



Genetic diversity of central and peripheral populations of *Toona ciliata* var. *pubescens*, an endangered tree species endemic to China

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ABSTRACT. Our objective was to examine the genetic diversity of central and peripheral populations of *Toona ciliata* var. *pubescens*, to elucidate whether the central-peripheral hypothesis applies to these populations. We analyzed 392 individuals from nine natural populations using eight pairs of polymorphic SSR primers. The results showed that the mean numbers of observed and expected alleles in peripheral populations were higher than in central populations. Common widespread and rare local (RL) alleles were observed in all populations. However, common local alleles were found in five populations and rare widespread alleles were only found in three. The total numbers of the four types of alleles were higher in peripheral than in central populations, and the quantity of the RL allele was obviously higher in the peripheral populations than in the central populations. Both the observed and expected heterozygosities were higher in peripheral populations compared with the central populations. The coefficient of gene differentiation of the peripheral populations was 0.3045, which was significantly higher than that of the central

populations. The gene flow between central populations was greater than one, but less than one between peripheral populations. This indicates that frequent gene flow exists between central populations, while terrain and habitat fragmentation prevent gene flow between peripheral populations. A Mantel test indicated that there was no relationship between genetic and geographical distance of *T. ciliata* var. *pubescens*.

Key words: Central population; *Toona ciliata* var. *pubescens*; Conservation strategies; Genetic diversity; Peripheral population

INTRODUCTION

Habitat fragmentation has a huge impact on plant reproduction success and the survival of populations (Grime, 1977; Goverde et al., 2002). The direct result of this is the loss of pollinators (Matsumura and Washitani, 2000) and the formation of central and peripheral populations (Wang et al., 2002). Central populations are usually large and continuous in their distribution, occupying suitable habitat conditions (Huang et al., 2005), while peripheral populations occupy less suitable habitats and are more sensitive to climate change. The response of species to environmental changes depends largely on the response of the populations inhabiting the periphery (Ledig, 1986). When conservation strategies on the genetic diversity of plants are formulated, only conservation of the central populations is generally considered, with that of peripheral populations being largely ignored (Coates, 2000; Channell and Lomolino, 2000). Therefore, against the backdrop of global climate change, studies on peripheral populations are extremely valuable (Lesica and Allendorf, 1995; Van Rossum et al., 2003).

Studies on the genetic structure of peripheral and central populations have become hot issues in the field of species conservation and evolution research. Based on the central-rich model, Mayr (1963) proposed the central-peripheral hypothesis. This hypothesis states that compared with central populations, peripheral populations are small and have experienced a severe bottleneck effect; genetic drift and selection pressure would lead to reduced genetic diversity with increased genetic differentiation eventually leading to extinction (Hampe and Petit, 2005). Although many studies on central and peripheral populations support the central-peripheral hypothesis, e.g., in *Pinus rigida* Mill (Guries and Ledig et al., 1982), *Lychnis viscaria* (Lammi et al., 1999), and *Viola elatior* (Eckstein et al., 2006), some controversies remain. Some researchers believe that migration from central populations can partly compensate for low density and reproductive success rates, thereby maintaining relatively high genetic diversity in peripheral populations (Kirkpatrick and Ravigne, 2002; Hampe and Petit, 2005). Hamann et al. (1998) and Gapare et al. (2005) conducted studies on *Picea sitchensis* (Bong.) Carr and *Alnus rubra* (Bong), respectively, and showed that because of high levels of gene flow between populations, their peripheral and central populations have high levels of genetic diversity.

Toona ciliata var. *pubescens* is a deciduous, broad-leaved tree species belonging to the genus *Toona* in the family Meliaceae. It grows rapidly, with a straight trunk, red

