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Sampling strategies for natural *Toona ciliata* populations

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ABSTRACT. The genetic diversity and spatial autocorrelation of *Toona ciliata* populations were analyzed with eight microsatellite markers to determine an appropriate sampling strategy for the effective conservation of natural *T. ciliata* populations. The average number of alleles and effective number of alleles were 6.1 and 2.7, respectively. The mean expected heterozygosity was 0.6006. Overall, 96.6% of the genetic variation existed in two natural populations, which was concluded from the coefficient of genetic differentiation (F_{ST} = 0.1854). Therefore, two natural populations were selected for conservation and sampling. The mean effective number of alleles and expected heterozygosity increased with increasing sample number. The mean expected heterozygosity tended to be stable when the sampling number exceeded 30. The maximum value of expected heterozygosity was 0.4770 when the sampling number was 45. Therefore, 45 sampled individuals were sufficient for conservation and sampling. Similarity relationships existed between individuals within 240 m. There were marked differences among individuals over 240 m away. The distance

between individuals exceeded 240 m when natural populations were sampled.

Key words: *Toona ciliata*; Genetic diversity; Sampling strategy; Spatial autocorrelation analysis

INTRODUCTION

An appropriate sampling strategy is fundamental for the conserved samples that represent the total genetic diversity of a population or species. Therefore, for effective conservation to be accompanied by a proper strategy, the principle of strategic conservation is to capture the greatest diversity of a given population or species using the minimum number of samples. Two factors must be considered for strategic sampling - the number of samples, including the number of populations in a given area and the number of individuals within a given population, and the sampling methods in relation to the genetic structure of the populations under consideration (Jin and Lu, 2003). Research on sampling strategies is fundamental for the collection of germplasm and for the conservation of biological diversity. Resources usually limit the species, populations, and individuals be sampled, and only a proportion of them can be collected and protected. However, samples should contain as much genetic variation as possible in order to reflect the evolutionary history of the species or populations. It is difficult to objectively assess the genetic diversity of a species being studied and protected without a reasonable sampling strategy to protect resources. Plant sampling strategy is affected by the plant's biological characteristics, environmental conditions, and the purpose of sampling (Gilbert et al., 1999; Li and Zeng, 2000; Zhu et al., 2006). Therefore, sampling strategies differ depending on the plant species, and suitable sampling strategies have been developed based on species' characteristics. Current research on plant sampling strategy has focused mainly on herbs (Ceska et al., 1997; Malosetti and Abadie, 2001; Li et al., 2003), with limited reports available for woody plants. A reasonable sampling strategy should include the following: the number of populations, by sampling a number of individuals within a population, and the distance between individuals. Therefore, it is important to understand the genetic diversity and spatial genetic structure of species that are being studied. Genetic diversity can reveal the level of genetic diversity and genetic differentiation among populations. Spatial genetic structure of natural populations is important for establishing sampling strategies (Mashall and Brown, 1975). The spatial genetic structure of genetic variation should be helpful to improve the efficiency of sampling, in order to capture more genetic diversity of species. Spatial autocorrelation analysis is an effective method used to study the spatial structure of genetic variation, especially in the context of small-scale areas, and can be used to detect and quantify spatial dependence for sampling values at calibration points (Sokal and Oden, 1978; Liu et al., 2008). *Toona ciliata* is a deciduous, broad-leaved, fast-growing tree species belonging to the *Toona* genus in Meliaceae. Flowers of this species are small, and pollen is dispersed mainly by the wind. This species is known as "Chinese mahogany", since the color of the wood is red, and the wood grain is very beautiful; therefore, *T. ciliata* is a valuable timber species with high economic value and development prospects (Liu et al., 2006; Zhang et al., 2006). Numbers of this species have declined due to environmental changes, logging, and its slow regeneration. The tree species is classified as second-class protection of endangered species in the Wildlife Conservation and Nature Reserve Management Department of the State Forestry

Administration, Institute of Botany in Chinese Academy of Sciences (2013). It is also listed as a rare and endangered species throughout the provinces (Lou and Jin, 2000; Zhang, 2000; Liu and Wu, 2005). Therefore, genetic studies on the conservation of species diversity are urgent. Preliminary reports on the genetic diversity of this species include the genetic structure of natural populations (Liu et al., 2009), spatial genetic structure (Liu et al., 2008), and community structure (Liu et al., 2010). However, sampling strategies were not investigated in these studies, and no suitable sampling strategy was developed to conserve natural populations of *T. ciliata*. In view of this, materials collected in natural populations were used to analyze genetic diversity and spatial genetic structure with microsatellite loci. A reasonable sampling strategy in natural populations based on the results was developed, including the number of sampled populations, sampled individuals within populations, and the distance among sampled individuals. The study will guide the conservation of genetic diversity, germplasm collections, and preservation.

