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RESEARCH PAPER

Low within-population genetic diversity and high genetic differentiation among populations of the endangered plant *Tetracentron sinense* Oliver revealed by inter-simple sequence repeat analysis

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Abstract

• ***Key message*** ***Tetracentron sinense*** **Oliver, an endangered species from China, displays a low within-population genetic diversity and high genetic differentiation among populations, and the existing populations could be divided into three conservation and management units.**

• ***Context*** The endangered tree *Tetracentron sinense* Oliver has great value; however, little is known regarding the within-population genetic diversity and differentiation among *T. sinense* populations.

• ***Aims*** We examined the genetic diversity and differentiation of *T. sinense* wild populations, and we tested the effect of small-size population on the level of genetic diversity within these populations.

• ***Methods*** Using inter-simple sequence repeat (ISSR), we assessed the genetic variation and structure among 174 individuals from 26 natural populations of *T. sinense* sampled across its distribution range in China.

• ***Results*** The ISSR primers yielded 180 amplified loci (123 were polymorphic). At the species level, the percentage of polymorphic loci (*PPL*), Nei's gene diversity (*H*), and Shannon's information index (*I*) were 68.3%, 0.196 and 0.300, respectively. The average population level *PPL* was 20.0%, and the *Na*, *Ne*, *H*, and *I* were 1.20, 1.13, 0.076, and 0.112, respectively. AMOVA revealed high genetic differentiation among populations (52.0% of total variance, $P = 0.001$), consistent with the gene differentiation coefficient ($Gst = 0.607$) and gene flow ($Nm = 0.326$). The 174 individuals of the 26 *T. sinense* populations clustered into three groups, and *T. sinense* geographic and genetic distance were significantly correlated.

• ***Conclusions*** *T. sinense* exhibited intermediate within-species genetic diversity, indicating preserved evolutionary potential. The low within-population genetic diversity and high genetic differentiation among *T. sinense* populations may be one of important factors causing endangerment. Three conservation units were determined based on genetic difference and structure. Inter-population introduction of individuals within units via appropriate propagation and seedling management might be an effective strategy for increasing *T. sinense* within-population genetic diversity and population size.

Keywords *Tetracentron sinense* Oliver · Genetic variation · Genetic structure · Molecular marker · ISSR · Conservation strategy, China

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Contribution of the co-authors Xiaohong Gan conceived the project, designed the experiments, and performed the experiment partly; Shan Li wrote the manuscript and performed the experiment; and Hongyan Han, Xuemei Zhang, and Zongqiong Tian performed the experiment and statistical analysis.

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1 Introduction

Tetracentron sinense Oliver, the only living species in the Tetracentraceae family, is mainly distributed in moist-temperate-deciduous or mixed evergreen and deciduous forests in central and southwest China (Fu and Bruce 1992). As an Arcto-Tertiary relict with a rich fossil record (Wu 2004), *T. sinense* is important for the study of ancient flora and the origin and evolution of angiosperms. Because of the high demand for medicinal (Wang et al. 2006), timber, and ornamental purposes (Zhang and Gao 1990), *T. sinense* has been subject to excessive destruction resulting in scattered distribution and poor regeneration. The climate change during the

Quaternary ice age and human disturbances in recent years have resulted in a sharp decline in *T. sinense* population size, and its existing populations have consequently become small-sized (Frankel and Soulé 1981). Therefore, *T. sinense* is currently listed in Appendix III of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora, https://cites.org/eng/node/41216), and is also listed as a National Second-grade Key Preserved Wild Plant in China (Fu 1992). Thus, collecting and conserving *T. sinense* germplasm resources is very urgent and necessary.

In recent years, sporogenesis and gametophyte development (Gan et al. 2012), pollination ecology (Gan et al. 2013), and seed and seedling ecology (Zhou 2007, Luo et al. 2010, Cao et al. 2012, Gan et al. 2012, 2013, Han et al. 2015, Li et al. 2015) have been studied to explore the causes of environment-related endangerment and suggest conservation strategies for the germplasm resources of *T. sinense.* Gan et al. (2013) have hypothesized that small population size limits the insect and wind pollination efficiency during *T. sinense* flowering and pollination, resulting in reduced genetic diversity within the population, which might be a crucial factor leading to species endangerment. However, to date, this hypothesis has not been confirmed by genetic diversity assessments.

Long-term preservation of endangered species requires the establishment of scientific conservation and management systems combining biological and environmental factors (Zhou et al. 2016). Genetic diversity is the basis for species evolution, and sufficient genetic variability is required for endangered species to evolve in response to changing environments (Frankel and Soulé 1981); thus, its maintenance is crucial for long-term survival and evolution (Stockwell et al. 2003, Yang et al. 2014). Therefore, knowledge regarding the genetic diversity and differentiation of the endangered plant *T. sinense* in different environments is a necessary prerequisite for a better understanding of the evolutionary history and endangerment mechanism of the species, as well as providing practical strategies for conservation and genetic management (Gordon et al. 2012, Gaafar et al. 2014, Lopes et al. 2014).

A series of genetic markers have been developed to analyze and evaluate the genetic diversity of endangered species. Compared with other genetic markers, DNA-based markers have superior reproducibility for the evaluation of genetic diversity (Gaafar et al. 2014). Sun et al. (2014) examined the phylogeography of *T. sinense* and described its current patterns of genetic diversity based on chloroplast DNA markers. However, because of slower evolutionary rates and insufficient polymorphism sites (Muir and Filatov 2007), maternally inherited chloroplast genes cannot accurately reveal the genetic variation within *T. sinense* populations without being combined with nuclear DNA markers; however, thus far, no studies have examined the genetic diversity within *T. sinense* populations based on nuclear DNA markers.

