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Genetic diversity and population structure of *Castanopsis eyrei* based on simple sequence repeat markers

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ABSTRACT. *Castanopsis eyrei* (Fagaceae) is one of the dominant tree species in mid-subtropical, evergreen, broad-leaved forests. We obtained 14 pairs of simple sequence repeat (SSR) primers from previous studies, which were used to analyze 90 *C. eyrei* individuals from three populations at different altitudes. Low heterozygosity was detected (F_{is} = 0.6124), and the observed heterozygosity was lower than the expected heterozygosity, possibly because of inbreeding and/or the population substructure. The genetic differentiation between populations was relatively low (F_{st} = 0.0645); only 7% of the total genetic variation occurred between populations. The medium-altitude population had higher genetic diversity than the low-altitude or high-altitude populations.

Key words: *Castanopsis eyrei*; Genetic diversity; Population structure; SSR

INTRODUCTION

The mid-subtropical zone of China is covered with forests dominated by broad-leaved evergreen trees. Wuyishan National Nature Reserve is a world cultural and natural heritage site, and contains a number of tree species in the well-preserved, broad-leaved evergreen forest (Fang, 2005). *Castanopsis eyrei* (Fagaceae) is one of the dominant species in mid-subtropical evergreen broad-leaved forests (Hu et al., 2009), and is an important part of forest ecosystems in the subtropical zone of China. *C. eyrei* is important for water conservation, biodiversity protection, biomass maintenance, and local climate regulation (Ren and Xue, 2008; Ren et al., 2010).

Research on *C. eyrei* has primarily focused on its community characteristics (Jin, 1998), population structure and dynamics (Xu et al., 2005), and intra- and interspecific competition (Xu et al., 2005). Li and Jin (2006) studied the population genetic diversity of this species at different successional stages of communities on Tiantai Mountain using random amplified polymorphic DNA (RAPD) markers. They found that 75% of the genetic variation was within populations and 25% was between populations. Based on inter simple sequence repeat (ISSR) markers, Jin et al. (2007) also reported higher genetic variation within populations than between populations in this species, and that genetic distance was significantly positively correlated with geographical distance.

Simple sequence repeats (SSRs) are tandem-repeated units that are abundant and widely distributed in genomes (Tóth et al., 2000). SSR markers appear to be variable and, because of their co-dominance and high reproducibility, are greatly superior to other markers such as RAPD, ISSR, and amplified fragment length polymorphism (AFLP) (Wang and Szmidt, 2001). Many studies based on SSR markers have proved that they are highly efficient for the assessment of genetic variation within and between populations of plants (Cao et al., 2006; Torres-Díaz et al., 2007; Emanuelli et al., 2013).

With changing altitude, habitats change due to the changing climate, terrain, and vegetation (Sáenz-Romero and Tapia-Olivares, 2003; Sáenz-Romero et al., 2006), and the genetic pattern of a single species may differ with altitude. The genetic differentiation of populations at different altitudes has been detected in some species (Bellusci et al., 2005; Zhang, 2006); however, some studies have reported little or no genetic variation between populations at different altitudes (Aradhya et al., 1993; Oyama et al., 1993; Gehring and Delph, 1999). In this study, SSR markers, combined with population genetic theory, were used to study the genetic diversity and population genetic structure of *C. eyrei* with changing altitude. By understanding the relationship between altitude and population genetic differentiation, suitable strategies can be developed for the production and usage of this species.

MATERIAL AND METHODS

Sampling

Naturally grown *C. eyrei* individuals were collected from three populations at three altitudes in Wuyishan National Nature Reserve, where there is relatively little human activity. Leaves of 30 randomly selected plants were sampled from each population, and the interval between adjacent individuals was at least 30 m. After collection, the leaves were dried and stored in silica gel. The locations of the populations studied are presented in Table 1.

Table 1. Sampling locations for *Castanopsis eyrei* populations.