heartwood, and beautiful wood grain, and it is known as Chinese mahogany. It is a valuable timber species with high economic value and development prospects. It is an economically and ecologically important native tree species in Southern China. Because of environmental change, logging, and slow natural regeneration, its numbers have gradually declined. *T. ciliata* var. *pubescens* is listed as a second-class state conservation endangered species endemic to China (Liu et al., 2008). It occurs naturally throughout a belt along a subtropical monsoon region over 2000 km from northeast Zhejiang Province through Jiangxi, Anhui, Fujian, Hubei, Hunan, Guizhou Provinces, and up to southwest Yunnan and Guangxi Provinces. The species is limited to the subtropical monsoon region. In northeast Zhejiang, Fujian, Anhui, and Jiangxi Provinces, the species has large and small populations. In southwest Guizhou, Guangxi and Yunnan Provinces, the range is more attenuated, and then becomes discontinuous. Based on the terrain and topography of its distribution area, vast areas to the south of the Dabieshan Mountains, north of the Nanling Mountains, and east of the Xuefeng Mountains extending to the eastern coast form the central populations of *T. ciliata* var. *pubescens*. These include the Xianju (XJ) population in Zhejiang Province, Nanping (NP) population in Fujian Province, Jingxian (JX) population in Anhui Province, and Yifeng (YF) population in Jiangxi Province. Those beyond the above range form the peripheral populations, mainly including the Yuanmou (YM) population in Yunnan Province, Enshi (ES) population in Hubei Province, Xiangxi (XX) population in Hunan Province, Baise (BS) population in Guangxi Province, and Ceheng (CH) population in Guizhou Province. These populations provide good material for the study of the genetic diversity of central and peripheral populations of *T. ciliata* var. *pubescens*.

In this study, we employed microsatellite (SSR) marker techniques to elucidate the differences in genetic diversity between the central and peripheral populations of *T. ciliata* var. *pubescens*. We looked at the expected heterozygosity, the distribution of rare alleles within and between the populations, gene flow, inbreeding, and genetic differentiation to verify whether the conclusions of the central-peripheral hypothesis apply to *T. ciliata* var. *pubescens*, that is, whether or not the peripheral populations have low genetic diversity and high genetic differentiation.

MATERIAL AND METHODS

Materials

The field investigation on the nine *T. ciliata* var. *pubescens* populations in nine provinces were performed in 2011 using the direct sampling method (Eckert et al., 2008). The conditions and sampling quantity of the nine populations are shown in Figure 1. There were fewer individuals in the YF, XJ, NP, JX, YM, and XX populations, according to the investigation conditions where all individual plant materials were collected. There were more individuals in the CH, ES, and BS populations; at first, the distance between individuals was over 50 m. Upper leaves were collected from individual trees and 392 individual samples were collected in total. The leaves were rapidly dried with silica gel; the ratio of silica gel to leaf was 10:1. The location of each individual sampled was recorded by GPS (Magellan, eXporist 600).

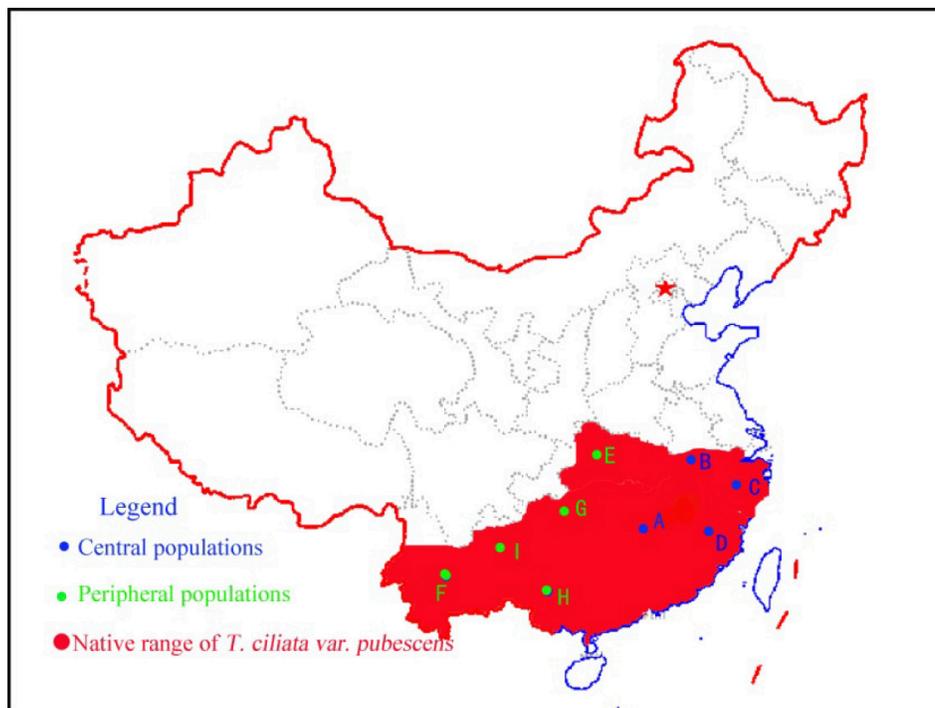


Figure 1. Native range of *Toona ciliata* var. *pubescens* and locations of populations sampled.

