Original article

Genetic variation of isolated *Picea balfouriana* populations from the southeast of the Qinghai-Tibet Plateau

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Abstract
• The objective of this work is to estimate the level of genetic variation and pattern of genetic structure of isolated *Picea balfouriana* populations.
• Nine SSR markers and six STS markers were assayed in ten natural populations of *P. balfouriana*, which is a regionally distributed conifer species in the southeast of the Qinghai-Tibet Plateau.
• Expected heterozygosity ranged from 0.592 to 0.710 based on SSRs, and from 0.489 to 0.635 based on STS markers. The SSR and STS markers revealed that 11% and 12% of variation, respectively, was present among populations. However, the SSRs showed no deviation from the Hardy-Weinberg equilibrium ($F_{IS} = -0.030$), unlike the STS markers ($F_{IS} = 0.249$). In addition, assignment methods showed that individuals from the same sampling site usually cluster together.
• Our results indicated that the distribution of genetic variation and population genetic structure of *P. balfouriana* may be attributed to habitat fragmentation and heterogeneous environments caused by the complex topographic environment in the Qinghai-Tibet Plateau. The population genetic information obtained in our study will benefit the development and utilization of appropriate conservation and breeding strategies for *P. balfouriana*.

Résumé – Variation génétique des populations isolées de *Picea balfouriana* du sud-est du plateau Qinghai-Tibet.
• L'objectif de ce travail a été d'estimer le niveau de variation génétique et le modèle de la structure génétique de populations isolées de *Picea balfouriana*.
• Neuf marqueurs SSR et six marqueurs STS ont été testés sur dix populations naturelles de *P. balfouriana* qui est un conifère distribué au niveau régional dans le sud-est du plateau Qinghai-Tibet.
• L'hétérozygotie attendue allait de 0,592 à 0,710 sur la base des marqueurs SSR, et de 0,489 à 0,635 sur la base des marqueurs STS. Les marqueurs SSR et STS ont révélé que 11 % et 12 % de la variation, respectivement, étaient présentes parmi les populations. Toutefois, les marqueurs SSR ne montraient aucun écart par rapport à l'équilibre de Hardy-Weinberg ($F_I = -0,030$) à la différence des marqueurs STS ($F_I = 0,249$). Les méthodes d'assignation bayésiennes ont montré que les individus d'un même site d'échantillonnage étaient habituellement groupés ensemble.
• Nos résultats pourraient indiquer que la distribution de la variation génétique et la structure génétique de la population de *Pinus balfouriana* peuvent être attribuées à la fragmentation de l'habitat et à des environnements hétérogènes causés par un environnement topographique complexe dans le sud-est du plateau Qinghai-Tibet. L'information génétique obtenue sur la population dans notre étude sera bénéfique pour le développement et l'utilisation appropriés des stratégies de conservation et de sélection de *Pinus balfouriana*.

1. INTRODUCTION

The Qinghai-Tibetan Plateau is the highest and largest plateau in the world, and it has experienced a perplexing history of geological events and environmental fluctuations (Liu et al., 2006; Zhang et al., 2005). The Hengduan Mountains are located in the southeast of the Qinghai-Tibet Plateau and extend from northwestern Yunnan, through western Sichuan and eastern Tibet, to southern Qinghai. Due to the extremely complex topography and climatic conditions, the Hengduan Mountains have the highest species richness in the area and they are widely regarded as a core region of the Himalayas,

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which is one of the world's biodiversity hotspots (Myers et al., 2000). China is considered to have a high diversity of spruce species, with 16 species and 9 varieties of spruce, and, furthermore, 11 species and 6 varieties being endemic (Ying, 1989). Because 9 species and 5 varieties of spruce have been recorded in the southeast of the Qinghai-Tibet Plateau alone, this region is regarded as the center of the natural range for the genus *Picea* in China (Yuan et al., 2007).

