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Comparative analysis of genetic diversity and population genetic structure in *Abies chensiensis* and *Abies fargesii* inferred from microsatellite markers

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ABSTRACT

Abies chensiensis Tieghem and *Abies fargesii* Franchet are two closely related tree species of Pinaceae endemic to China. *A. chensiensis* is usually found scattered in small forest fragments, whereas *A. fargesii* is a dominant member of coniferous forest. To evaluate the genetic effect of fragmentation on *A. chensiensis*, a total of 24 populations were sampled from the whole distribution of the two species. Seven nuclear microsatellite loci were employed to analyze comparatively the genetic diversity and population genetic differentiation. Both *A. chensiensis* and *A. fargesii* have high level within-population genetic diversity and low inter-population genetic differentiation. Low microsatellite differentiation (2.1%) between *A. fargesii* and *A. chensiensis* was observed. But microsatellite marker was able to discriminate most populations of these two species. Compared to *A. fargesii*, *A. chensiensi* has lower allelic diversity and higher genetic differentiation among populations. It suggested the existence of negative genetic impacts of habitat fragmentation on *A. chensiensis*.

1. Introduction

Abies chensiensis Tieghem and *Abies fargesii* Franchet are two closely related tree species of Pinaceae endemic to China (Fu et al., 1999). They have similar geographical distribution; these are found in Qinling Mountains, Bashan Mountains and southeast Tibet Plateau (Zhang et al., 2005; Shi et al., 2009). *A. chensiensis* is usually found scattered in small forest fragments at elevations from 1500 to 2300 m, whereas *A. fargesii* is a dominant member of coniferous forests found at elevations between 2100 and 3700 m (Zhang et al., 2005; Shi et al., 2009). Timbers of these two species are used for construction, furniture, and wood pulp (Fu et al., 1999). Because of excessive use, the *A. chensiensis* forest has been degraded. Recently *A. chensiensis* is listed as “vulnerable” in the China Species Red List and has been included in the Checklist of State Protection Category II in China (Wang and Xie, 2004). Morphologically, the two species are distinguished by *A. chensiensis* having bracts of seed cones not exserted, whereas *A. fargesii* has bract of seed cones exserted with much longer cusps.

Wang et al. (2011) had investigated species delimitation and biogeography of these two fir species using mitochondrial (mt) and plastid (pt) DNA sequences (Wang et al., 2011). MtDNA haplotypes showed no obvious species bias in terms of

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relative frequency. In contrast, a high level of ptDNA variation was recorded in both species. Maternally inherited genomes (mt DNA) experience considerably more subdivision than paternally inherited genomes (ptDNA) (Wang et al., 2011). Mode of inheritance appears to have a major effect on the estimates of genetic differentiation (Petit et al., 2005). Nuclear microsatellites (SSRs) have widely used in plant genetics and breeding owing to many desirable genetic attributes including hypervariability, multiallelic nature, codominant inheritance and reproducibility (Kalia et al., 2011).

Population genetics theory predicts that fragmentation of continuous population into small, isolated stand may lead to an erosion of genetic variation and increase inter-population genetic divergence due to increased random genetic drift, elevated inbreeding and reduced gene flow (Young et al., 1996). But long-distance pollination and sometimes seed dispersal prevent genetic isolation in many tree species (Kramer et al., 2008). In this study, we used biparentally inherited nuclear microsatellites to examine comparatively the levels of genetic variability and genetic differentiation in *A. chensiensis* and *A. fargesii*. The aim is to estimate if there is a negative genetic impact of fragmentation on *A. chensiensis*.

2. Materials and methods

2.1. Population sampling

Nine populations of *A. chensiensis* and 15 of *A. fargesii* were sampled, which covered the whole distribution (Table 1, Fig. 1). Six to 52 were collected from each population during June 2011 to October 2012. For each individual, approximately 0.5 g of young leaves were collected and immediately stored in plastic bags containing silica gel prior to transport to the lab.