Genomic DNA extraction and PCR

Genomic DNA was extracted with the improved CTAB method (Liu et al., 2006). A 15- μ L reaction mix was used, comprising 1.5 μ L 10X buffer, 0.3 μ L 2 U/ μ L Taq, 2.5 mM dNTP, 10 mM primers, and 30 ng template DNA. Amplification was performed with the MYCYCLE PCR Apparatus (BIORED, USA). The reaction program was as follows: pre-denaturation at 94°C for 4 min, then 35 cycles (94°C denaturation for 1 min, 52°-55°C annealing for 0.5 min, 72°C extension for 1 min), and a final extension at 72°C for 10 min. The eight SSR loci used have been previously designed and screened by our laboratory and feature good repeatability, clear and stable amplification bands (Table 1) (Liu et al., 2006). The PCR amplification product was added with about 5 μ L sampling buffer, and analyzed on an 8% polyacrylamide gel at 120 V for 3 h. The electrophoretic buffer was 1X TBE. PCR amplifications were visualized by silver staining according to manufacturer guidelines (Promega, 1995). A 10-bp DNA ladder (Invitrogen, USA) was used as a size standard; gels were photographed with a digital camera (Canon, Japan).

Data analysis

The observed number of alleles (N_A), number of effective alleles (N_E), observed het-

Table 1. Primers for amplifying 8 SSR loci in *Toona ciliata* var. *pubescens*.

Locus accession No.	Primer sequence (5'-3')	Repeat motif	Tm (°C)	Allele size (bp)
Tc01	DQ778303 F: TCAATGCAATTAGGAGGAA R: TGCTTGTGAACCTGTG	(GA) ₈	52	240-291
Tc02	DQ453904 F: TAGGAAAGGCAAGGTGGG R: GGGTGGTCGATGAGGGTT	(AG) ₁₄	55	109-120
Tc03	DQ453907 F: GATTACGCCAGGCAAACG R: TTGAATATGGGAGAAGGT	(CT) ₆	55	230-320
Tc04	DQ453906 F: GAAACCAGCAGGCAGAGC R: GAAGAAGGGTGAGCGAGA	(AG) ₁₀	55	110-230
Tc05	DQ453905 F: AGTAATAGCCTGTAGAGCAG R: AGAGTGGGGTGGTCGATGAG	(AG) ₁₃	55	120-242
Tc06	DQ453903 F: GACTCGTGACACTTAGCCTGTA R: CTGGCGTAATCATGGTCATAC	(TTTCTC) ₇	55	121-231
Tc07	DQ453912 F: ATGGATGAGTGTCCGATAGG R: TGTGATGTAGGAGTCTGAAC	(TC) ₇	55	182-280
Tc08	DQ453914 F: TGTCTCAGTTATGCTGGCGT R: CTGCCAATCAACAAGAG	(TC) ₈	55	170-260

erozygosity (H_o), and expected heterozygosity (H_E) of various loci, inbreeding coefficient (F_{IS}) and coefficients of gene differentiation (F_{ST}), and gene flow (N_m) of the populations were calculated with POPGENE32 (Yeh and Boyle, 1997). Analysis of molecular variance (AMOVA) of the genetic variation between the central and peripheral populations was conducted with ARLEQUIN2.0 (Excoffier and Lischer, 2010). The significance of the genetic differentiation coefficient between populations was tested by TFPGA (Miller, 1997). Genetic structure was analyzed with STRUCTURE 2.2 (Pritchard et al., 2000).

