifera* T. Chen (*Ho* = 0.28, *He* = 0.37) (Liu et al. 2019), *Pseudotsuga menziesii* (Mirbel) Franco (*Ho* = 0.230, *He* = 0.302) (Montiel et al. 2019), but lower than that of *Quercus variabilis* Bl. (*He* = 0.707) (Shi et al. 2017). It might be related to the species distribution range and sampling size. For example, *D. odorifera* is indigenous to Hainan Island in China, and 42 wild trees from seven populations covering its whole native distribution were typed by 19 SSR markers (Liu et al. 2019). A total of 12 *P. menziesii* populations (234 trees) from the central region of Mexico were used to determine the genetic diversity by 12 SSR markers (Montiel et al. 2019). However, *Q. variabilis* is widely distributed in China, and 879 samples from 19 populations were evaluated using 25 SSR markers (Shi et al. 2017).

Both *Nei's* and *I* were used to reflect the level of genetic diversity, such that greater values indicate higher genetic diversity within the population. In this study, the average values of *Nei's* and *I* were 0.376 and 0.576, respectively, indicating that natural populations of *P. sheareri* contain rich genetic diversity. There is an inseparable relationship between the genetic diversity of a species and its living habits and life history characteristics (Hamrick et al. 1979). High genetic diversity has also been detected in other subtropical tree species, which is likely a consequence of life history traits of these trees, such as a long life span and a predominantly outcrossing mating system (Hamrick et al. 1996; Petit et al. 2006). Genetic diversity is also affected by geographical distribution, population size, and climate change caused by glaciers (Angela et al. 2012). Populations that are continuously distributed over a large area have more opportunities to maintain the level of allelic diversity than niche populations (Michele et al. 2014). *P. sheareri* is currently distributed in southern China, with a relatively wide distribution region, which is consistent with the medium genetic diversity. In addition, *P. sheareri* harbored higher genetic diversity than other *Phoebe* species, such as *P. chekiangensis* (Ding et al. 2015) and *Phoebe bournei* (Hemsl.) Yang (Jiang et al. 2009). It was consistent with the wider distribution region in *P. sheareri* than other two *Phoebe* species.

Genetic structure among P. sheareri populations

Analysis of genetic diversity and population structure is of great significance for plant molecular breeding and protection of genetic resources. *Fst* is an effective way to measure genetic differentiation and gene flow between populations (Peng et al. 2017). It commonly considered *Fst* < 0.05 between populations to be a low level of genetic differentiation, 0.05 < *Fst* < 0.15 a medium level, 0.15 < *Fst* < 0.25 a high level, and *Fst* > 0.25 a very high level, when calculating *Fst* value using SSR data (Pearse et al. 2004). The average *Fst* of the *P. sheareri* population was 0.227, indicating that a high degree of genetic differentiation between populations. AMOVA also showed that genetic differentiation among populations was extremely significant, and 21.2% of genetic variation was among population (Table 3). Similar results have been reported for *P. chekiangensis* (Ding et al. 2015), *P. bournei* (Jiang et al. 2009), and *Betula luminifera* H. Winkl (Zhang et al. 2010).

Previous studies showed that woody species with large geographic ranges and outcrossing mating system usually harbored more abundant genetic variation within population than those of among populations (Hamrick et al. 1992). *P. sheareri* with the ten-years old began flowering, which is the hermaphrodite flower with diverse volatile secondary compounds and insect pollination. The levels of inbreeding was expected to low in *P. sheareri* populations, as estimated by *Fis* = 0.036 (Table S3), which was lower than other insect-pollinated forest tree species, such as *Dalbergia nigra* Fr. Allem. (*Fis* = 0.08) (Buzatti et al. 2012), *Cabralea canjerana* (Vell.) Mart. (*Fis* = 0.06) (Melo et al. 2014), and *Prunus africana* (Hook. f.) (*Fis* = 0.08–0.19) (Berens et al. 2014). It might attribute to the abundant flower fragrance attracting insect pollination, which increase the outcrossing ratio.