2.2. DNA extraction and microsatellite genotyping

Genomic DNA was extracted from dry leaves according to the modified CTAB method (Doyle, 1987). Genotypes of all samples were determined using 7 nuclear SSR markers: Ach1, Ach2, Afa2, Afa3, Afa4 and Afa5 developed for *A. chensiensis* and *A. fargesii* (Zhan et al., 2014) and As09 developed for *Abies sachalinensis* (Lian et al., 2007). PCR amplification was conducted according to the method as described in Zhan et al. (2014). Fragments were separated by a 6% denaturing polyacrylamide gel. The fragments were visualized by silver staining.

2.3. Data analysis

GENEPOP version 4.0 (Raymond and Rousset, 1995) was used to test for conformation to Hardy–Weinberg equilibrium (HWE) in each population using the Markov chain method (5000 dememorizations, 100 batches, and 1000 iterations) and

Table 1
Collecting information and genetic parameters revealed by 7 SSR loci in 24 populations of *A. chenisensis* and *A. fargesii*.

Species	Population code	Longitude	Latitude	Sample	*A*	A_e	H_O	H_E	F_{IS}
		(E)	(N)	Size					
A. chensiensis	SNQ	110°28′8″	31°47′4″	25	8.9	4.8	0.697	0.709	0.037
	SJQ	108°50′10″	32°2′31″	30	8.0	4.9	0.719	0.761	0.072
	SRQ	112°15′1″	33°44′30″	7	5.3	4.2	0.653	0.671	0.103
	BTQ	111°47′37″	33°38′13″	6	5.7	4.1	0.738	0.704	0.043
	HDQ	108°27′7″	33°26′4″	30	8.7	5.2	0.743	0.742	0.016
	LCQ	107°54′9″	33°41′23″	30	8.9	4.4	0.629	0.705	0.125
	GEQ	104°20′12″	33°33′9″	26	10.4	6.5	0.659	0.780	0.174
	LZQ	103°53′42″	34°7′28″	30	8.7	5.9	0.676	0.773	0.142
	WZQ	103°37′1″	33°54′35″	30	10.1	6.7	0.652	0.759	0.157
	Average				8.3	5.2	0.685	0.734	0.097
A. fargesii	SNB	110°23′56″	31°43′29″	29	10.3	6.5	0.685	0.736	0.087
	SCB	109°50′15″	31°39′23″	24	10.3	4.7	0.661	0.718	0.100
	HLB	109°20′39″	31°59′59″	30	10.9	6.4	0.629	0.737	0.163
	XQB	110°28′32″	34°25′20″	28	8.7	5.8	0.676	0.755	0.123
	YZB	108°36′40″	33°24′39″	51	11.0	6.9	0.650	0.747	0.139
	PHB	108°29′6″	33°28′2″	28	10.0	5.3	0.673	0.707	0.066
	HDB	108°27′7″	33°26′4″	18	8.0	5. 4	0.619	0.751	0.203
	LCB	107°54′10″	33°41′23″	30	10.0	6.9	0.676	0.810	0.182
	LLB	107°47′41″	33°54′22″	30	9.7	5.7	0.705	0.727	0.048
	HSB	106°46′46″	33°35′28″	30	9.7	6.2	0.685	0.717	0.061
	STB	104°10′42″	33°40′43″	52	12.6	7.1	0.678	0.742	0.097
	ZNB	103°19′44″	34°26′54″	30	9.7	6.3	0.624	0.733	0.165
	XCB	104°2′49″	34°13′20″	44	11.1	6.8	0.597	0.731	0.194
	THB	103°34′8″	34°54′12″	30	11.1	6.4	0.633	0.762	0.185
	DLB	103°41′14″	34°54′21″	30	10.9	7.2	0.705	0.767	0.098
	Average				10.3	6.2	0.660	0.743	0.127

A: number of alleles; A_e: number of effective alleles per locus; H_O: observed heterozygosity; H_E: expected heterozygosity; and F_{IS}: inbreeding coefficient.