According to the allele frequency and distribution within and between the populations, alleles were classified into four types (Marshall and Brown, 1975): common widespread allele (CW) (frequency ≥ 0.05 , existing in $\geq 25\%$ of populations), common local allele (CL) (frequency ≥ 0.05 , existing among $\leq 25\%$ of populations), rare widespread allele (RW) (frequency ≤ 0.05 , existing among $\geq 25\%$ of populations), and rare local allele (RL) (frequency ≤ 0.05 , existing among $\leq 25\%$ of populations). The allele frequency was calculated with GenAlix 6.41 (Peakall and Smouse, 2006) to conduct statistics on the distribution conditions of various types of alleles among the populations.

RESULTS

Number and distribution of alleles of natural populations of *T. ciliata* var. *pubescens*

PCR amplification was performed for 392 individuals with eight SSR loci among nine natural populations of *T. ciliata* var. *pubescens*, and all eight primers were polymorphic. N_A per SSR locus varied from six to eight. Fifty-six observed alleles were detected among all populations and loci, and an average amplification of seven alleles per locus was observed. The average N_E was 3.01. In the central populations, N_A was at least three and at most seven with the average being 4.75, and the average N_E being 2.53. For the peripheral populations, the average N_A was 6.38 and that of N_E was 2.87 (Table 2).

Table 2. Observed (N_A) and effective (N_E) number of alleles in populations of *Toona ciliata* var. *pubescens*.

Loci	All populations		Central populations		Peripheral populations	
	N_A	N_E	N_A	N_E	N_A	N_E
Tc01	6.00	3.37	3.00	1.98	5.00	3.64
Tc02	6.00	1.60	3.00	1.73	6.00	1.48
Tc03	6.00	2.57	4.00	2.94	6.00	2.27
Tc04	8.00	3.42	4.00	3.00	6.00	2.57
Tc05	8.00	3.36	3.00	2.93	8.00	2.57
Tc06	8.00	3.87	6.00	3.23	8.00	4.25
Tc07	8.00	3.44	7.00	2.58	8.00	4.06
Tc08	6.00	2.45	6.00	1.82	4.00	2.09
Mean	7.00	3.01	4.75	2.53	6.38	2.87
Standard deviation	1.07	0.74	1.58	0.59	1.51	1.00

The distribution of the four types of alleles in nine populations of *T. ciliata* var. *pubescens* is shown in Figure 2. The CW alleles were present in all nine populations; these were the most frequent of the four types of alleles. CW alleles were highest in the YF population (CW = 23) and lowest in the CH population (CW = 13). CL alleles were highest in the CH population but were absent in BS, XX, YM, and ES. The RW alleles only occurred in the YF, ES, and XX populations. The RL alleles were found in all nine populations of *T. ciliata* var. *pubescens*, and were highest in the ES population; only one RL allele was found in the CH, XX, and BS populations. Considering all four types of alleles, the ES and JX populations had the highest number of alleles, and the CH population had the least.