The genetic structure of a plant population is determined by interactions among processes such as gene flow, mutation, selection, and mating strategy (Schaal et al. 2010). Understanding population genetic structure is critical to elucidating hazard mechanisms and identifying ways to protect endangered species (Barbara et al. 2013). Both Bayesian clustering using STRUCTURE and UPGMA analysis based on genetic distance showed that these 21 populations divided into eastern populations and western populations, which Tianmu Mountain and Nanling Mountain might be the corresponding potential distribution centers. However, these two clustering results had somewhat difference, which might attribute to the distinct algorithms. For example, the Bayesian clustering ascertains spatial population structure and estimates the ancestry of individuals in these clusters (Chen et al. 2007). Intriguingly, most of individuals in XN and several trees in FJS harbored the same ancestry with QY and SY. The geographic distance between XN and QY is more than 870 km. What triggers the current spatial population structure? It needs further study to elucidate. UPGMA analysis distinguished three main groups approximately in line with the geographic area of occurrence, which consistent with Mantel test result ($r = 0.624$; $P < 0.0001$). Similar phenomenon was observed in *P. chekiangensis* (Ding et al. 2015), *Liquidambar formosana* Hance (Bi et al. 2010), *P. bournei* (Jiang et al. 2009). Thus, it determined that geographical isolation is a cause of genetic differentiation among *P. sheareri* populations. It suggests that certain geographical distribution characteristics exist in those populations, indicating that the genetic differentiation of *Phoebe* populations conforms to the geographic isolation model (Bohonak 2002).

Geographic isolation restricts gene flow. The structuring of diversity within and among populations is expected to be related to effective population size and gene flow (Hamrick et al. 1992). Gene flow between populations is thought to be existed when $Nm > 1$, indicating that alleles are distributed among different populations, reducing the probability of genetic drift (Slatkin 1987). The average *Nm* among *P. sheareri* populations were 1.322 (Table S3), and the *Nm* calculating from *Fst* was 0.927, suggesting that there is relatively low gene flow among *P. sheareri* populations. This value is smaller than those reported for *P. chekiangensis* (1.992) (Ding et al. 2015) and other widespread species such as *B. luminifera* (3.596) (Zhang et al. 2010), *L. formosana* (3.051) (Sun et al. 2016), and *Sorbus pohuashanensis* (Hance) Hedl. (3.047) (Zheng et al. 2008). In plant species, pollen and seeds often disperse at different scales (Anderson et al. 2010). In the case of *P. sheareri*, the small bees are probably participating in the pollen movement, and seeds depend on autochory for dispersal. Flight distances for small bees can be as long as 621-951m (Araújo et al. 2004), and restricted seed dispersal triggers the offspring localized close to the maternal plants (Kalisz et al. 2001), which limit the gene flow within population and among populations. Restricted gene flow, genetic differentiation can occur at short distances, which may be the cause of genetic variation in natural populations of *P. sheareri*.

In situ survey of the natural distribution of *P. sheareri* showed that there are fragmented biomes in the distribution range. Previous studies showed that the fragmented biomes were the interaction of the factors, such as biological characteristics, habitat heterogeneity and artificial disturbance (Liu et al. 2015).

The key factor limiting population development was low natural regeneration ability. The reproduction of *P. sheareri* is characterized by the production of many seeds, while the germination was affected by soil moisture condition. The germination rates of seeds are high (75%) under suitable moisture, such as under big trees. However, the seedling growth was significantly affected by light condition, exemplified by when the young trees were older than three years, the shading

limited the growth, even causing the death (Chen et al. 2013). Only a few seedlings of *P. sheareri* survive to become adults, increasing the difficulty to enlarge the population size. It was consistent with the varied distribution patterns of diameter-class in 21 populations (Fig.S1).