Picea balfouriana is one of the regionally distributed conifer species in the southeast of the Qinghai-Tibet Plateau and considered as a constructive species within its distribution area (Collaborating Group for Vegetation of Sichuan, 1980). There is a long-standing dispute about the taxonomic status of *P. balfouriana*. Although it is usually considered as a variety of *P. likiangensis* (ECCAS, 1978), some botanists consider that *P. balfouriana* should be considered as an independent species, as many differences in geographical distribution and ecological characteristics exist between *P. balfouriana* and *P. likiangensis* (Editorial committee of the Sichuan Flora, 1983). In China, *P. balfouriana* has a large distribution region, which is inferior only to that of *P. wilsonii* Mast and *P. brachytyla* var. *complanata* (Liu et al., 2002). It occurs in the western part of the Sichuan province, eastern Tibet and southern part of the Qinghai province. The distribution center of *P. balfouriana* is located in the middle reaches of the Yalong River (Editorial Committee of the Sichuan Flora, 1983). Its vertical distribution mainly ranges from an altitude of 3 000 to 4 100 m, some trees even occurring at above 4 300 m (Yuan et al., 2007). It is an optimal species for the production of biomass used as a source of, e.g., fuel, fiber and lumber (ECCAS, 1978; Editorial Committee of the Sichuan Flora, 1983). Moreover, it is well adapted to stressful environments at high altitude, especially to cold and drought conditions, which are generally harsh for other trees (Editorial Committee of the Sichuan Flora, 1983). In alpine regions, *P. balfouriana* can form patchy forests, which effectively prevent meadows from expanding and improve the efficiency of water resource conservation (Editorial Committee of Forest of China, 1999). Owing to the above reasons, *P. balfouriana* plays a significant role in the alpine regions. Therefore, the conservation and maintenance of the *P. balfouriana* populations is an important objective. Better knowledge of the level and distribution of genetic diversity enables the development and utilization of appropriate conservation and breeding strategies. However, so far, no published genetic information of *P. balfouriana* based on molecular markers has been available. In-depth investigations of the genetic diversity and population characteristics within its native range are greatly needed.

Studies on genetic diversity based on molecular markers have the potential to provide information with crucial implications for evolutionary biology and ecology as well as for conservation biology. Microsatellite markers (SSR), which are short tandem repeats, mutate frequently by slippage and proofreading errors during DNA replication (reviewed, e.g., by Oliveira et al., 2006). It follows that SSR markers are highly variable with multiple co-dominant alleles, and they have emerged as the most popular and versatile marker type for ecological applications at present (Li et al., 2002; Rajora

et al., 2000; Selkoe and Toonen, 2006). Another marker type, sequence tagged site markers (STS), represents the amplification of well-characterized specific loci and reveal co-dominant polymorphisms (Bouillé and Bousquet, 2005).

In our study, two types of molecular markers (SSR and STS) were used to estimate the genetic structure of *P. balfouriana* populations originating from the southeast of the Qinghai-Tibet Plateau with varying climatic and geographical conditions. The results will not only provide a deep insight into its genetic diversity and population genetic structure but also valuable information for further management and breeding programs of *P. balfouriana*.

2. MATERIALS AND METHODS

The distribution of *P. balfouriana* is discontinuous and patchy throughout the southeastern region of the Qinghai-Tibet Plateau. Ten *P. balfouriana* populations, which occur in various natural habitats with different climates and topographies within the major distribution area, were selected for the study (Fig. 1 and Tab. I). All populations were sampled from natural forests occupying an area above 10 hm² and isolated by mountains and rivers. Mature cones were collected from approximately 28 nonadjacent individuals in each population. Sampled trees were separated by a minimum of 50 m. DNA was extracted from megagametophytes using Gen EluteTM Plant Genomic DNA Miniprep Kits (Sigma). About 60 to 90 megagametophytes per tree were pooled to obtain enough DNA. DNA concentrations were determined by comparison with a serial dilution of standard lambda DNA. The quality of DNA was checked by a DNA-Protein instrument (Bio-RAD).

From a set of SSR markers previously developed for a genetic study in *Picea* species (Pfeiffer et al., 1997; Scotti et al., 2002a, 2002b), six dinucleotide microsatellites (SpAC1F7, EAC7B09, EAC1D10, EAC1F04, EAC6B01 and EAC7H07) and three trinucleotide microsatellites (EATC1B02, EATC1E03 and EATC3G04) were selected. The SSR reactions were based on the procedure described by Scotti et al. (2002b) with some slight modifications. Each amplification reaction was performed in a volume of 25 μL containing 2.5 μL 10 × reaction buffer (TaKaRa, Dalian, Mg²⁺ free Dalian), 1.5–2.5 mM Mg²⁺ (TaKaRa, Dalian), 150 μM dNTP (Promega), 0.25 μM primer, 1.0 U Taq polymerase (TaKaRa, Dalian) and 20–40 ng of genomic DNA. The reaction mixtures were overlaid with mineral oil. For each primer, amplifications were carried out in 48-well plates using the following program: an initial step of 4 min at 94 °C, 7 cycles of touchdown consisting of 94 °C for 30 s, $(T_a + 7)$ °C for 30 s △↓ and 72 °C for 30 s, 30 cycles of amplification consisting of 94 °C for 45 s, T_a for 45 s, 72 °C for 45 s, and a final extension of 72 °C for 10 min. Following electrophoresis, the gels were silver-stained using the procedure of Panaud et al. (1996) and photographed using the Gel Doc 2000™ image analysis system (Bio-RAD) following the manufacturer's instructions. To ensure the validity of the analyses, the PCR reactions were performed at least twice in all cases. The fragment sizes were estimated using a standard molecular weight marker pUC 19 DNA / *Msp* (*Hpa*).