MATERIAL AND METHODS

Materials

Seven populations were surveyed in Zhejiang, Jiangxi, and Yunnan Provinces China in 2005 and 2006. Detailed information on the seven populations is listed in Table 1. Every individual was recorded using GPS positioning in the Yifeng population in Jiangxi Province. The distance between each individual was more than 50 m. Upper leaves were collected from trees, separated, and dried rapidly with silica gel at a leaf mass ratio of 10:1.

Table 1. Geographic location and number of individuals sampled from seven natural populations of *Toona ciliata*.

Population	Abbreviation	Longitude (E)	Latitude (N)	Altitude (m)	Individuals
Yifeng	YF	114°29'-114°45'	28°30'-28°40'	220-475	65
Binchuan	BC	100°16'-101°23'	25°02'-25°22'	1404-1820	60
Yuanmou	YM	101°49'-101°52'	25°17'-25°40'	1112-1230	30
Wuding	WD	102°08'-102°12'	25°47'-25°51'	1405-1802	29
Shizong	SZ	103°42'-104°34'	24°21'-25°00'	812-912	84
Xianju	XJ	120°32'-120°56'	28°48'-28°56'	600-820	27
Suichang	SC	119°12'-119°23'	28°30'-28°36'	510-1220	25

Genomic DNA extraction and PCR amplification

Genomic DNA was extracted using a modified CTAB (cetyltrimethyl ammonium bromide) method (Doyle, 1991; Liu et al., 2006). SSR (simple sequence repeat marker) primers were designed and selected in our lab, and eight polymorphic primer pairs, which gave reproducible, clear, and stable amplified bands, were used (Liu et al., 2006). The reaction system, PCR procedures, gel electrophoresis, and silver staining methods were performed as previously described (Liu et al., 2009).

Data analysis

Genetic diversity parameters were estimated using the POPGENE version 1.31 software. The following parameters were estimated to determine population-level variation: alleles (N_A), effective number of alleles (N_E), observed heterozygosity (H_O), expected

heterozygosity (H_E), and genetic differentiation coefficient (F_{ST}). A total of 5, 10, 15, 30, 45, and 65 individuals were randomly selected, and this was repeated four times to obtain data for PopGen32 analysis in order to detect how the number of individuals sampled number affects genetic diversity in Yifeng population. Genetic diversity parameters were calculated with different sampling numbers. Spatial autocorrelation analysis was performed as described by Chen (2001) and Liu et al. (2008).

RESULTS

Number of sampled populations based on analysis of genetic diversity

Locus Tc06 and Tc07 have eight alleles, and locus Tc02 has four alleles. The average N_A was 6.1. N_E ranged from 1.4 to 3.6, the average N_E was 2.7. The average H_O was 0.6275, which ranged from 0.3344 to 0.8656 (Table 2). The average H_E was 0.6006, ranging from 0.2922 to 0.7199. The average F_{ST} was 0.1854, ranging from 0.631 to 0.4192, which indicates that 81.46% of the genetic variation occurs within populations and 18.54% occurs between populations.

Table 2. Genetic diversity of *Toona ciliata*.

Locus	N_A	N_E	F_{IS}	F_{ST}	H_E	H_O
Tc01	5	2.7	-0.1389	0.2690	0.6299	0.5969
Tc02	4	1.4	-0.3401	0.1149	0.2922	0.3344
Tc03	6	2.0	-0.0975	0.0631	0.5080	0.5375
Tc04	7	2.9	-0.5791	0.1830	0.6605	0.8656
Tc05	6	3.3	-0.4900	0.4192	0.6968	0.6020
Tc06	8	3.5	-0.2628	0.1025	0.7145	0.8065
Tc07	8	3.6	0.1396	0.0631	0.7199	0.6187
Tc08	5	2.4	-0.4074	0.2131	0.5826	0.6586
Average	6.1	2.7	-0.2479	0.1854	0.6006	0.6275
Standard Deviation	1.4577	0.7667	0.2337	0.1217	0.1439	0.1627

N_A: allele number; N_E: effective allele number; F_{IS}: inbreeding coefficient; F_{ST}: genetic divergence coefficient; H_E: expected heterozygosity; H_O: observed homozygosity.