In addition, further research found that the genetic distance between populations was insignificantly correlated with the temperature difference but was significantly correlated with precipitation difference, indicating that precipitation has a role in population differentiation. It was consistent with biological characteristics of *P. sheareri*, which enjoys humid environment and has a certain cold tolerance, so the difference in water is more significant for population differentiation.

Conservation strategy of P. sheareri **natural population**

An important component of biodiversity is the diversity of forest genetic resources. Due to the deterioration of the ecological environment and the frequent intensification of human activities, the natural population of *P. sheareri* has been gradually decreased and is fragment-shaped, which is not conducive to maintaining the genetic diversity of *P. sheareri*. The ability of a population or species to evolve and adapt to the environment depends on its level of genetic diversity. Low levels of genetic diversity are not beneficial for areas of increased distribution and may increase the likelihood of disease or pests (Li et al. 2018). Environmental conditions and species characteristics are also considered key factors that may affect genetic diversity. Therefore, understanding the value of genetic diversity and population genetic differentiation is critical to identifying current threats to conservation and elucidating the mechanisms for protecting endangered species (Petit et al. 2010). As a tree species with high ecological and economic value, it is urgent to formulate a reasonable and effective protection strategy. In the present study, the natural population of *P. sheareri* has a medium degree of genetic diversity, with obvious genetic differentiation. The diversity level within the population is much higher than that of among the populations. Therefore, *in situ* conservation is the main strategy for protection. We found that the distribution patterns of diameter-class varied significantly in 21 populations (Fig.S1). For these populations locating in nature reserves and harboring more than one hundred individuals, such as TMS, FJS, and THY harboring relative high genetic diversity, the key is to effectively protect the native forest ecosystem. None class V tree was observed in THY, and other eight populations, indicating that the artificial disturbance might cause limitations for the population self-propagation. So we suggest that *P. sheareri* should be included in the endangered species protection list Redbook, thus more people know this species and increase people's protection awareness. In the nature reserves, such as THY, TMS, FJS, and LS, the *P. sheareri* population and its biodiversity, and ecological environment should be further studied, then we can expand the population and its distribution are by thinning forest stand, cultivating seedling reintroduction, and helping population growth. Although ZJ harbored less than 200 individuals, the genetic diversity index ranked second. Five diameter-class individuals were detected and the class IV trees and class II saplings were predominantly in this population. We should improve the population environment to help the class I and class III individuals' growth, to sustain the genetic diversity and structure in ZJ.

Due to the seed germination of *P. sheareri* requires certain shade conditions and has certain difficulties, in addition to *in situ* conservation, we can collect *P. sheareri* seeds in the main distribution region. Through artificial breeding, seedlings with three-years old were planted in different places, such as the original population, thereby expanding the weak *P. sheareri* populations, and can also establish offspring test and genetic resource orchards to protect the genetic diversity of *P. sheareri*.

On the other hand, the existing individuals by through vegetative propagation and their offsprings can be collected in the gene reservation center of *P. sheareri.* In addition, seed orchards, and collection nurseries of superior tree should be established to preserve the diversified genotypes to ensure requirement of long-term breeding in *P. sheareri*.

Conclusion

The genetic diversity and genetic structure of 21 natural population of *P. sheareri* involving in 428 individuals were evaluated using 32 pairs of EST-SSR primers. The results showed that *P. sheareri* had a medium genetic diversity, indicating that those populations with high genetic diversity should be conserved *in situ*. However, there was significant genetic differentiation among populations, and the gene flow was relatively low, indicating that gene flow between populations might be blocked and genetic drift might have been existed. Genetic structure revealed that significant genetic differences among populations were observed, which correlated with geographical distances. It suggested that habitat fragmentation and geographical isolation, and precipitation difference contribute to the genetic differentiation of natural populations in *P. sheareri*.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication: Not applicable.

Availability of data and material: We have deposited the raw data into the public repository, and the DOI was https://doi.org/10.6084/m9.figshare.12332552.v4

Competing interests: The authors declare that they have no conflict of interest.