Six STS primer pairs (Sb17, Sb21, Sb29, Sb32, Sb49 and Sb62) developed for *P. mariana* and previously characterized in *P. glauca* and *P. sitchensis* (Perry and Bousquet, 1998a; 1998b) were used. The amplification program was as follows: an initial step of 5 min at 94 °C, 38 cycles of 45 s at 94 °C, 45 s at 50–56 °C and 90 s at
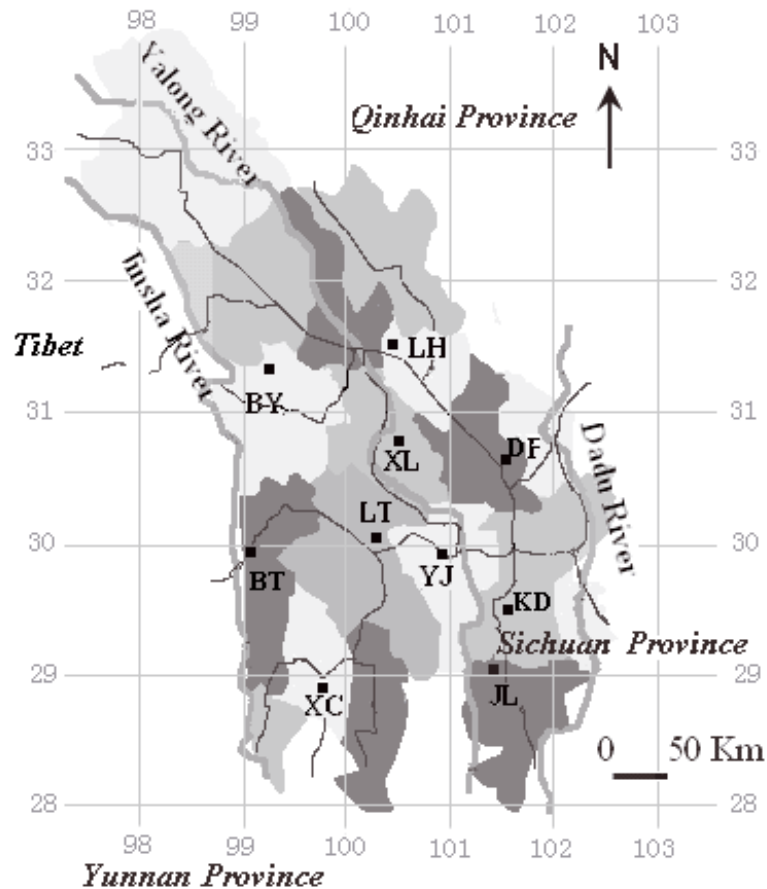
Figure 1. The locations of the ten *P. balfouriana* populations from the southeast of the Qinghai-Tibet plateau. Different gray shades and lines stand for the territorial districts of local counties of selected populations and main roads.

Table I. The ecological and geographical parameters of the *P. balfouriana* populations sampled.

Population	No. of samples	Coordinates	Altitude (m)	Landform feature	Annual rainfall (mm)	Annual average temperature (°C)
JL	27	29° 03′ N / 101° 25′ E	3 180	Alpine and canyon	892	8.8
DF	30	30° 28′ N / 101° 39′ E	3 650	Plateau and mountain	579	7.8
XC	31	28° 54′ N / 99° 48′ E	3 580	Alpine and canyon	462	10.7
LH	29	31° 32′ N / 100° 22′ E	3 500	Plateau and mountain	652	6.3
YJ	29	29° 59′ N / 100° 56′ E	3 430	Alpine and valley	706	10.9
BY	28	31° 20′ N / 99° 30′ E	4 010	Alpine and valley	599	7.7
BT	29	30° 00′ N / 99° 03′ E	3 500	Alpine and valley	474	12.6
KD	28	29° 27′ N / 101° 26′ E	3 410	Alpine and canyon	905	7.0
XL	29	30° 48′ N / 100° 30′ E	4 020	Alpine and valley	602	7.4
LT	21	30° 03′ N / 100° 28′ E	4 014	Plateau and mountain	726	3.0

72 °C, and a final extension step of 7 min at 72 °C. The PCR products were resolved by electrophoresis on 2% agarose gels and stained with 0.1% ethidium bromide. The molecular weights were estimated using the GeneRuler™ 100 bp DNA Ladder Plus (Fermentas). The gel images were recorded and the band sizes were quantified using the Gel Doc 2000™ image analysis system (Bio-RAD).