To date, a number of molecular markers related to the nuclear DNA have been used to examine genetic diversity. Among various marker systems, the inter-simple sequence repeat (ISSR) marker system is a technique that uses repeat-anchored primers to amplify DNA sequences between two inverted SSRs (Zietkiewicz et al. 1994, Culley and Wolfe 2001). By combining most of the benefits of amplified fragment length polymorphism (AFLP) and SSR with the universality of Random Amplification of Polymorphic DNA (RAPD) (Reddy et al. 2002), ISSR markers are reproducible under different conditions at a relatively low cost, and enable the use of higher primer annealing temperatures and the generation of longer sequences than other markers (Gaafar et al. 2014). ISSR markers have been successfully used to evaluate the genetic diversity of several endangered plant species (Lu et al. 2006, Tileye et al. 2007, Trindade et al. 2012, Zhang et al. 2013, Gaafar et al. 2014, Yang et al. 2014).

In this study, the genetic diversity and differentiation of 26 wild *T. sinense* populations were assessed using ISSR molecular markers. The aims of the study were (1) to assess the level of genetic diversity within populations and genetic differentiation among populations of *T. sinense*, (2) to test the effect of small-size population on the level of genetic diversity within these populations, and (3) to provide suggestions for conservation and management of *T. sinense*.

2 Materials and methods

2.1 Plant species and sampled populations

Samples were obtained from 174 individuals from 26 *T. sinense* populations across the *T. sinense* distribution range in China from July to August 2015 (Table 1; Fig. 1). Because of excessive destruction and poor regeneration, only a small sample size of these populations was available for this study. Thus, although only a small number of individuals in these populations were analyzed in this study, they can reflect the actual situation of this species. Fresh young leaves were collected, dried immediately with silica gel in zip-lock plastic bags, transported back to the laboratory, and stored in a freezer at − 80 °C. The sampled individuals were at least 50 m apart, to avoid sampling clonal individuals or individuals from the same family. Parameters such as longitude, latitude, altitude, and voucher number were recorded for each population (Table 1).

2.2 DNA extraction and detection

Total genomic DNA was extracted from approximately 80 mg of dry leaf materials using the no. LB1238 dry plant sample genome extraction kit (LABGENE Biotechnology, Chengdu, China). DNA was dissolved in 80 μl deionized water. Concentration and purity were measured using a NanoDrop

Table 1 The geographical location, population code, and sample size of 26 *T. sinense* populations

Population code	Population name	Location	Sample size	Total amount of adult trees	Longitude (E)	Latitude (N)	Altitude (m)
P1	Jinfo Mountain	Nanchuan, Chongqing	5	16	107.18	29.02	2077–2125
P2	Huangguan	Ningshan, Shanxi	11	30	108.48	33.58	1667–1710
P3	Foping	Foping, Shanxi	5	17	107.78	33.65	1602–1658
P4	Badagongshan Mountain	Sangzhi, Hunan	10	25	110.06	29.76	1209–1502
P5	Mulinzi	Hefeng, Hubei	6	17	110.20	30.04	1435–1608
P6	Tangjiahe	Qingchuan, Sichuan	10	36	104.40	32.58	1943–2041
P7	Longxi-Hongkou	Dujiangyan, Sichuan	7	25	104.37	32.90	1720–1782
P8	Kangpu	Weixi, Yunnan	4	12	99.13	27.55	2561–2583
P9	Baimaxueshan Mountain	Weixi, Yunnan	8	18	99.35	27.63	2562–2902
P10	Qizimeishan Mountain	Xuanen, Hubei	5	17	109.74	30.04	1444–1552
P11	Leigongshan Mountain	Leishan, Guizhou	6	30	108.20	26.38	1644–1976
P12	Micangshan Mountain	Wangcang, Sichuan	5	14	106.55	32.65	1873–1934
P13	Fanjingshan Mountain	Tongren, Guizhou	4	13	108.69	27.91	2079–2103
P14	Wolong	Wenchuan, Sichuan	9	20	103.11	30.96	2305–2502
P15	Baihe	Jiuzhaigou, Sichuan	7	18	104.14	33.25	1867–1945
P16	Dafengding	Meigu, Sichuan	6	32	103.14	28.77	2163–2318
P17	Shunhuangshan	Dongan, Hunan	7	20	110.01	26.37	1492–1704
P18	Shennongjia	Shennongjia, Hubei	5	21	110.36	31.41	1256–1737
P19	Huangboyuan	Taibai, Shanxi	8	32	104.12	33.24	2037–2060
P20	Kuankuoshui	Suiyang, Guizhou	5	17	107.17	28.23	1614–1626
P21	Dashahe	Daozhen, Guizhou	3	12	107.76	29.15	1627–1637
P22	Baishuijiang	Wenxian, Gansu	10	28	104.33	32.90	2182–2302
P23	Emei	Emeishan, Sichuan	4	15	103.36	29.56	1616–1847
P24	Wufenghouhe	Wufeng, Hubei	9	25	110.54	30.07	935–1449
P25	Gaoligongshan	Tengchong, Yunnan	5	13	98.71	25.97	2472–2499
P26	Ailaoshan	Shuangbai, Yunnan	10	32	101.11	24.44	2452–2530

2000 Spectrophotometer (Thermo, Fisher Scientific, Waltham, MA, USA) and integrity was determined by electrophoresis on 1.0% (*w*/*v*) agarose gels. The DNA was stored at − 20 °C until use.

2.3 ISSR-PCR amplification

ISSR primers (UBC primers 100, University of British Columbia, Vancouver, BC, Canada) were synthesized by Shanghai Sangon Biological Company. Fourteen polymorphic primers (Table 2) that produced clear and reproducible bands were selected for testing ISSR genetic diversity. The PCR reaction mixture contained 12.5 μl 2 × Taq PCR Master Mix, 0.6 μM primer, 70 ng genomic DNA, and double distilled water (to 12.5 μl), in a total volume of 25 μl. PCR conditions were as follows: 94 °C for 5 min; followed by 40 cycles of denaturation at 94 °C for 70 s, annealing for 70 s, and DNA elongation at 72 °C for 1.5 min; and a final cycle of 70 s at 94 °C, 70 s at annealing temperature, and 5 min at 72 °C. Amplified products were separated by electrophoresis on 1.5% agarose gels. The 100 to 2000 bp DNA ladder (LABGENE Biotechnology) was used as a standard molecular weight. Photographs were taken using the UVP-gel documentation system (LABGENE Biotechnology).