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Authors' contributions

Conceptualization: JHZ; Methodology: JHZ; Software: YW; Validation: YFL and WTX; Formal Analysis: YFL and XH; Investigation: YFL and ANY; Resources: LHL; Data curation: WTX and YFL; Writing – original draft: YW; Writing – review & editing: JHZ; Visualisation: ZKT; Supervision: ZKT; Project Administration: ZKT and JHZ; Funding acquisition: ZKT.

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Figures

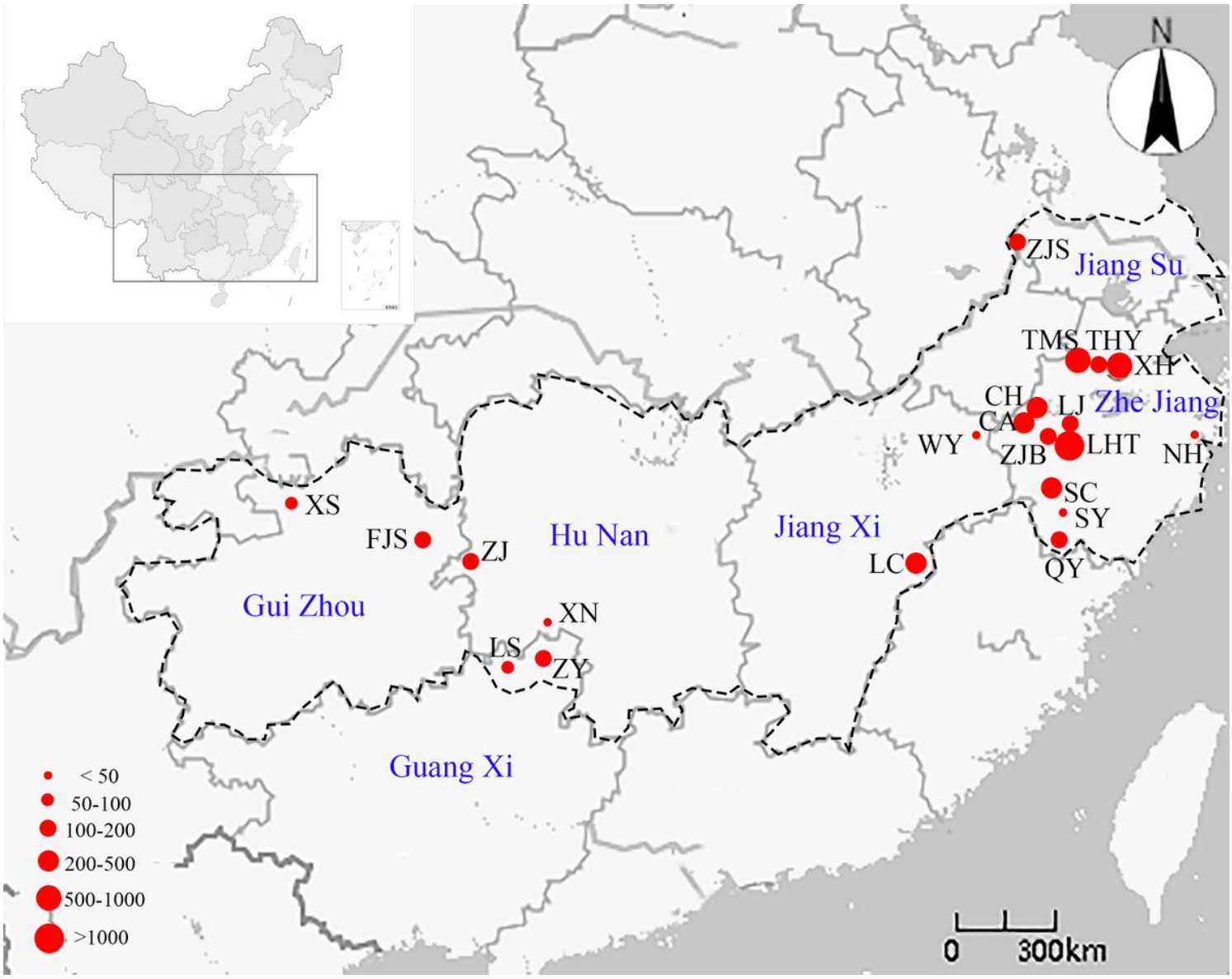

Figure 1

Distribution map of collection samples of P. sheareri. The distribution region is marked by black dotted line, and the sampling locations are marked by red round dot and the size of the dot represents the number of population. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