Observed and expected heterozygosity (H_o and H_e) at each locus for all populations were estimated by using the program ARLEQUIN (3.11) (Excoffier et al., 2005). GENEPOP version 3.4 (Raymond and Rousset, 1995) was used to estimate the *p*-values for the exact tests of departures from the Hardy-Weinberg equilibrium using the Markov chain method with 1 000 iterations (Guo and Thompson, 1992), and the significance level of each test was determined by applying the sequential Bonferroni procedure (Hochberg, 1988). Null alleles may lead to heterozygote deficiencies. Therefore, the proportion of null alleles was calculated as $(H_e - H_o)/(1 + H_e)$ (Brookfield, 1996). Genetic divergence between populations was investigated using the estimator F_{ST} (Weir and Cockerham, 1984). Statistical significance of pairwise

F_{ST} values was tested using the program FSTAT 2.9.3 (Goudet, 2001) after the calculation of Bonferroni corrections (Rice, 1989). The analysis of molecular variance (AMOVA) was performed to estimate the relative magnitude of genetic differentiation among populations using the program GenAlEx version 6 (Peakall and Smouse, 2006). The number of effective migrants (Nm) was estimated by Wright's (1969) $0.25(1 - F_{ST}) / F_{ST}$. UPGMA clustering was based on Nei's (1972) standard genetic distance. Bootstrapping across loci (1000 replicates) was conducted using Populations 1.2.28 (Olivier, 2002) and visualized using TreeView (Page, 1996). A principal coordinate analysis (PCA) based on Nei's (1972) genetic distance matrices was applied to obtain an additional representation of genetic relationships among populations using the program GenAlEx version 6. Mantel (1967) tests were conducted to investigate the possible relationship between the genetic and geographical distances. A Bayesian cluster analysis was performed using the Structure 2.2 software (Falush et al., 2003; Pritchard et al., 2000), which applies an approach to assign individuals into their populations based on a Markov chain Monte Carlo (MCMC) algorithm. We analyzed the data using a "linkage model", except for one SSR locus (EATC3G04, for which there is no linkage information). The data of genetic map distances between markers came from Acheré et al. (2004) and Pelgas et al. (2006). We analyzed data by a run consisting of 100 000 burn-in iterations followed by 500 000 further iterations without phased information. Twenty runs were carried out for each K value from 1 to 13, suggested by Evanno et al. (2005).

3. RESULTS

Nine SSR markers and six STS markers were used to characterize genetic diversity in ten populations of *P. balfouriana*. As a result of the SSR genotyping, a total of 106 alleles was detected. The number of alleles per locus across all populations ranged from 4 (locus EATC3G04) to 16 (loci SpAC1F7 and EAC1F04), with the mean number of alleles per locus equaling 11.8. Among the SSR loci, 82 alleles were detected across the six dinucleotide microsatellites with the average number of alleles per locus equaling 13.7, and 24 alleles were detected across the three trinucleotide microsatellites with the average number of alleles per locus equaling 8.0 (Tab. II). In the STS genotyping, the number of detected alleles per locus across all populations varied from 6 (Sb29) to 16 (Sb17), with a total of 60 distinct alleles and the mean number per locus equaling 10.0 (Tab. III).

Based on the SSR markers, the average H_e values of individual populations showed a relatively narrow range, with the values varying from 0.592 in population XC to 0.710 in population BT (Tab. II). Across all populations, the mean expected heterozygosity (H_e) was 0.631, with the H_e values ranging from 0.068 at locus EATC3G04 in population LH to 0.909 at locus SpAC1F7 in population XC. When the nine SSR loci were classified into two classes, the dinucleotide microsatellites showed H_e values varying from 0.681 to 0.852, the average value equaling 0.752, while the trinucleotide microsatellites possessed lower values, the average equaling 0.416. Based on the STS markers, the average H_e values of individual populations varied from 0.489 in population

DF to 0.635 in population BT (Tab. III). Across all populations, the mean value of H_e was 0.553, with the individual H_e values varying from 0 at locus Sb21 in populations LH and LT to 0.887 at locus Sb17 in population XL.

The Hardy-Weinberg equilibrium was verified for all loci and populations by testing the departure of F_{IS} from zero. Based on the SSR markers, all loci, except for EATC1B02, exhibited significant deviations. Four loci (EATC3G04, EAC7H07, EAC7B09 and SpAC1F7) deviating from the Hardy-Weinberg equilibrium were associated with positive values of F_{IS}, while another four loci (EAC1F04, EAC6B01, EATC1E03 and EAC1D10) were associated with negative F_{IS} values. Based on the STS genotyping, all six loci significantly departed from the Hardy-Weinberg equilibrium and mostly expressed positive F_{IS} values. When calculated across all populations, the mean F_{IS} value based on the SSR loci equaled – 0.030, while the mean value based on the STS loci equaled 0.249, indicating a deficiency of heterozygosity. In addition, the proportion of null alleles was calculated. Among all loci, only one SSR locus (EAC7B09) showed a positive value equaling 0.055 but four STS loci (Sb21, Sb32, Sb49 and Sb62) presented positive values equaling 0.117, 0.126, 0.0167 and 0.163, respectively.