Population	Latitude (N)	Longitude (E)	Altitude (m)	Sampling size
SYG	27º41.1'	117º53.9'	492	30
DAY	27º53'	117º50.7'	699	30
WSK	27º43.3'	117º40.1'	1200	30

DNA extraction and polymerase chain reaction (PCR) amplification

Total DNA was extracted from the silica gel-dried leaves using a modified 2X CTAB procedure (Doyle and Doyle, 1987). The 14 SSR markers (Table 2) used in the present study were selected based on their ability to amplify DNA in this species, and the reproducibility of their products. DNA amplifications were performed in 20-mL reaction volumes containing 1X reaction buffer (TaKaRa), 10-15 ng genomic DNA, 250 mM each dNTP, 20 mM each primer, and 1 U *Taq* DNA polymerase (Takara). The PCR was programmed according to the following profile: 94°C for 5 min, followed by 30 cycles at 94°C for 45 s, annealing temperature for 45 s, 72°C for 45 s, and a final extension at 72°C for 5 min. A 1-µL aliquot of each sample was separated on 8% denaturing polyacrylamide gel using silver staining. A 50-bp DNA ladder (Takara) was used to identify alleles (Figure 1).

Table 2. Characteristic of the fourteen SSRs studied.

Locus	Size rang (bp)	Annealing temperature (° C)	Repeat sequence	References
Ccu62F15	141-163	52	(TC)17	Ueno et al., 2003
Ccu90T17	156-190	65	(TC)23	Ueno et al., 2003
Ccu87F23	267-289	58	(TC)19	Ueno et al., 2003
Cch10	184-199	62	(GTTTTG)3(GT)8	Huang et al., 2009
Cch12	150-175	60	CAAC(CA)2GAAC	Huang et al., 2009
Cch14	153-157	62	(CACCCA)5	Huang et al., 2009
CcC01471	110-173	55	(TC)3ATTCTT(TC)14	Ueno et al., 2009
CcC02014	201-220	55	(TC)11TGTGATCGATCGCCGAGAAA(GAA)6	Ueno et al., 2009
CcC02069	117-129	55	(CAA)6	Ueno et al., 2009
CC4323	240	60	(TGT)7	Li and Sun, 2012
CC3754	294	57	(CAC)6	Li and Sun, 2012
CC2448	175	56	(GT)8	Li and Sun, 2012
CC1944	239	57	(CAT)6	Li and Sun, 2012
CC125	281	54	(AGCT)4	Li and Sun, 2012

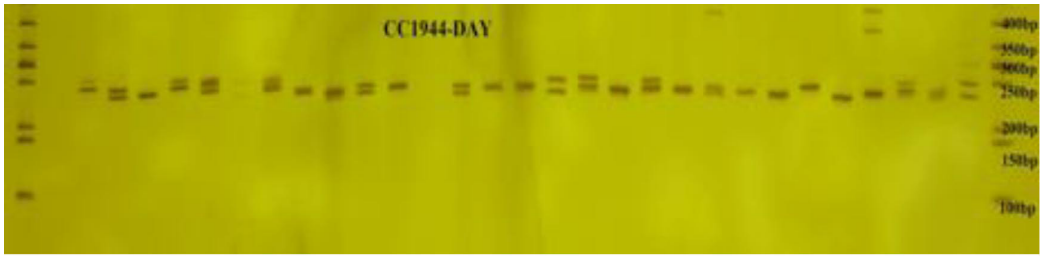

Figure 1. Amplification patterns of primer CC1944 in population DAY.

Data analysis

The number of alleles (*A*), the number of effective alleles (*Ae*), the observed

heterozygosity (*Ho*), the expected heterozygosity (H_E), heterozygosis deficiency (F_{is}), and the gene differentiation coefficient (F_{st}) were all generated using the program POPGENE1.32 (Yeh and Boyle, 1997). We conducted an analysis of molecular variance (AMOVA) using the software program GenAlEx 6.5 (Peakall and Smouse, 2012). A principal coordinates analysis (PCoA) based on the Nei and Li (1979) distance coefficient was performed in GenAlEx 6.5.

RESULTS

Microsatellite diversity

The total number of alleles per locus is listed in Table 3. A total of 93 alleles were detected, and the mean number of alleles per locus was 6.6. All 14 of the loci assayed exhibited a high level of polymorphism, with the number of effective alleles per locus ranging from 2.4487 at CC2448 to 8.5906 at CC1944. The observed heterozygosity ranged from 0.0625 to 0.4699, with an average of 0.2750. The average expected heterozygosity had slightly higher values than *Ho*, and ranged from 0.5312 to 0.8197, with a mean of 0.7590.