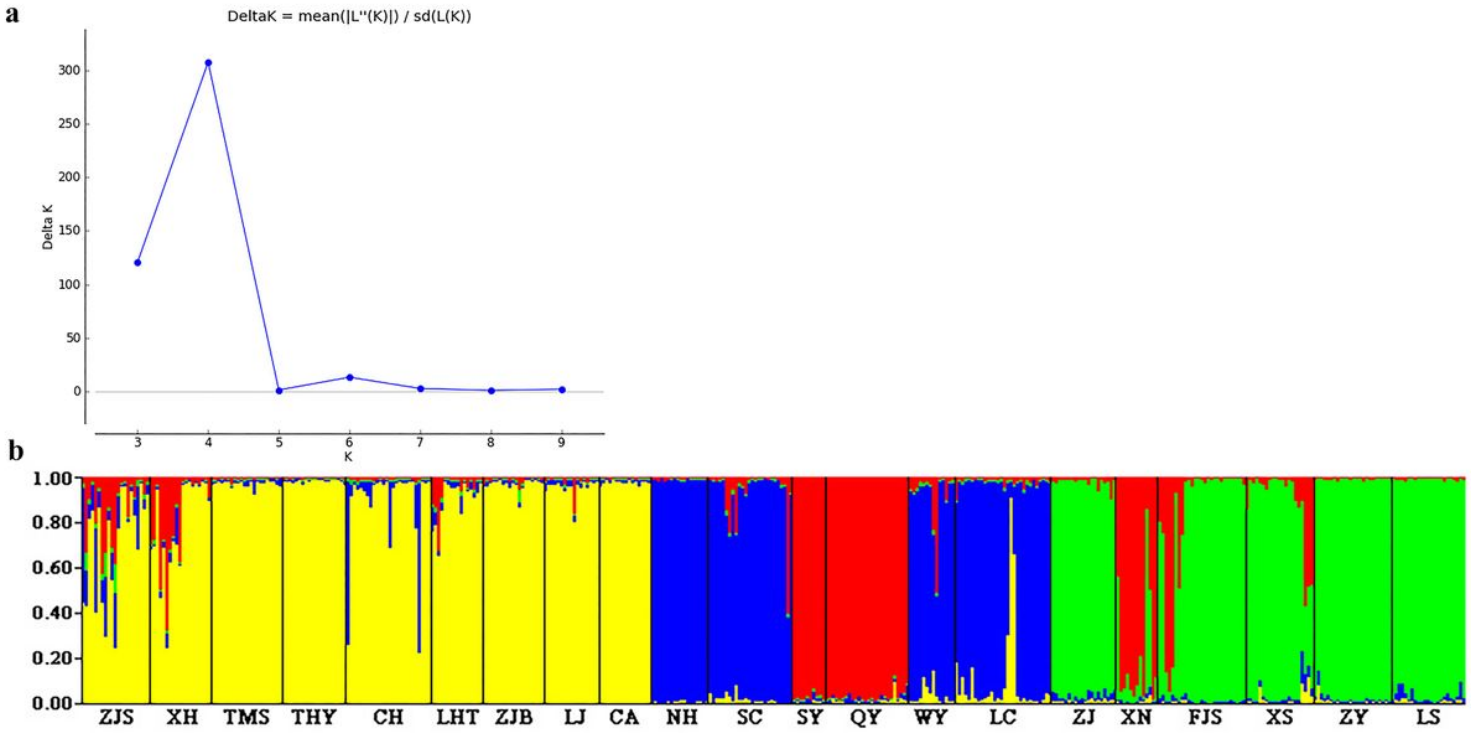

Figure 2

Genetic structure analysis of P. sheareri populations. a) The relationship between the number of clusters (K) and the corresponding ΔK statistic was calculated from ΔK according to structural analysis. b) Results of the structure analysis of P. sheareri populations when K = 4. Each individual is represented by a single vertical bar, which is partitioned into four different colors, each representing a genetic cluster; colored segments show the estimated ancestor ratio of the individual to each genetic cluster.