The genetic divergence among populations was measured by the F_{ST} estimator (Tab. IV). Based on the SSR markers, the maximum F_{ST} value was found between populations BY and DF ($F_{ST} = 0.197$), while the minimum F_{ST} value was found between populations BT and KD ($F_{ST} = 0.044$). Based on the STS markers, the maximum and minimum genetic divergences were observed between the same population pairs as based on the SSR markers, with F_{ST} values equaling 0.217 and 0.004, respectively. The Mantel test between the two matrices of F_{ST} values based on SSR and STS markers showed a significant positive association ($r = 0.468$, $P < 0.05$). The analysis of molecular variance (AMOVA) showed that 11% and 12% of the total variation existed among the populations as detected by using SSR and STS markers, respectively. The overall gene flow (Nm) obtained from F_{ST} based on SSR and STS markers equaled 1.97 and 1.96, respectively.

The UPGMA dendrograms based on Nei's (1972) standard genetic distances between populations are shown in Figure 2. The SSR dendrogram showed that the main split is between population BY and the rest of the samples. Within the main group, there are three significant sub-groups (populations JL and YJ, BT and KD, and DF and LH) (Fig. 2a). The UPGMA dendrogram based on STS markers showed a similar trend between population BY and other populations, but the subgroups do not follow an identical pattern (Fig. 2b). Remarkably, the UPGMA cluster analyses, based on the two marker types, congruently revealed that population BY (with relatively high bootstrap values, 72% and 65%), a marginal population from the northwestern part of the natural distribution region, is genetically most distant. Additionally, the relationships among populations were further illustrated by the results of a principal coordinate analysis based on the SSR and STS matrices. The first two coordinates accounted for 30.6 and 25.7%, and 39.4 and 23.3% of the total variance, respectively (Figs. 3a, 3b). Based on the SSR markers, the difference

Table II. Number of alleles (in brackets), observed (H_o) and expected (H_e) heterozygosity, inbreeding coefficient (F_{IS}) at each locus for all populations, and average H_e in each population based on SSR markers.

	JL	DF	XC	LH	YJ	BY	BT	KD	XL	LT	Average H_e
SpAC1F7 (16)											
H_o	1.000	0.933	0.936	0.897	0.897	1.000	0.966	1.000	0.966	0.857	
H_e	0.771	0.776	0.909	0.86	0.906	0.837	0.828	0.879	0.883	0.873	0.852
F_{IS}	0.230**	0.053*	0.210**	0.096**	0.247**	0.121**	0.064*	−0.003**	−0.081**	0.028**	
EAC7B09 (8)											
H_o	0.741	0.567	0.677	0.414	0.517	0.607	0.655	0.571	0.517	0.619	
H_e	0.708	0.698	0.615	0.757	0.686	0.699	0.739	0.731	0.664	0.512	0.681
F_{IS}	0.008**	0.036**	−0.026*	0.256**	0.109**	0.100	0.039**	0.045**	0.155**	−0.121	
EAC1D10 (15)											
H_o	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.964	0.931	0.952	
H_e	0.713	0.508	0.632	0.509	0.556	0.606	0.875	0.879	0.890	0.891	0.706
F_{IS}	−0.126**	−1.000**	−0.492**	−1.000**	−0.491**	−0.319**	−0.060**	−0.065**	−0.033**	−0.039**	
EAC1F04 (16)											
H_o	1.000	0.967	1.000	0.966	1.000	1.000	1.000	1.000	1.000	1.000	
H_e	0.509	0.825	0.588	0.885	0.819	0.544	0.844	0.635	0.837	0.614	0.710
F_{IS}	−1.021**	−0.039**	−0.492**	−0.050**	−0.084**	−0.321**	−0.109**	−0.151**	−0.061**	−0.317**	
EAC6B01 (15)											
H_o	1.000	0.967	0.968	0.862	0.931	0.929	0.966	0.964	0.966	0.952	
H_e	0.836	0.740	0.775	0.696	0.828	0.769	0.792	0.779	0.707	0.710	0.763
F_{IS}	−0.108**	−0.186**	−0.129**	1.020**	−0.079*	−0.180**	−0.159**	−0.144**	−0.084**	−0.103**	
EAC7H07 (12)											
H_o	0.963	1.000	0.968	1.000	1.000	1.000	1.000	1.000	0.966	1.000	
H_e	0.769	0.838	0.806	0.792	0.828	0.831	0.806	0.629	0.870	0.810	0.798
F_{IS}	0.030*	0.192**	0.127**	0.046**	0.169**	0.069*	0.172**	−0.064*	0.054**	0.036**	
EATC1B02 (6)											
H_o	0.259	0.733	0.194	0.793	0.345	0.357	0.483	0.321	0.483	0.667	
H_e	0.237	0.504	0.184	0.549	0.324	0.314	0.402	0.280	0.405	0.455	0.365
F_{IS}	−0.057	−0.277**	−0.011	−0.317**	−0.047	−0.092	−0.144	−0.079	−0.063	−0.487*	
EATC1E03 (14)											
H_o	1.000	1.000	0.742	0.966	1.000	0.964	0.828	0.929	1.000	1.000	
H_e	0.509	0.640	0.672	0.622	0.610	0.541	0.737	0.720	0.590	0.512	0.615
F_{IS}	−1.000**	−0.322**	−0.037	−0.443**	−0.491**	−0.456**	−0.110	−0.151**	−0.186**	−1.000**	
EATC3G04 (4)											
H_o	0.333	0.600	0.161	0.069	0.103	0.429	0.414	0.393	0.345	0.333	
H_e	0.283	0.437	0.151	0.068	0.100	0.343	0.361	0.340	0.290	0.302	0.267
F_{IS}	0.087	0.220*	−0.073	−0.018	−0.038	0.389	0.057	0.785**	−0.194	−0.043	
Average H_e	0.593	0.663	0.592	0.638	0.629	0.609	0.710	0.652	0.682	0.631	0.640