Table 3. Parameters of variability calculated for the 14 pairs of SSR markers in 90 accessions.

Locus	*A*	*Ae*	*Ho*	*He*
Ccu62F15	5	3.150	0.2683	0.7379
Ccu90T17	7	4.7302	0.0625	0.7785
Ccu87F23	7	4.7149	0.4382	0.7589
Cch10	5	3.0706	0.1034	0.5312
Cch12	7	6.4712	0.3750	0.8197
Cch14	5	3.1420	0.0805	0.6676
CcC01471	8	5.6008	0.4699	0.7767
CcC02014	7	4.7964	0.1549	0.7274
CcC02069	8	4.5234	0.4205	0.7252
CC4323	6	4.9959	0.2809	0.7705
CC3754	5	4.3493	0.5000	0.6998
CC2448	6	2.4487	0.0714	0.5645
CC1944	11	8.5906	0.3500	0.8004
CC125	6	3.0652	0.2738	0.6444
Mean	6.6429	4.5939	0.2750	0.7590
St Dev	1.6458	1.5881	0.1570	0.0788

A = Number of different alleles; *Ae* = effective number of alleles; H_O = observed heterozygosity; H_E = unbiased expected heterozygosity.

The fixation index indicates whether there is a heterozygosis deficiency ($F_{is}>0$) or excess ($F_{is}< 0$). The F_{is} of all of the 14 loci was higher than 0, indicating a heterozygote deficiency in the three populations (Table 4). The mean value of H_O (0.2750) was significantly lower than the mean value of H_E (0.7590), indicating that mating in this species deviates from a random pattern (Table 5). The F_{st}, which indicates genetic differentiation between populations, varied between loci (0.0139-0.3140), with a mean of 0.0645.

Genetic variation within and between populations

We estimated the genetic variation between populations using Wright's analysis of hierarchical F-statistics (Wright, 1965). The mean value of F_{st} (0.0645) suggested that this species has most genetic variation within, rather than between, its populations. The AMOVA

analysis (Table 6) suggested that 7% of the total molecular variance was attributable to between-population diversity ($P < 0.001$), while the rest (93%) was associated with differences within populations. The PCoA (Figure 2) also indicated that individuals from different populations were not distinct from each other.

Table 4. *F statistics* (Wright,1965) following the method of Nei (1987) for fourteen polymorphic loci across three natural populations of *Castanopsis eyrie*.

Locus	F_{is}	F_{st}	N_m
Ccu62F15	0.6033	0.0788	2.9233
Ccu90T17	0.9219	0.0139	17.7655
Ccu87F23	0.4209	0.0372	6.4774
Cch10	0.8001	0.3140	0.9184
Cch12	0.5397	0.0311	7.7969
Cch14	0.8818	0.0223	10.9394
CcC01471	0.3979	0.0528	4.4838
CcC02014	0.7835	0.0803	2.8635
CcC02069	0.4199	0.0698	3.3328
CC4323	0.6350	0.0368	6.5429
CC3754	0.2810	0.0920	2.4681
CC2448	0.8742	0.0466	5.1155
CC1944	0.5600	0.0934	2.4253
CC125	0.5836	0.0432	5.5323
Mean	0.6124	0.0645	3.6286

Table 5. Genetic variation within populations based on fourteen microsatellite loci.

Population	*A*	*Ae*	H_O	H_E	*Pa*
SYG	5.5714	3.4572	0.2566	0.6885	4
DAY	6.0714	4.3748	0.2901	0.7674	23
WSK	5.8571	3.8455	0.2792	0.7144	3
Across population	5.8333	3.8925	0.2753	0.7234	
Species level	6.6429	4.5939	0.2750	0.7636	30

A = Mean number of alleles; *Ae* = Mean effective number of alleles; H_O = observed heterozygosity; H_E = unbiased expected heterozygousity; *Pa* = number of samples with one or more private alleles.

Table 6. AMOVA analysis of genetic variances within and among populations.