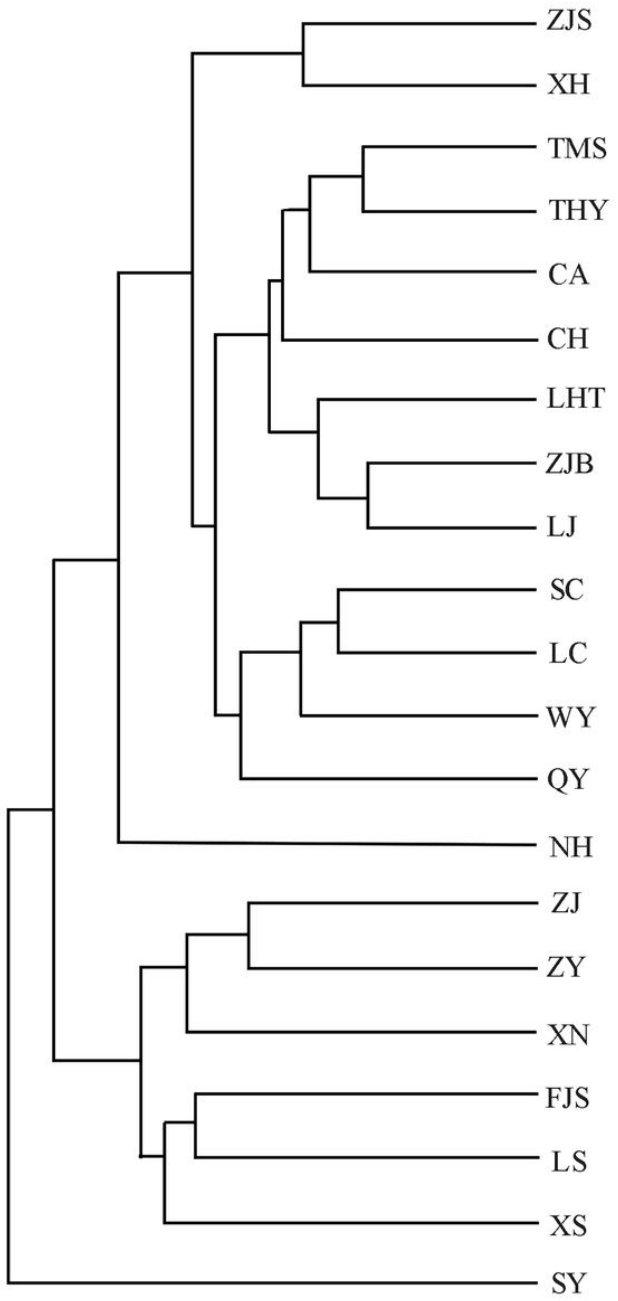

Figure 3

The unweighted pair group method with arithmetic mean (UPGMA) dendrogram for P. sheareri populations based on Nei's genetic distance. The 21 populations were divided into three categories. The first major category included the following sampling sites: ZJS, XH, TMS, THY, CA, CH, LHT, ZJB, LJ, SC, LC, WY, QY, and NH. The second category comprised ZJ, ZY, XN, FJS, LS, and XS. SY formed the third category.