The number of sampled populations was estimated with the coefficient of gene differentiation according to the method described by Hamrick et al. (1991). If the number of sampled populations is n, the genetic variation contained in these populations is 1 - $(F_{ST})^n$. The average coefficient of gene differentiation among populations was 0.1854; therefore 96.6% variance occurred in two populations of species in this study. Therefore, populations with higher levels of genetic diversity can be sampled from the natural population. Seven populations were clustered using UPGMA (unweighted pair-group method with arithmetic means) (Figure 1) according to Nei's (1978) genetic distance between seven populations. Three populations from Yifeng, Xianju, and Suichang (SC) from northern areas clustered together, while four populations from Yuanmou (YM), Binchuan, Wuding, and Shizong from the southern area clustered together in the dendrogram. Thus, two populations should be selected, one from the northern area, and one from the southern area. Populations SC and YM were suitable for sampling and protection.

Effects of sampling number on genetic diversity

To determine values for genetic diversity parameters, samples of 5, 10, 15, 30, 45,

and 65 individuals were analyzed. The N_E and H_E are shown in Tables 3 and 4. The mean N_E increased significantly with sampling number (Table 3). When the number of plant samples was five, the N_E was 1.6927. When the sampling number was 65, the N_E was 2.0753. H_E with different numbers of samples is shown in Table 4. The H_E was 0.3987 when the sample number was five. H_E also increased with increasing sample number and stabilized between 0.4641 and 0.4770 when the sample number increased to 30 individuals. When the sampling number was 45, H_E was at the maximum observed level of 0.4770. In summary, the number of samples for each population should exceed 30 individuals, with 45 samples per population being the most appropriate number to ensure genetic protection when natural populations are sampled.

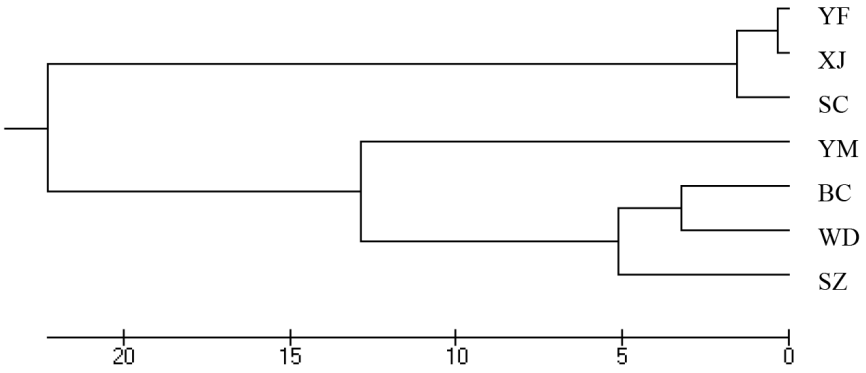

Figure 1. UPGMA clustering according to Nei's (1978) genetic distance for seven populations. YF = Yifeng, XJ = Xianju, SC = Suichang, YM = Yuanmou, BC = Binchuan, WD = Wuding, and SZ = Shizong.

Table 3. Effect of sampling number on the number of effective alleles.

Sampling number	Replication				Mean
	1	2	3	4	
5	1.6070	1.5871	1.6377	1.9388	1.6927
10	1.7106	1.7233	1.7644	1.8728	1.7678
15	1.8537	1.8322	1.7901	1.7915	1.8169
30	2.0375	1.9167	1.9037	2.0172	1.9688
45	1.9939	2.0170	2.0520	2.0363	2.0248
65	2.0753	2.0753	2.0753	2.0753	2.0753

Table 4. Effect of sampling number on expected heterozygosity.