2.4 Data analysis

The experiment was repeated three times to ensure accuracy, and only clear and reproducible bands were recorded. The bands were scored as present (1) or absent (0) for each sample, and a matrix of different ISSR phenotypes was assembled for data analysis (Yang et al. 2014). Parameters, including the percentage of polymorphic loci (*PPL*), observed number of alleles (*Na*), effective number of alleles (*Ne*), Nei's gene diversity (*H*), Shannon's information index (*I*), and total gene diversity (*Ht*), gene diversity within populations (*Hs*), coefficient of genetic differentiation ($Gst = [Ht\text{-}Hs]/Ht$), and gene flow among populations (*Nm*), were calculated to evaluate genetic diversity using the POPGENE1.32 software (Nei 1974, Wright, 1978, Yeh et al. 1997, Balloux and

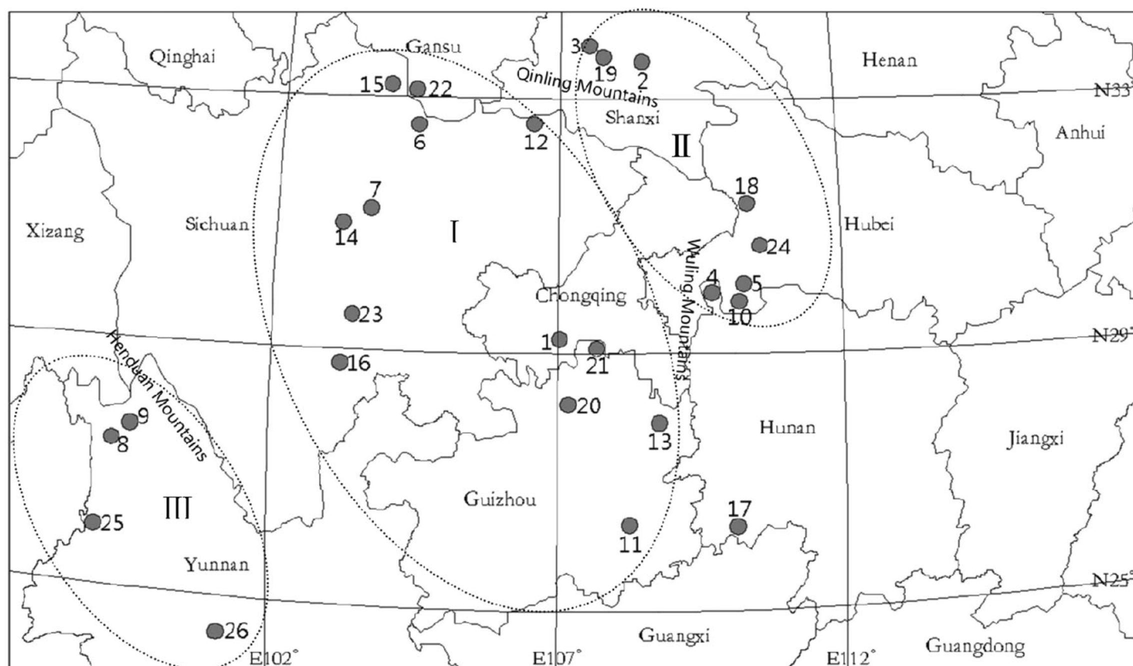

Fig. 1 Geographic distribution of 26 *Tetracentron sinense* populations

Lugonmoulin 2002). Each diversity index was compared among 26 populations using ANOVA and multiple comparison analysis, and the correlation between within-population genetic diversity and sample size was analyzed using Pearson correlation analysis. Both analyses were conducted in SPSS statistic program v23.0.

For further estimation of the genetic variation among individuals within populations and among populations, DCFA and WINAMOVA 1.55 software were used for analysis of molecular variance (AMOVA) (Excoffier et al. 1992, Zhang and Ge 2002). To determine the genetic relationships between the 26 *T. sinense* populations, the genetic structure of populations and individuals was obtained by UPGMA (unweighted pair-group method with arithmetic means) cluster analysis using NTSYS software (Rohlf 2000). A Bayesian-clustering method based on the

Table 2 The sequences and amplification of 14 ISSR primers

The number of primer	Primer sequence (5′-3′)	Annealing temperature (°C)	Total number of loci	Number of polymorphic loci	Percentage of polymorphic loci (%)
UBC808	AGA GAG AGA GAG AGA GC	54.6	18	15	83.3
UBC811	GAG AGA GAG AGA GAG AC	54.6	15	12	80.0
UBC817	CAC ACA CAC ACA CAC AA	52.2	7	5	71.4
UBC825	ACA CAC ACA CAC ACA CT	52.2	12	8	66.7
UBC827	ACA CAC ACA CAC ACA CG	54.6	9	4	44.4
UBC828	TGT GTG TGT GTG TGT GA	52.2	12	7	58.3
UBC834	AGA GAG AGA GAG AGA GYT	53.9	16	13	81.3
UBC835	AGA GAG AGA GAG AGA GYC	56.2	11	9	81.8
UBC836	AGA GAG AGA GAG AGA GYA	53.9	15	8	53.3
UBC840	GAG AGA GAG AGA GAG AYT	53.9	14	12	85.7
UBC841	GAG AGA GAG AGA GAG AYC	56.2	15	5	33.3
UBC842	GAG AGA GAG AGA GAG AYG	56.2	13	8	61.5
UBC848	CAC ACA CAC ACA CAC ARG	56.2	13	10	76.9
UBC849	GTG TGT GTG TGT GTG TYA	53.9	10	7	70.0
Total	–	–	180	123	68.3

admixture model and correlated with the allele frequency, utilizing STRUCTURE version 2.3.3 (Pritchard et al. 2000), was also applied to infer how many clusters (*K*) were most appropriate for interpreting the data without prior information regarding the number of locations at which the individuals were sampled (Thriveni et al. 2014). All clusters derived from either cluster analysis or model-based structure analysis were also validated by principal coordinate analysis (PCoA) using the MVSP 32 program (Zhang et al. 2010). A Mantel test was performed to assess the correlation between geographic distance and genetic distance for the 26 populations using TFPGA software (Miller 1997, Wang et al. 2011).