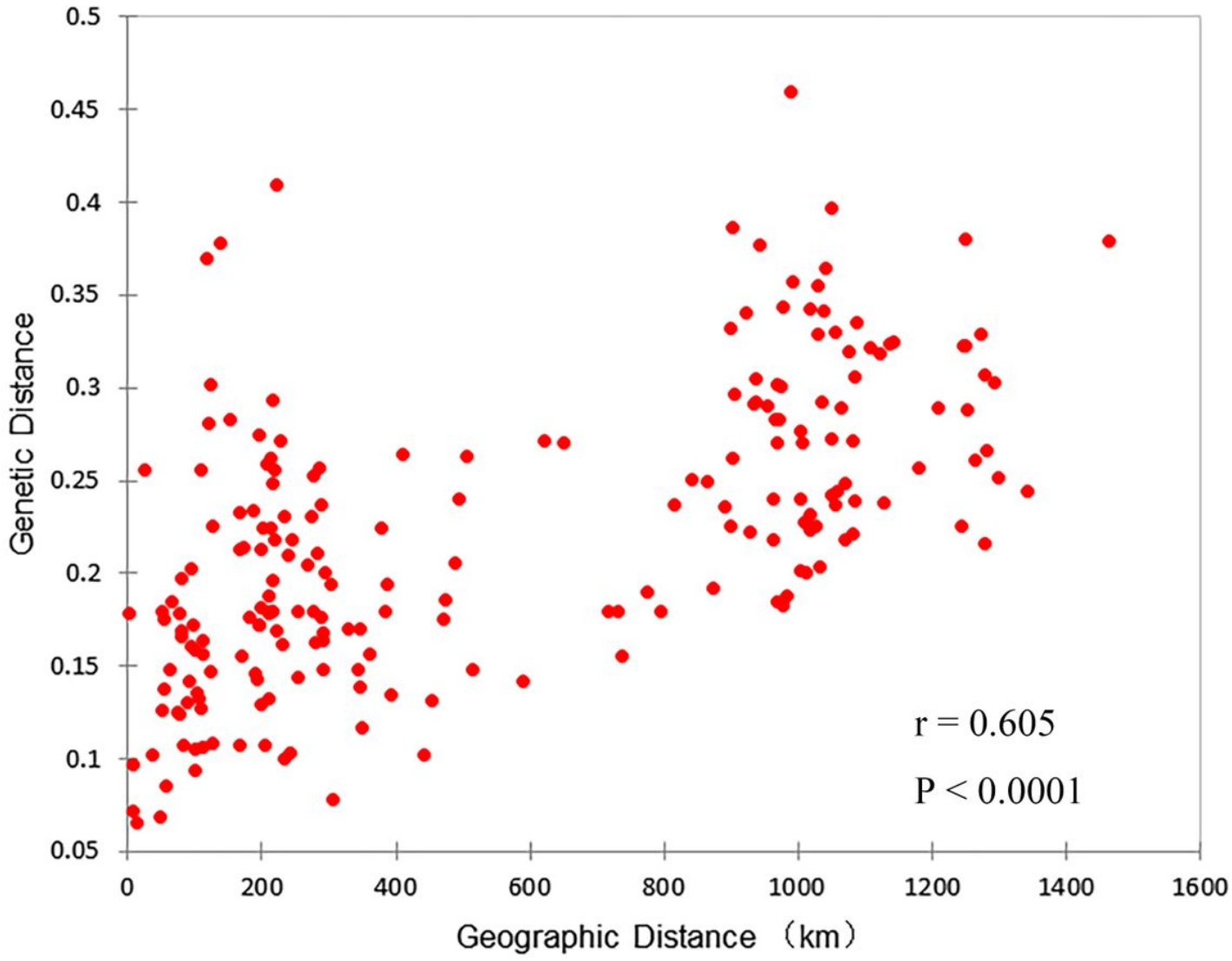

Figure 4

Mantel test on the correlation between Nei's genetic distance and geographic distance (km) among P. sheareri populations. Genetic distance was significantly positively correlated with geographic distance.

Supplementary Files

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