NS: Not significant; * significant at $P < 0.05$; ** significant at $P < 0.01$.

when compared with the dendrogram was that PCA classified populations BY, DF and YJ as the main cluster. On the other hand, based on the STS markers, PCA classified populations BY, XC, BT and KD as the main cluster and populations BT and XC clustered closely but clearly distinctly from the main cluster. Mantel's test showed that there was no significant correlation between genetic distances (measured by F_{ST}) and the natural logarithm of geographic distances, based on either SSR ($r = 0.104$, $P = 0.255$) or STS data ($r = 0.162$, $P = 0.179$). A Bayesian cluster analysis revealed that the highest value of (P/D) was at $K = 10$ by SSR markers and at $K = 8$ by STS markers. The result of the analysis showed that, based on SSR markers, individuals from the same sampling site mostly belong to the same cluster, while, based on the STS markers, individuals from some populations (XL and LH; BT and KD) derive a large part of their genetic composition from another inferred cluster.

4. DISCUSSION

The main objective of this study was to assess the level and pattern of genetic variation among *P. balfouriana* populations originating from the southeast of the Qinghai-Tibet Plateau, in order to assist practical conservation actions and resource exploitation of this endemic spruce tree. Compared with previous studies on conifers based on SSR markers, such as investigations on the discontinuously distributed *P. asperata* (mean $H_e = 0.707$, Wang et al., 2006) and widely distributed *P. abies* (mean $H_e = 0.604$, Meloni et al., 2007; mean $H_e = 0.675$, Scotti et al., 2006), *P. balfouriana* populations possess a moderate degree of genetic diversity (mean $H_e = 0.640$). Based on STS markers, the variation level of *P. balfouriana* populations was similar to that reported previously for both continuously (mean $H_e = 0.573$) and disjointedly (mean $H_e = 0.566$) distributed Sitka spruce populations (Gapare et al., 2005). Thus,

Table III. Number of alleles (in brackets), observed (H_o) and expected (H_e) heterozygosity, inbreeding coefficient (F_{IS}) at each locus for all populations, and average H_e in each population based on STS markers.