2.5 Data availability

The datasets generated and/or analyzed during the current study are available in the GitHub repository, https://github.com/bhgan/Low-within-population-genetic-diversity-and-high-genetic-differentiation-among-populations-of-the-en [accessed 21 June 2018] or from the corresponding author on reasonable request.

3 Results

3.1 ISSR polymorphisms

Fourteen primers that produced clear and reproducible amplicon profiles were chosen for genomic DNA amplification of all 174 individuals of the 26 *T. sinense* populations (Table 2). In total, 180 bright and discernible DNA fragments ranging in size from 100 to 2000 bp were scored, of which 123 bands (68.3%) were polymorphic among the 174 individuals, indicating that the average *PPL* was 68.3%. Bands and polymorphic bands per primer ranged from 7 to18 and 4 to 15, respectively. The *PPL* per primer ranged from 33.3 to 85.7%. As wild *T. sinense* individuals are rare, the amplification results of the samples in this experiment can reflect the actual genetic situation of the species.

3.2 Genetic diversity and differentiation

At the population level, the *PPL* ranged from 9.44 to 30.6% with an average of 20.0% (Table 3). *H* and *I* ranged from 0.041 to 0.122 and 0.059 to 0.177, respectively, with an average of 0.076 and 0.112, respectively. *Na* ranged from 1.094 to 1.306 with an average of 1.197. *Ne* ranged from 1.074 to 1.218 with an average of 1.134. The genetic diversity parameters differed among the 26 populations. The maximum values of the five abovementioned parameters were found in the P22 population, while the minimum values appeared in the P8 population. Overall, the genetic diversity of the P22, P6, P4, P15, and P2 populations was relatively high, while that of P9 and P8 was relatively low. ANOVA analysis showed that there were significant difference in within-population genetic diversity indices among the 26 *T. sinense* populations ($P = 0.000 < 0.01$, Table 3). All genetic diversity indices had a very significant positive correlation with sample size based on Pearson correlation analysis (Table 4). At the species level, the *PPL*, *H*, and *I* were 68.3%, 0.196, and 0.300, respectively (Table 4). *Ht* was 0.194 and the within-population gene diversity (*Hs*) was 0.076, which is < 50% of the *Ht* (Table 5).

The coefficient of genetic differentiation (*Gst*) and gene flow among populations (*Nm*) were 0.607 and 0.324, respectively (Table 5). AMOVA (Fig. 2) demonstrated extremely significant genetic differentiation among populations and within populations ($P < 0.001$); 52.0% of the total variation was due to differences among populations, while the remaining 48.0% was caused within-population differences.

3.3 Genetic structure

A dendrogram of the 26 populations was generated based on Nei's genetic distances using UPGMA cluster analysis (Fig. 3). Using a genetic distance of 0.140, UPGMA analysis clustered the 26 populations into three groups. Group I included thirteen populations (P1, P6, P7, P11, P13, P14, P15, P16, P17, P20, P21, P22, and P23), group II consisted of nine populations (P2, P3, P4, P5, P10, P12, P18, P19, and P24), and the remaining four populations (P8, P9, P25, and P26) formed group III.

The genetic structure of 174 individuals from 26 populations was analyzed by structure analysis according to differences in allele frequency. Because 26 populations were examined in this study, the *K* value was set as 2 to 26. The relationship between the Ln P (D) value and *K* value was analyzed based on the methods described by Pritchard et al. (2000); however, no obvious inflection point was observed (Fig. 4a). Therefore, the ΔK value corresponding to each *K* value was further calculated based on the criteria reported by Evanno et al. (2005) and the related calculation formula ($\Delta K = M$ (|L (k + 1)−2 L(k) + L (k-1) | (/S) L (k)). As the maximum likelihood values showed a typical curvilinear response to increasing *K* (Fig. 4b), $K = 3$ (three groups) was defined to provide the optimal structure for further analysis. Structure cluster analysis showed that group I (blue) included fourteen populations (P1, P6, P7, P11, P12, P13, P14, P15, P16, P17, P20, P21, P22, and P23), group II (green) consisted of eight populations (P2, P3, P4, P5, P10, P18, P19, and P24), and the remaining four populations(P8, P9, P25, and P26) formed group III (red) (Fig. 4c). Topology was completely consistent with the PCoA plot (Fig. 5).