Source	d.f.	Sum of squares	Variance component (%)	P
Between populations	2	62.628	7	<0.001
Within populations	177	1038.683	93	<0.001

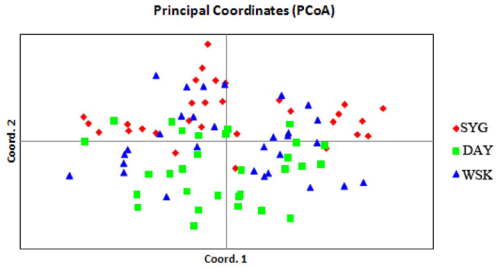

Figure 2. Principal coordinates analysis (PCoA) of all individuals. SYG, DAY and WSK stands for three populations.

The observed number of alleles for one population varied from 5.5714 to 6.0714, with an average of 5.8333, and the average effective number of alleles for one population varied from 3.4572 to 4.3748, with a cross-population value of 3.8925 and a species-level value of 8. The observed and expected heterozygosities are presented in Table 5. The DAY population at medium altitude contained the highest genetic diversity, while the SYG population at low altitude had the lowest genetic diversity (Table 5). The number of samples with one or more private alleles in DAY was 23, which was much higher than in the other two populations (4 and 3). The high number of samples with private alleles in DAY was consistent with the high genetic diversity observed in this population.

DISCUSSION

Using 14 SSRs, 93 alleles were detected in 90 individuals from three populations of *C. eyrei*. The mean number of alleles per locus was 6.6, and the ratio of polymorphic loci was 100%. SSRs are used because of their codominant inheritance, multiallelic nature, abundance in the genome, and high reproducibility, but SSR marker development is time-consuming and costly. In this study, polymorphic SSRs were screened from the literature, indicating the transferability of SSRs from closely related species, which has also been found in other studies (Cui et al., 2008; Bhawna et al., 2015). Therefore, screening SSR markers from closely related species is a time- and money-saving method.

The polymorphism level of *C. eyrei* (H_E = 0.7590) was similar to that of other tree species in the *Castanopsis* genus, such as *C. acuminatissima* (Blakesley et al., 2004) and *C. fargesii* (Xu et al., 2001). The average observed heterozygosity was lower than the average expected heterozygosity, which is consistent with results from other species in this genus (Vidhanaarachchi et al., 2005; Yamada et al., 2006). Our results suggest that genetic diversity in this species is relatively high (H_E = 0.7590) compared to in other species. For example, the expected heterozygosity for *Cyclobalanopsis myrsinaefolia* is 0.553 (Liu et al., 2008), for *Lithocarpus densiflorus* it is 0.535 (Nettel et al., 2009), and for *C. sclerophylla* it is 0.568 (Wang et al., 2012). The high genetic diversity of *C. eyrei* is closely related to its biological characteristics. *C. eyrei* is a wind-pollinated species, with a long lifespan and wide geographical distribution, which have resulted in its high genetic diversity.

A large and significant deficit of heterozygosity was detected (0.612), which was consistent with the observed heterozygosity being lower than the expected heterozygosity. Positive F_{is} values have also been reported in several other species, such as *Dactylis glomerata* (Madesis et al., 2014) and *Sesamum indicum* (Wang et al., 2012). Significant deficits of heterozygosity may be indicative of inbreeding and/or the population substructure (Hartl and Clark, 1997).

The low mean F_{st} value (0.0645) between the populations indicates that there is low genetic differentiation between them, and the AMOVA showed that 93% of the molecular variation was between all of the individuals while only 7% was attributable to variation between individuals from different locations.

We found that the medium-altitude population had the highest genetic diversity; a similar result was obtained for *Quercus aquifolioides* in Wolong Nature Reserve (Zhang, 2006). The high genetic diversity observed in the medium-altitude population may be attributable to the better habitat there. At medium altitude, there is less human disturbance than at low altitude, and the climate is not as harsh as at high altitude. In addition, population sizes at high and low altitudes are lower than at medium altitude, which would result in greater inbreeding; therefore, the medium-altitude populations of many species have the highest genetic diversity.

Conflicts of interest

The authors declare no conflict of interest.

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