	JL	DF	XC	LH	YJ	BY	BT	KD	XL	LT	Average H_e
Sb29 (6)											
H_o	0.259	0.767	0.484	0.724	0.379	0.929	0.621	0.429	0.828	0.619	
H_e	0.301	0.688	0.474	0.620	0.390	0.685	0.625	0.528	0.656	0.632	0.560
F_{IS}	0.136	–0.014	0.044	Mono	–0.033	0.037**	0.033	0.124**	–0.208	–0.06	
Sb49 (7)											
H_o	0.260	0.400	0.290	0.172	0.241	0.536	0.413	0.679	0.207	0.381	
H_e	0.477	0.341	0.290	0.164	0.276	0.464	0.622	0.670	0.194	0.316	0.381
F_{IS}	0.210**	–0.110	–0.035	–0.030	0.066	0.077*	0.150**	–0.077**	–0.041	–0.216	
Sb21 (10)											
H_o	0.407	0.000	0.194	0.000	0.207	0.535	0.241	0.214	0.172	0.000	
H_e	0.526	0.282	0.543	0.000	0.420	0.518	0.533	0.413	0.313	0.000	0.355
F_{IS}	–0.009**	1.000**	0.572**	Mono	0.180**	–0.062*	0.566**	0.352**	0.018**	Mono	
Sb17 (16)											
H_o	0.889	0.833	0.871	0.931	0.897	0.929	0.828	0.929	0.966	0.905	
H_e	0.831	0.852	0.878	0.870	0.844	0.792	0.837	0.834	0.887	0.870	0.850
F_{IS}	–0.036**	0.081**	0.022**	–0.056**	–0.052**	–0.086**	–0.033*	–0.098	–0.086**	–0.046**	
Sb62 (8)											
H_o	0.185	0.200	0.387	0.241	0.276	0.500	0.345	0.214	0.207	0.610	
H_e	0.516	0.554	0.608	0.655	0.584	0.764	0.430	0.318	0.498	0.796	0.572
F_{IS}	0.462**	0.368**	0.116**	0.486**	0.351**	0.11**	0.302**	0.007**	0.171**	0.011**	
Sb32 (13)											
H_o	0.296	0.167	0.419	0.483	0.310	0.214	0.621	0.679	0.517	0.286	
H_e	0.479	0.219	0.597	0.723	0.661	0.496	0.764	0.799	0.643	0.619	0.600
F_{IS}	0.019**	0.211	0.073**	0.08**	0.248**	0.304**	0.051**	0.032**	0.064**	0.387**	
Average H_e	0.522	0.489	0.565	0.505	0.529	0.620	0.635	0.594	0.532	0.539	

NS: Not significant; * significant at $P < 0.05$; ** significant at $P < 0.01$.

Table IV. Pairwise F_{ST} values among populations of *P. balfouriana* based on SSR (below) and STS data (above).

	JL	DF	XC	LH	YJ	BY	BT	KD	XL	LT
JL	—	0.088***	0.125***	0.117***	0.045***	0.200***	0.081***	0.065***	0.124***	0.094***
DF	0.138***	—	0.172***	0.097***	0.059***	0.217***	0.144***	0.131***	0.143***	0.080***
XC	0.143***	0.166***	—	0.197***	0.126***	0.215***	0.148***	0.139***	0.178***	0.152***
LH	0.116***	0.078***	0.127***	—	0.057***	0.109***	0.134***	0.109***	0.029**	0.086***
YJ	0.086***	0.088***	0.169***	0.109***	—	0.144***	0.072***	0.064***	0.075***	0.064***
BY	0.167***	0.197***	0.180***	0.160***	0.137***	—	0.136***	0.141***	0.083***	0.128***
BT	0.096***	0.090***	0.092***	0.051***	0.075***	0.103***	—	0.004NS	0.098***	0.11***
KD	0.109***	0.118***	0.063***	0.076***	0.127***	0.144***	0.044***	—	0.082***	0.104***
XL	0.124***	0.117***	0.115***	0.096***	0.105***	0.108***	0.052***	0.062***	—	0.116***
LT	0.104***	0.169***	0.123***	0.116***	0.156***	0.175***	0.056***	0.056***	0.080***	—

NS: Not significant; ** significant at $P < 0.01$; *** significant at $P < 0.001$.

the *P. balfouriana* populations have similar levels of diversity to other spruce species. Moreover, when we tested the relationship between the genetic diversity of *P. balfouriana* populations and altitude, we found that there was no clear relationship. This result is consistent with studies on other tree species in mountainous regions (Ohsawa, 2008). The result may relate to the complex interaction of ecological and geographical factors in the southeast of the Qinghai-Tibet Plateau. When comparing variation levels, the STS and trinucleotide microsatellite loci showed, on average, a considerably lower diversity level than the dinucleotide microsatellites, both as alleles per locus and as H_e values. This result is likely due to differences in mutation rates (Scotti et al., 2002a).