Table 3 Genetic diversity parameters of 26 *T. sinense* populations

Population code	PPL (%)	*Na*		*Ne*		*H*		*I*	
		Mean	Std error	Mean	Std error	Mean	Std error	Mean	Std error
P1	21.11	1.1211^{abcdef}	0.0305	1.1489^{abcdefg}	0.0238	0.0830^{abcdefgh}	0.0127	0.1214^{abcdef}	0.0181
P2	26.11	1.2611^{abcd}	0.0328	1.1738^{abcd}	0.0243	0.0992^{abcd}	0.0133	0.1462^{abcd}	0.0192
P3	15.00	1.1500^{efg}	0.0267	1.1024^{defg}	0.0203	0.0574^{efghi}	0.0108	0.0844^{efg}	0.0156
P4	28.89	1.2889^{abc}	0.0339	1.1880^{abc}	0.0252	0.1068^{abc}	0.0136	0.1577^{abc}	0.0196
P5	19.44	1.1944^{cdef}	0.0296	1.1482^{abcdefg}	0.0240	0.0812^{bcdefghi}	0.0128	0.1172^{bcdefg}	0.0183
P6	29.44	1.2944^{ab}	0.0341	1.2071^{ab}	0.0260	0.1170^{ab}	0.0142	0.1710^{ab}	0.0204
P7	23.33	1.2333^{abcde}	0.0316	1.1456^{abcdefg}	0.0230	0.0830^{abcdefgh}	0.0124	0.1234^{abcdef}	0.0178
P8	9.44	1.0944^{g}	0.0219	1.0743^{g}	0.0177	0.0410^{i}	0.0096	0.0589^{g}	0.0137
P9	11.67	1.1167^{fg}	0.0240	1.0767^{g}	0.0174	0.0438^{hi}	0.0095	0.0647^{fg}	0.0139
P10	21.11	1.2111^{abcdef}	0.0305	1.1415^{bcdefg}	0.0226	0.0808^{bcdefghi}	0.0123	0.1190^{bcdef}	0.0178
P11	18.33	1.1833^{defg}	0.0290	1.1264^{cdefg}	0.0220	0.0712^{cdefghi}	0.0118	0.1045^{cdefg}	0.0170
P12	14.44	1.1444^{efg}	0.0263	1.1051^{defg}	0.0207	0.0583^{defghi}	0.0111	0.0849^{efg}	0.0158
P13	13.89	1.1389^{efg}	0.0258	1.0942^{efg}	0.0195	0.0532^{fghi}	0.0104	0.0784^{efg}	0.0150
P14	22.78	1.2278^{abcde}	0.0313	1.1648^{abcdef}	0.0246	0.0916^{abcdefg}	0.0133	0.1332^{abcde}	0.0190
P15	26.67	1.2667^{abcd}	0.0331	1.1694^{abcd}	0.0238	0.0977^{abcde}	0.0131	0.1450^{abcd}	0.0189
P16	20.00	1.2000^{bcdef}	0.0299	1.1092^{defg}	0.0199	0.0642^{defghi}	0.0108	0.0977^{defg}	0.0157
P17	14.44	1.1444^{efg}	0.0263	1.0872^{g}	0.0180	0.0511^{ghi}	0.0100	0.0766^{efg}	0.0145
P18	19.44	1.1944^{cdef}	0.0296	1.1279^{cdefg}	0.0217	0.0731^{cdefghi}	0.0118	0.1080^{cdefg}	0.0171
P19	22.22	1.2222^{efg}	0.0311	1.1430^{h}	0.0225	0.0818^{bcdefghi}	0.0123	0.1211^{abcdef}	0.0178
P20	16.11	1.1611^{efg}	0.0275	1.0903^{fg}	0.0183	0.0532^{fghi}	0.0100	0.0809^{efg}	0.0146
P21	19.44	1.1944^{cdef}	0.0296	1.1270^{cdefg}	0.0211	0.0740^{cdefghi}	0.0117	0.1097^{cdefg}	0.0170
P22	30.56	1.3056^{a}	0.0344	1.2177^{a}	0.0269	0.1217^{a}	0.0145	0.1774^{a}	0.0207
P23	18.33	1.1833^{defg}	0.0290	1.1215^{cdefg}	0.0216	0.0690^{cdefghi}	0.0115	0.1020^{cdefg}	0.0167
P24	20.00	1.2000^{bcdef}	0.0299	1.1213^{cdefg}	0.0206	0.0707^{cdefghi}	0.0115	0.1055^{cdefg}	0.0167
P25	15.00	1.1500^{efg}	0.0267	1.1156^{cdefg}	0.0222	0.0624^{defghi}	0.0116	0.0900^{defg}	0.0165
P26	23.89	1.2389^{abcde}	0.0319	1.1655^{abcde}	0.0243	0.0929^{abcdef}	0.0669	0.1358^{abcde}	0.0189
Mean	20.04	1.1976	0.0059	1.1343	0.0044	0.0761	0.0715	0.1121	0.0034
Sum of Squares		13.577		6.573		2.064		4.377	
Mean Square		0.543		0.263		0.083		0.175	
F		3.432		2.980		3.189		3.262	
P		0.000		0.000		0.000		0.000	

Values with different letters are significantly different

PPL, the percentage of polymorphic loci; *Na*, observed number of alleles; *Ne*, effective number of alleles; *H*, Nei's gene diversity; *I*, Shannon's information index. The same as followed

The Mantel test showed that there was an extremely significant correlation between genetic distance and geographic distance ($r = 0.687$, $P = 0.001 < 0.01$; Fig. 6).

Table 4 Pearson correlation analysis between population size and genetic diversity parameters of *T. sinense* populations

Factor	*PPL* (%)	*Na*	*Ne*	*H*	*I*
Population size	0.686**	0.707**	0.666**	0.673**	0.676**

**$P \leq 0.01$

4 Discussion

4.1 Genetic diversity within species and population level

In this study, the *PPL* of *T. sinense* at the species level was 68.33%, which was lower than the average value (*PPL* = 71.02%) based on 107 plant species (Nybom and Bartish 2000). A comparison with other endangered woody plants showed that the *PPL* of *T. sinense* was obviously higher than that of *Loropetalum subcordatum* (Benth.) Oliv. (*PPL* = 62.27%) (Gong et al. 2010), *Ostrya rehderiana* Chun

Table 5 Genetic variation at species level and genetic differentiation among populations

Parameter	*PPL*	*H*	*I*	*Ht*	*Hs*	*Gst*	*Nm*
Value	68.3%	0.1957	0.3004	0.1938	0.0761	0.6072 ± 0.0170	0.3235 ± 0.0455

H, Nei's gene diversity; *I*, Shannon's information index; *Ht*, total gene diversity; *Hs*, gene diversity within populations; $Gst = (Ht\text{-}Hs)/Ht$, coefficient of genetic differentiation; *Nm*, gene flow among populations

(PPL = 29.90%) (Li et al. 2012), and *Metrosideros boninensis* Tuyama (*PPL* = 12.90%) (Shingo et al. 2010), but lower than that of *Pteroceltis tatarinowii* Maxim (*PPL* = 95.45%) (Li et al. 2013) and *Tsoongiodendron odorum* Chun (*PPL* = 79.67%) (Xu et al. 2014). These results indicate an intermediate level of within-species genetic diversity in *T. sinense*, and a relatively high level of genetic variation compared to other endangered woody plants.