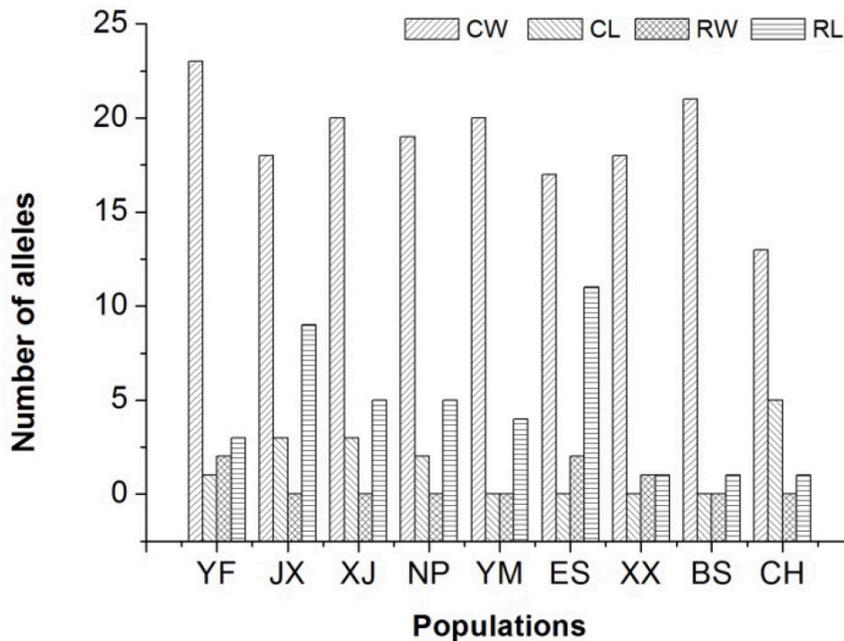


Figure 2. Distribution of different types of alleles in nine populations of *Toona ciliata* var. *pubescens*. CW = common widespread allele; CL = common local allele; RW = rare widespread allele; RL = rare local allele; YF = Yifeng; JX = Jingxian; XJ = Xianju; NP = Nanping; YM = Yuanmou; ES = Enshi; XX = Xiangxi; BS = Baise; CH = Ceheng.

Genetic diversity of natural populations of *T. ciliata* var. *pubescens*

Heterozygosity is one of the best parameters to measure genetic variation in populations (Nei et al., 1975). Population genetic diversity is reflected in the average expected heterozygosity. Studies conducted by Takezaki and Nei (1996) showed that the range of the H_E for microsatellite loci was 0.3-0.8. Populations with higher genetic diversity have higher H_E (Li et al., 2006). In this study, the H_E of eight microsatellite loci was within the range of 0.3762-0.7426 with the average being 0.6437 (Table 3); this is consistent with the results obtained by Takezaki and Nei (1996). The average H_E of the central populations was 0.5842, while the average H_E of the peripheral populations was 0.6103. The average H_E of different populations was 0.4304-0.6030. The highest H_E was in the YM population ($H_E = 0.6030$), and that of the BS population was the lowest ($H_E = 0.4304$) (Table 4). The numerical regularity of Nei's H_E (Nei's) was consistent with that of the H_E .

Table 3. Observed (H_o) and expected (H_E) heterozygosity of eight microsatellite loci in central and peripheral populations of *Toona ciliata* var. *pubescens*.

Loci	H_o			H_E			Nei's		
	All populations	Central populations	Peripheral populations	All populations	Central populations	Peripheral populations	All populations	Central populations	Peripheral populations
Tc01	0.5911	0.2229	0.8458	0.7044	0.4964	0.7269	0.7035	0.4948	0.7253
Tc02	0.3542	0.5605	0.2115	0.3762	0.4258	0.3243	0.3757	0.4244	0.3236
Tc03	0.4922	0.5987	0.4185	0.6113	0.6615	0.5611	0.6105	0.6594	0.5598
Tc04	0.7839	0.8089	0.7665	0.7090	0.6695	0.6121	0.7081	0.6674	0.6107
Tc05	0.5042	0.5629	0.4615	0.7031	0.6614	0.6128	0.7022	0.6592	0.6113
Tc06	0.7659	0.7080	0.8148	0.7426	0.6930	0.7673	0.7413	0.6905	0.7650
Tc07	0.5224	0.3448	0.6579	0.7101	0.6142	0.7559	0.7090	0.6120	0.7539
Tc08	0.6730	0.4724	0.8085	0.5926	0.4522	0.5222	0.5917	0.4504	0.5208
Mean	0.5859	0.5349	0.6231	0.6437	0.5842	0.6103	0.6427	0.5823	0.6088

Table 4. Genetic diversity in populations of *Toona ciliata* var. *pubescens*.

Population	H_o	H_E	Nei's
YF	0.5811	0.4920	0.4880
JX	0.4862	0.5023	0.4964
XJ	0.6103	0.4674	0.4548
NP	0.5019	0.5140	0.5029
YM	0.6692	0.6030	0.5899
ES	0.7596	0.5025	0.4977
XX	0.6848	0.4900	0.4805
BS	0.5926	0.4304	0.4276
CH	0.2143	0.4357	0.4266
Mean	0.5859	0.6437	0.6427