Sampling number	Replication				Mean
	1	2	3	4	
5	0.3248	0.3562	0.4163	0.4975	0.3987
10	0.3803	0.4216	0.4224	0.4392	0.4159
15	0.4113	0.4328	0.4224	0.4216	0.4220
30	0.4506	0.4616	0.4480	0.4960	0.4641
45	0.4606	0.4763	0.4903	0.4808	0.4770
65	0.4680	0.4680	0.4680	0.4680	0.4680

Spatial autocorrelation analysis to determine the distance between sampled individuals

Genetic variation within plant populations follows a patchy distribution rather than a completely random distribution, resulting in a spatial pattern determined by differences in the plant microenvironment between populations, plant breeding system, and the mechanism of seed and pollen transmission. Correlation coefficients were calculated between individuals in the Yifeng population, the distance between individuals was divided into eight levels, with each level being equal to 80 m. Results of the autocorrelation analysis are shown in Figure 2 and show that the spatial autocorrelation coefficient was a significant positive correlation when individuals were spaced within 240 m in Yifeng population. These results indicated that individuals located within 240 m in the Yifeng population were similar. The negative values were greater than the positive values, with the values increasing when the distance between individuals exceeded 240 m, which indicates that individuals differ greatly when the distance between them exceeds 240 m. Therefore, the distance between individual trees should exceed 240 m within populations during sampling in order to avoid duplication of sampling.

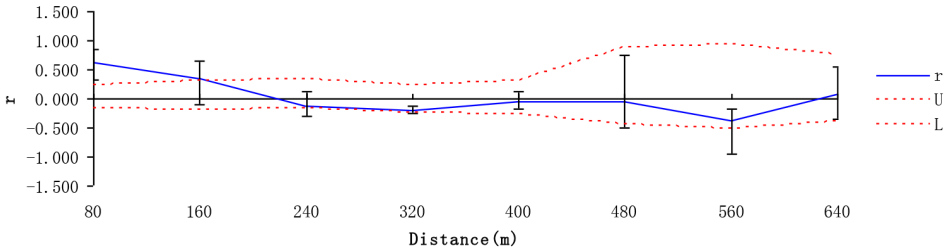

Figure 2. Spatial autocorrelation analysis of *Toona ciliata* population. r: spatial correlation coefficient; U, L: upper and lower error bars with 95% confidence interval, respectively.

DISCUSSION

Genetic diversity parameters for seven populations of *T. ciliata* in China were analyzed with eight SSR markers. The average N_A for seven populations was 3.375, the average N_E was 2.0753, and the average H_E was 0.492. The population genetic differentiation coefficient was 0.112. Two natural populations of the species accounted for 98.7% of the total variance according to the genetic differentiation coefficient. Therefore, two populations with a high level of genetic diversity were selected in order to sample natural populations to ensure their genetic protection. The results of this study are similar to those reported previously for *Baptisia arachnifera* (Ceska et al., 1997).

The mean N_E and expected heterozygosity increased significantly with sampling number. The H_E stabilized between 0.4641 and 0.4770 when 30 individuals were sampled. When the number of individuals sampled was 45, the value for H_E was the highest at 0.4770. Therefore, when sampling in natural populations or for protection, the number of individuals sampled from each population should exceed 30, and 45 samples per population is the most appropriate number. Zhu et al. (2006) and Jin et al. (2003) found that the sampling number was 27-52 when natural populations of wild soybean were sampled; this sampling number provides good results for captured the large proportion of population genetic diversity.

We found that individuals from the Yifeng population were similar when the distance between them was less 240 m by spatial autocorrelation analysis. Individuals varied greatly when the distance exceeded 240 m in the population. Therefore, the distance between individual trees should be more than 240 m when sampling within populations in order to ensure the collection of completely different genotypes. This will result in more genetic diversity being obtained for the same sampling number. The results of the present study are different to those obtained with herbal soybean (Zhu et al., 2006) and *Cyclobalanopsis glauca* (Chen, 2001). This difference may have occurred because of the biological characteristics of different species. *T. ciliata* has a small flower, and its pollen is mainly dispersed by the wind. The fruit is a capsule, which cracks naturally when mature. Its seeds have membranous wings, and are transmitted mainly by the wind meaning that the seeds can be transmitted over long distances. Soybean and *C. glauca* fruits are heavier than those of *T. ciliata*, and will be transmitted by animals and gravity over a relatively limited distance.

Conflicts of interest

The authors declare no conflict of interest.

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