The genetic diversity of species is the result of historical evolution and forms the basis for adaptation, survival, and evolution in changing environments (Stockwell et al. 2003, Yang et al. 2014). Abundant genetic variability can strengthen the ability of a species to respond to changing environments and consequently enhance its evolutionary potential. Genetic diversity is affected by many factors such as breeding system, seed dispersal, life form, geographical distribution, and historical origin (Hamrick et al. 1995, Nybom and Bartish 2000, Guo et al. 2016). Perennial plants with a longer life history usually demonstrate a higher level of genetic diversity compared to annual plants with a shorter life history (Nybom and Bartish 2000). As a tertiary relict plant (Fu and Bruce 1992), the long evolutionary history time scale of *T. sinense* has provided the possibility for gene mutation, recombination, and variation accumulation; in terms of spatial scale, the broad geographical distribution of *T. sinense* (Fu and Bruce 1992) has resulted in geographical isolation and habitat heterogeneity and thus increased genetic variation. The moderate within-species level genetic diversity of *T. sinense* indicates that this endangered species maintains a certain evolutionary potential.

The within-population level genetic diversity of *T. sinense* was obviously low compared to the within-species level. The *PPL* of the 26 *T. sinense* populations ranged from 9.44 to 30.56%, and the average was only 20.04%, indicating low level genetic variation within the populations. The gene diversity within populations (*Hs* = 0.076) was less than half of the total gene diversity (*Ht* = 0.194), suggesting that the genetic variation of *T. sinense* mainly existed among population, similar to the AMOVA analysis results. This result was also consistent with the cpDNA markers (Sun et al. 2014) and phenotypic traits (Han et al. 2017) results.

Fig. 2 Analysis of molecular variance for *T. sinense* populations

4.2 Genetic differentiation and structure

In population genetics, gene differentiation based on *Gst* values is classified as low (< 0.05), medium (0.05–0.15), or high (> 0.15). In the study, the *Gst* (0.607) and *Nm* (0.324) of *T. sinense* indicated a high level of genetic differentiation between populations and a low level of gene flow (Wright 1978, Balloux and Lugonmoulin 2002). The genetic diversity and structure of the plant populations were closely correlated with the following factors: the effective population size, breeding system, natural selection, and life history traits (including life form, ecological tolerance, seed dispersal, and gene flow) (Slatkin 1987, Hamrick and Godt 1990). For *T. sinense*, the growth characteristics of leafing out prior to flowering would hinder wind pollination to a certain extent; the rainy weather during flowering would restrict pollinator activity (Gan et al. 2013). These factors would promote selfing and inbreeding, which can increase the probability of homozygosity and reduce the probability of recombination, thus enhancing the similarity of individuals within a population and consequently increasing inter-population divergence (Loveless and Hamrick 1984). The relative statistical results showed that 51% of the total genetic variation in inbred species resulted from variation among populations (*Gst* = 0.510), which was more than five-fold that in outcrossing species (*Gst* = 0.099) (Hamrick and Godt 1990). Therefore, a breeding system mixed with selfing and outcrossing and shorter distance seed dispersal in *T. sinense* (Gan et al. 2013) had a negative impact on gene flow between individuals within populations or among populations, which eventually led to low level genetic variation within populations and high level genetic differentiation among populations.

It is generally believed that *Nm* > 1 is enough to inhibit genetic drift and prevent genetic differentiation among populations, while *Nm* < 1 is insufficient to counteract the effects of genetic drift, the dominant factor leading to genetic

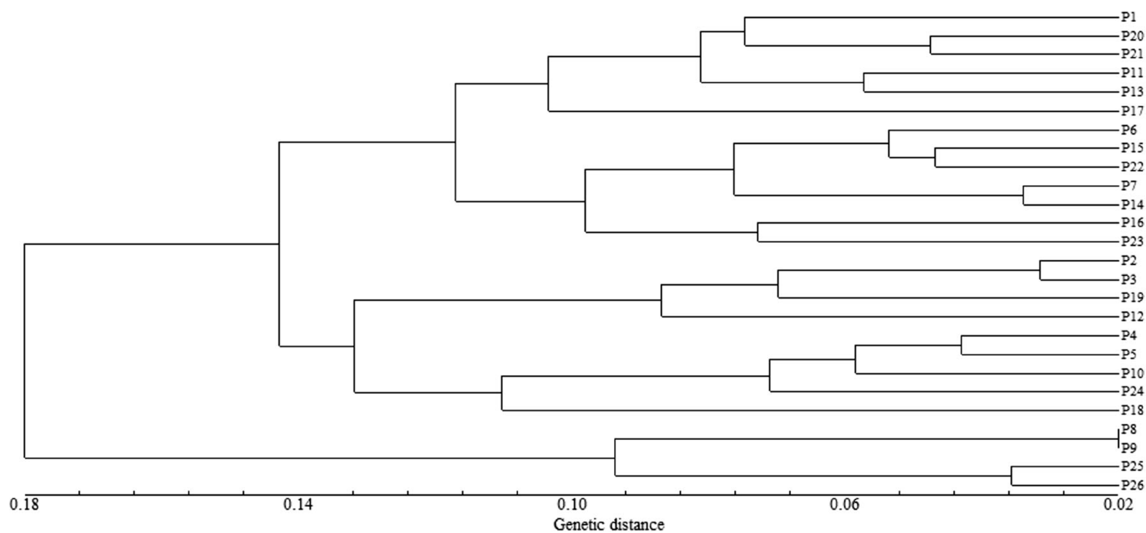

Fig. 3 UPGMA dendrogram based on Nei's genetic distance of 26 *T. sinense* populations