Genetic structure of natural populations of *T. ciliata* var. *pubescens*

Wright (1965) established an F -statistical method to describe the degree differentiation of populations with three parameters F_{IS} , F_{IT} , F_{ST} . Among these, F_{ST} is an important pa-

parameter for detecting the degree of differentiation of populations and it is extensively used in the study of population genetic structure. F_{IT} and F_{IS} represent the inbreeding index of the overall level and individuals in single populations, respectively. The average value of F_{IS} for the nine populations was -0.1631 (Table 5), indicating high heterozygosity and frequent genetic exchange between individuals. The average F_{IT} value for all populations was 0.1750, suggesting that the genetic exchange between the populations was infrequent, and the overall level of homozygosity in *T. ciliata* var. *pubescens* was higher. According to the results in Table 5, the F_{ST} values of all loci were within the range of 0.0678-0.5601, the average differentiation coefficient was 0.2907. The genetic variation between populations accounted for 29.07% of the total variation of the populations and the degree of differentiation between various populations was high. The average value of the genetic differentiation coefficient F_{ST} for the central population was 0.152, while that of the peripheral populations was 0.3045, indicating greater differentiation in the peripheral populations. The genetic differentiation coefficient did not reach a significant level between the CH, YF, NP, and XJ populations; however, it was significant between the other populations ($P < 0.01$) (Table 6). AMOVA showed that there was significant genetic difference between the central and peripheral populations ($P < 0.05$).

Table 5. Estimated F -statistics in central and peripheral populations of *Toona ciliata* var. *pubescens*.

Loci	F_{IS}			F_{IT}			F_{ST}		
	All populations	Central populations	Peripheral populations	All populations	Central populations	Peripheral populations	All populations	Central populations	Peripheral populations
Tc01	-0.1585	0.4797	-0.5316	0.3125	0.6036	0.0434	0.4066	0.2381	0.3754
Tc02	-0.3160	-0.4606	-0.1579	0.0638	-0.3495	0.3177	0.2886	0.0761	0.4108
Tc03	0.1149	-0.0799	0.2857	0.2815	0.0765	0.4389	0.1883	0.1448	0.2145
Tc04	-0.3975	-0.4430	-0.3569	-0.0470	-0.2678	-0.0346	0.2508	0.1214	0.2375
Tc05	-0.5859	-0.7503	-0.4372	0.3024	0.0343	0.3686	0.5601	0.4438	0.5607
Tc06	-0.2351	-0.0869	-0.3722	0.0054	-0.0340	0.0059	0.1947	0.0487	0.2755
Tc07	0.2670	0.4299	0.1598	0.3167	0.4525	0.2082	0.0678	0.0396	0.0577
Tc08	-0.3936	-0.1106	-0.6203	0.1294	-0.0414	0.0513	0.3753	0.0623	0.4145
Mean	-0.1631	-0.1085	-0.2099	0.1750	0.0600	0.1586	0.2907	0.1520	0.3045

The range of N_m between two populations of *T. ciliata* var. *pubescens* was great; the maximum N_m between populations reached 46.046 and the minimum was 0.1798 (Table 6). The average, N_m among the nine natural populations was 0.610, that of the central populations was 1.395, and that of the peripheral populations was 0.5710.

Table 6. Gene flow value (upper triangle) and F_{ST} value (lower triangle) among populations of *Toona ciliata* var. *pubescens*.

Population	YF	JX	XJ	NP	YM	ES	XX	BS	CH
YF	0.0000	0.6178	1.4426	0.5477	0.7544	0.6509	46.0463	12.4404	0.2611
JX	0.2881**	0.0000	0.8691	0.6499	0.6509	0.5673	0.6381	0.6913	0.3085
XJ	0.1477 ^{ns}	0.2234**	0.0000	0.9889	1.4718	1.0139	1.3115	2.1242	0.3013
NP	0.3134 ^{ns}	0.2778**	0.2018**	0.0000	11.7692	3.9028	0.4740	0.6627	0.1905
YM	0.2489**	0.2775**	0.1452**	0.0208**	0.0000	7.8669	0.6664	0.9648	0.2203
ES	0.2775**	0.3059**	0.1978**	0.0602**	0.0308**	0.0000	0.5872	0.7027	0.1798
XX	0.0054**	0.2815**	0.1601**	0.3453**	0.2728**	0.2986**	0.0000	4.7701	0.2574
BS	0.0197**	0.2656**	0.1053**	0.2739**	0.2058**	0.2624**	0.0498**	0.0000	0.2553
CH	0.4891 ^{ns}	0.4476 ^{ns}	0.4535 ^{ns}	0.5676 ^{ns}	0.5316 ^{ns}	0.5816 ^{ns}	0.4927 ^{ns}	0.4948 ^{ns}	0.0000

** $P < 0.01$; ^{ns} = not significant. For abbreviations, see legend to Table 1.

Genetic structure of *T. ciliata* var. *pubescens* was analyzed by using STRUCTURE 2.2. The results showed that nine populations were divided into five groups (Figure 3). The central populations of YF, NP and XJ belonged to a group, and the peripheral populations of XX, BS and ES belonged to another group. The other three populations each comprised an individual group.