differentiation among populations (Slatkin 1985, Hamrick et al. 1995). For *T. sinense*, *Nm* was only 0.324, and there were significant differences in within-population genetic diversity among the 26 populations. Thus, small-size populations with remote geographical isolation due to the climate change during the Quaternary ice age and human disturbances (Frankel and Soulé 1981), contributed to a higher level of genetic drift and inbreeding, resulting in significant loss of genetic diversity within populations and an increase in genetic differentiation among populations (Ellstrand and Elam 1993, Freeland 2005, Willi et al. 2006). The Mantel Test showed that there was a significant correlation between geographic distance and genetic distance ($r = 0.687$, $P = 0.001$), indicating that geographical isolation played an important role in the high genetic

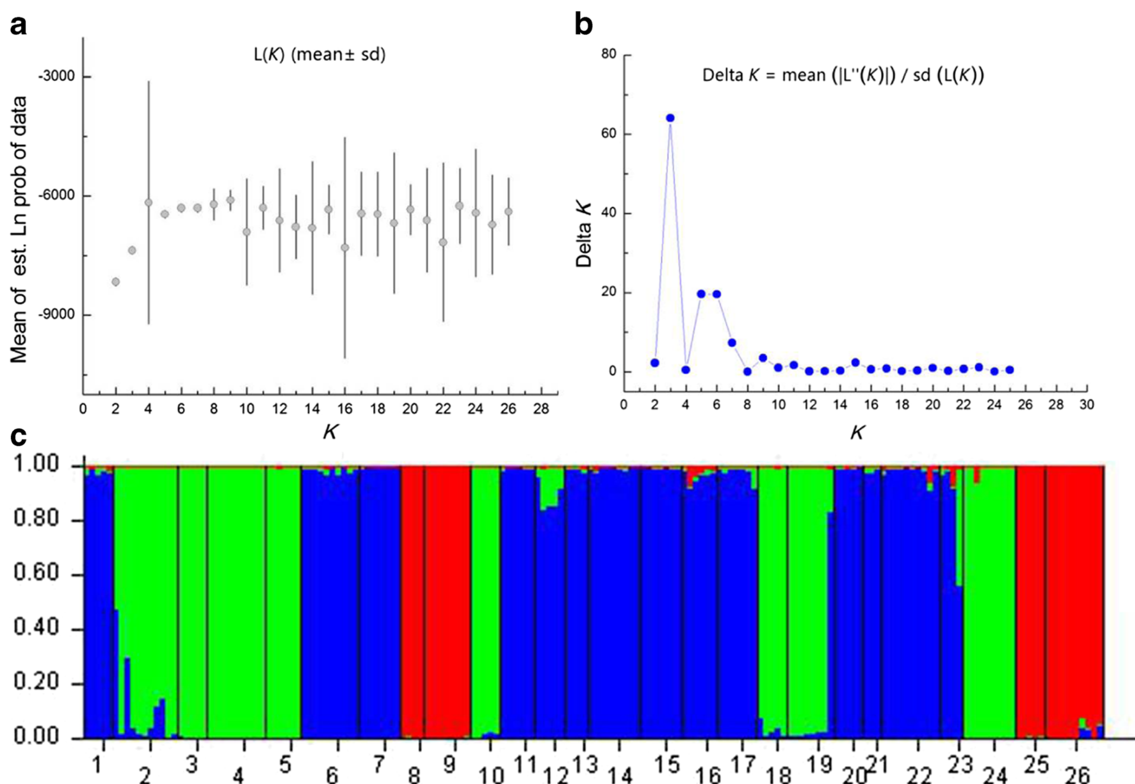

Fig. 4 Results of genetic structure analysis of 174 individuals from 26 *T. sinense* populations. **a** The probability of the data Ln P(D) (± SD) against the number of *K* clusters. **b** Δ*K* values from the mean log-likelihood probabilities from STRUCTURE runs where inferred clusters (*K*) ranged from 2 to 26. **c** Estimated genetic clustering ($K = 3$) for 174 individuals from 26 populations

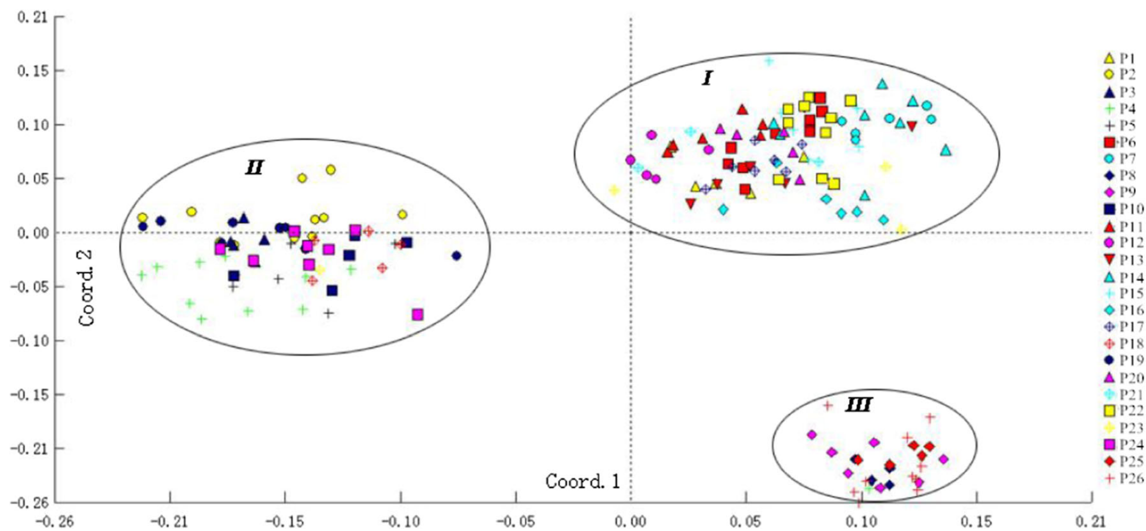

Fig. 5 Principal coordinate analysis (PCoA) of 174 *T. sinense* individuals

differentiation of *T. sinense* among populations. Therefore, the small population effect and geographical isolation may constitute the main factors causing low genetic diversity within populations and high genetic differentiation among populations of *T. sinense*. As population is the basic unit of species evolution, the low within-population genetic diversity of *T. sinense* could reduce the adaptability to changing environments, which is not conducive to long-term survival and evolution. In this study, the correlation analysis showed that the level of within-population genetic diversity was significantly positively correlated with the small number of sampled trees, indicating that the sample size in each *T. sinense* populations has a very important limitation to within-population genetic variation analysis. Fine scale spatial genetic structure (SGS) data based on genetic analysis of all individuals within a population would be helpful for elucidating the actual population history, genetic variation, and underlying processes of the

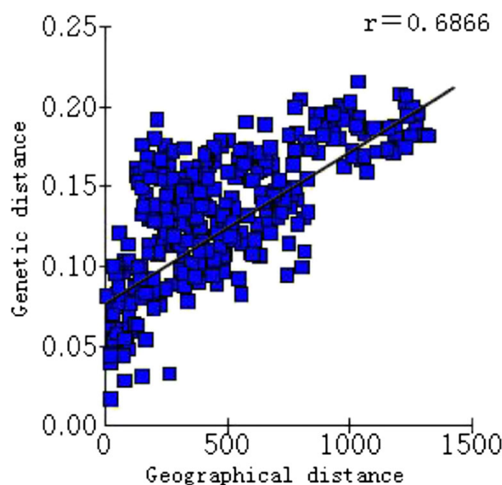

Fig. 6 Correlation between Nei's genetic distance and geographic distance by Mantel test

endangered plant *T. sinense* (Troupin et al. 2006), which needed to be further study.

The UPGMA and structure analyses both clustered the 26 *T. sinense* populations into three groups; this was further confirmed by the PCoA results. The extremely significant correlation between the geographic and genetic distance of *T. sinense* according to the Mantel test suggested a clear geographic tendency in the distribution of genetic variability. Notably, many neighboring populations clustered together so tightly that they were not obviously distinguishable in the PCoA plot (e.g., populations P4, P5, P10, P18, P19, and P24 in group II and populations P8, P9, P25, and P26 in group III). In contrast, the relationship between the populations in group I was relatively weak. Of the three groups, group III from the southwestern region of the Hengduan mountains is located in the southwestern zone of the *T. sinense* geographical distribution, group II from the eastern region of the Wuling mountains and northern region of the Qinling mountains is located in the northeastern zone of the geographical distribution, and group I is located between the Hengduan mountains and the Wuling or Qinling mountains in the central position (Fig. 1), suggesting obvious directional characteristics from southwest to northeast, which may be correlated with population recession to southwestern China (refuge) during the Quaternary ice age and population dispersion following the ice age (Sun et al. 2014). The three groups separated solely by the Hengduan, Qinling, and Wuling mountains, strongly indicate that geographical isolation played an important role in the genetic structure and distribution of *T. sinense*.

4.3 Implications for conservation

Our study results suggest that small populations due to human disturbance and habitat fragmentation (Gan et al. 2013) are an important cause of low genetic diversity within *T. sinense*

populations; thus, the primary strategy should be to conduct in situ conservation to preserve all existing populations and their habitats. In particular, populations P22, P6, P4, P15, and P2 should be prioritized for in situ conservation because of their relatively higher within-population genetic variation. Moreover, germplasm resource banks of *T. sinense* should also be constructed via ex situ conservation and collection of genetic resources, in order to avoid the loss of genetic resources because of population degradation.

In the study, the existing *T. sinense* populations clustered into three groups based on genetic structure analysis, and small population size and geographical isolation played important roles in low gene flow among the populations. Therefore, each group should be considered as a conservation and management unit. For the three conservation units, inter-population introduction of individuals within units via appropriate propagation and seedling management might be an effective strategy for increasing within-population genetic diversity and population size of *T. sinense* (Frankham et al. 2002), especially for populations P8 and P9 because of their lower within-population genetic diversity.

5 Conclusions

Comprehensive molecular analysis showed that *T. sinense* is characterized by low genetic diversity within populations and high genetic differentiation among populations, which is attributed to small population effect and geographic isolation. However, the genetic diversity of *T. sinense* within species was at an intermediate level, indicating that *T. sinense* maintains a relatively higher evolutionary potential. Of course, this evolutionary potential may only be achieved by effective conservation strategies, such as in situ conservation for each population and its habitat, and inter-population introduction of individuals via appropriate propagation and seedling management between populations in order to increase the heterozygosity within *T. sinense* populations and the population size. The genetic structure obtained in this study will provide the scientific basis for these conservation efforts.

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Compliance with ethical standards

The State Forestry Administration of the People's Republic of China granted permissions to Professor Xiaohong Gan for using the endangered species of plant (*Tetracentron sinense*).

Conflict of interest The authors declare that they have no conflict of interest.

Abbreviations AFLP, amplified fragment length polymorphisms; cpDNA, chroloplast DNA; *Gst*, coefficient of genetic differentiation; *H*, Nei's gene diversity; *H*s, gene diversity within populations; *Ht*, total gene diversity; *I*, Shannon's information index; ISSR, inter-simple sequence repeat; *N*a, observed number of alleles; *N*e, effective number of alleles; *Nm*, gene flow among populations; PPL, the percentage of polymorphic loci; RAPD, random amplified polymorphic DNA; SGS, spatial genetic structure; SSR, simple sequence repeat

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