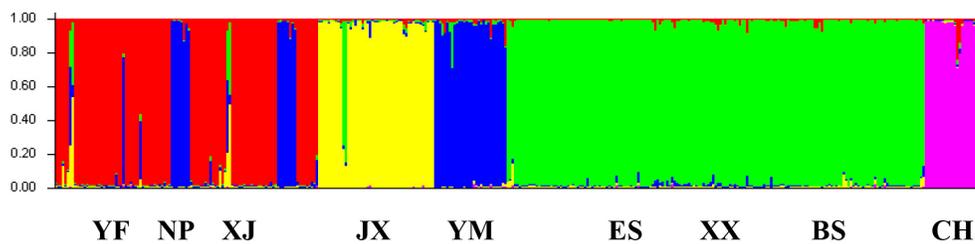


Figure 3. Estimated population structure of natural population for *Toona ciliata* var. *pubescens*. For abbreviations, see legend to Table 1.

Genetic and geographic distance among natural populations of *T. ciliata* var. *pubescens*

The genetic distance of nine populations of *T. ciliata* var. *pubescens* was within the range 0.0134-0.9136 (Table 7). The genetic distance between the NP and CH populations was the largest, and that between the YF and XX populations was the smallest. A *Mantel* test indicated no relationship between genetic and geographical distance of *T. ciliata* var. *pubescens* ($r = -0.1077$, $P = 0.2490-0.7520$).

Table 7. Genetic (lower triangle) and geographic distance (upper triangle) between natural populations of *Toona ciliata* var. *pubescens*.

Population	YF	JX	XJ	NP	YM	ES	XX	BS	CH
YF	0.0000	466.5504	669.4126	522.6245	1582.3536	1438.1204	1371.0503	1220.4042	1000.2136
JX	0.5458	0.0000	237.5536	123.7655	2042.5601	1898.9270	1831.9209	1683.5406	1464.7217
XJ	0.2519	0.6742	0.0000	146.7940	2251.4805	2107.1323	2040.0699	1888.4254	1667.2401
NP	0.5081	0.3900	0.4607	0.0000	2104.7085	1960.3726	1893.3087	1741.7866	1520.7142
YM	0.4805	0.3403	0.4392	0.0710	0.0000	144.6144	211.5460	367.2899	589.4589
ES	0.5249	0.3648	0.3951	0.1402	0.0741	0.0000	67.0702	223.4521	445.1481
XX	0.0134	0.5143	0.3162	0.5381	0.5139	0.5547	0.0000	158.4894	378.9916
BS	0.0461	0.6311	0.2206	0.5019	0.4753	0.5484	0.0922	0.0000	222.4602
CH	0.7474	0.7489	0.7915	0.9136	0.8965	0.9570	0.7532	0.7312	0.0000

DISCUSSION

Genetic diversity of central and peripheral populations of *T. ciliata* var. *pubescens*

The H_E of the central populations was lower than that of the peripheral populations, which indicated higher genetic diversity in peripheral populations. But no significant difference was found between central and peripheral populations by using the paired *t*-test (P

= 0.2536). These results do not agree with the central-peripheral hypothesis (Hampe and Petit, 2005). Possible reasons for the high genetic diversity observed in peripheral populations are: 1) *T. ciliata* var. *pubescens* is a long-living, deciduous tree species. The peripheral populations, such as YM and ES, have many ancient specimens, which contribute a large number of CW and RW alleles. According to the results of this study, the number of RW alleles in peripheral populations was significantly higher than that of central populations. The results are consistent with those of Gapare et al. (2005) regarding the RW alleles in the peripheral and central populations of North American spruces. 2) Van Rossum et al. (2003) conducted a study on allozyme variation of the central and peripheral populations of *Silene nutans* L. and discovered that both populations had high levels of genetic diversity, mainly related to their extensive distribution and great range of habitats. *T. ciliata* var. *pubescens* is widely distributed in subtropical regions from 24°-30°, across northern, central, and southern subtropical regions. The climates and habitat conditions across its distribution vary greatly; for instance, the peripheral populations of *T. ciliata* var. *pubescens* located on the Yunnan-Guizhou Plateau is in the process of adapting to the high altitude habitat conditions, and it may evolve into different ecotypes or genotypes with different ecological characteristics, leading to rich genetic variations.

Genetic structure and differentiation of *T. ciliata* var. *pubescens* populations

The genetic differentiation coefficient was significantly different between the central (0.1520) and peripheral populations (0.3045) ($P < 0.05$), thus conforming to the prediction of the central-peripheral hypothesis that the genetic differentiation of peripheral populations is greater than that of the central population. The results were similar to those for *S. nutans* L. (Van Rossum et al., 2003) and *P. sitchensis* (Bong.) Carr (Gapare et al., 2005). Lesica and Allendorf (1995) proposed that species with weak dispersion ability are more susceptible in peripheral locations compared with species with long distance dispersion, since limited pollen and fruit dispersion reduce effective N_m and result in a high proportion of self-pollination leading to reduced N_m . *T. ciliata* var. *pubescens* exhibits outcrossing insect-borne pollination with short dispersal distances and fruits spread by gravity. The peripheral populations of *T. ciliata* var. *pubescens* are located on the southwest Yunnan-Guizhou Plateau, with complex terrains and serious habitat fragmentation, resulting in low N_m between the peripheral populations ($N_m = 0.571$). The central populations are located in the subtropical monsoon climate zone, which is affected by the southeast monsoon, so gene exchange between populations is frequent ($N_m = 1.395$).

A Mantel test indicated no relationship between genetic and geographical distance of *T. ciliata* var. *pubescens*. Therefore, we suggest that terrain rather than geographical distance is the main cause of genetic differentiation between these populations. For example, the two populations of YM and XX in Yunnan province are geographically close, but because of the terrain and topography, the genetic differentiation between these populations is large.

Strategies for the conservation of *T. ciliata* var. *pubescens*

The main conservation strategies for forest genetic resources include *in situ* and *ex situ* conservation (Ledig, 1986). However, we believe the focus should be on *in situ* conserva-

tion. *In situ* conservation is the conservation of the ecosystems and natural habitats as well as the maintenance and recovery of populations of species that play vital roles in the natural environmental state (White et al., 2007). *In situ* conservation of forest genetic resources is mainly achieved by establishing nature reserves. The Yukeng provincial, Wuyishan, and Guanshan National Nature Reserves have been established, which play a key role in the conservation of XJ, NP, YF, and other central populations. In YM, XX, ES, and other peripheral populations, a large number of ancient *T. ciliata* var. *pubescens* trees are reserved; however, local people do not pay attention to them and they have poor conservation awareness. Implementing the protection and establishment of small nature reserves has been suggested. *Ex situ* conservation is supplementary to *in situ*. Populations are established and biodiversity components are protected outside of the natural habitat (White et al., 2007). *Ex situ* conservation should be combined with plantation management, tree breeding, and forest-introduction projects. According to this study, genetic variation in these populations accounts for 70.93% of the total variation; therefore, when performing *ex situ* conservation of *T. ciliata* var. *pubescens*, fewer populations should be selected and the sampling quantity within the population should be increased. Hamrick et al. (1991) proposed a method to estimate the number of populations that should be sampled for a species with the genetic differentiation coefficient F_{ST} . If the number of the populations sampled is n , then the ratio of genetic variation that was sampled is $1 - (F_{ST})^n$. In this study, the average coefficient of genetic differentiation between populations is 0.2907; therefore, more than three populations sampled can account for 97.5% of the total variance. As such, when performing sampling of natural populations and carrying out *ex situ* conservation of natural populations of *T. ciliata* var. *pubescens*, three populations with high levels of genetic diversity would suffice. In summary, our results indicate that the three populations of YM in Yunnan Province, NP in Fujian Province, and JX in Anhui Province are ideal for conservation and sampling.

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