The amount of genetic diversity varied among the *P. balfouriana* populations. Habitat fragmentation and variable climate conditions may be the main reasons for the uneven distribution of genetic diversity. Population BT possessed the highest level of genetic diversity based on both types of markers. It may be ascribed to the following three causes: (1) Population BT has a large range of tree sizes and a high density. (2) It is located on the eastern bank of the middle reaches of the Jinsha River, where it can easily exchange genetic material with other populations by seed or pollen dispersal. (3) Introgression from another species is also a possible reason since hybridization commonly occurs between many *Picea* species. Other spruce species, such as *P. likiangensis*, occur in
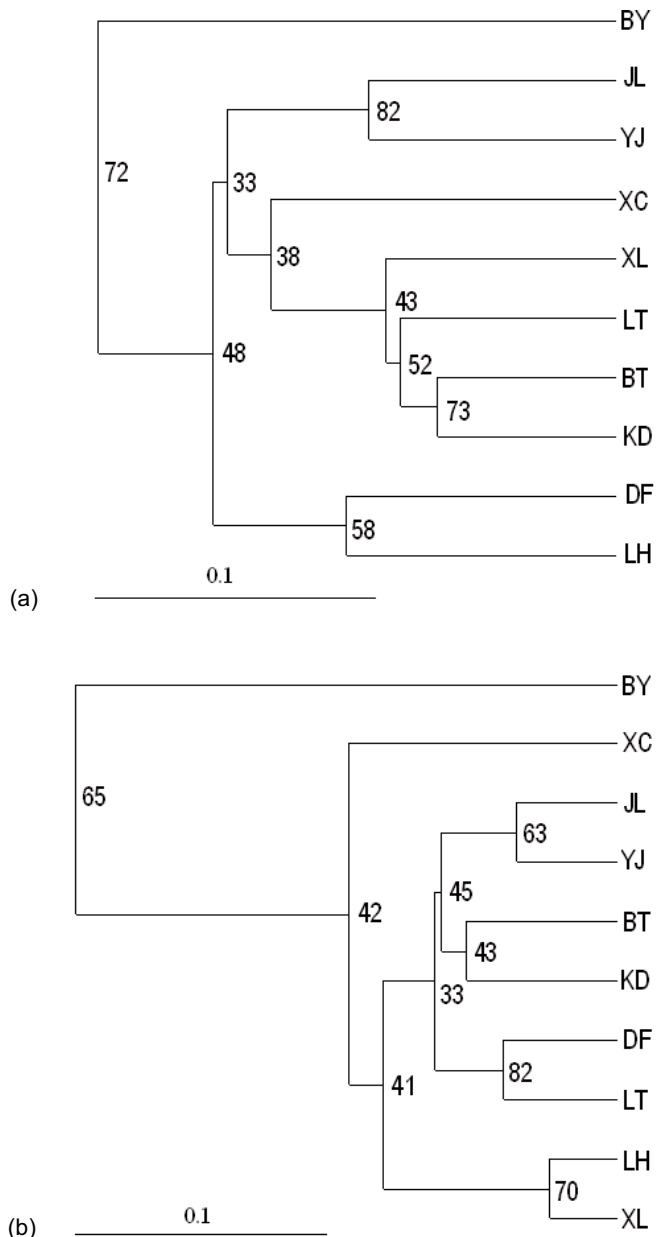
Figure 2. The dendrograms of *P. balfouriana* revealed by UPGMA cluster analysis based on Nei's (1972) standard genetic distances and bootstrapped across loci (1 000 replicates). UPGMA cluster analysis: (a) based on nine SSR markers; (b) based on six STS markers.

neighboring geographical regions of population BT. Thus, hybridization between species may happen in the overlapping region of distribution, as detected for some other Chinese conifer species as well (Luo et al., 2005; Szmidt and Wang, 1993).

When comparing the SSR and STS data sets, the inbreeding coefficients F_{IS} were not of the same order of magnitude for these two types of markers. The SSR markers showed that the whole set of populations did not deviate from the Hardy-Weinberg equilibrium ($F_{IS} = -0.030$), while the STS markers indicated a considerable deficiency of heterozygosity

($F_{IS} = 0.249$). A possible explanation for such a contradictory result is the different technical resolution capacity of the two types of markers. SSR markers represent repeat sequences which are usually outside gene coding regions and have high mutation rates, and thus, they are generally considered neutral. On the other hand, STS markers, which are derived from arbitrarily selected genes, are not adaptively neutral, and, accordingly, the STS loci may have lower mutation rates than the SSR loci. High levels of inbreeding have also been detected in some other coniferous tree species, e.g., *P. sitchensis* (Gapare et al., 2005), *P. asperata* (Wang et al., 2006) and *Pinus contorta* (Thomas et al., 1999), which may be related to population size or habitat fragmentation. In addition, the presence of null alleles may contribute to heterozygote deficiency, as detected especially by STS markers.

Both marker types showed that most genetic diversity existed within populations. Compared with previous studies, the amount of genetic differentiation of *P. balfouriana* was lower than that of *P. asperata*, which is distributed narrowly and discontinuously (Wang et al., 2006), but distinctly higher than the values detected among populations of widespread Norway spruce (Maghuly et al., 2006; Meloni et al., 2007), transcontinentally distributed black spruce (Perry and Bousquet, 2001) and both disjointedly and continuously distributed Sitka spruce (Gapare et al., 2005). Moreover, high levels of population differentiation have been commonly observed in plant species from the southeast of the Qinghai-Tibet Plateau when compared with other species belonging to the same genus, e.g., *Cupressus gigantean* (Xia et al., 2008), *P. asperata* (Wang et al., 2006), *Gentiana atuntsiensis* and *G. striolata* (Zhang et al., 2007), *Megacodon stylophorus* (Ge et al., 2005) and *Populus cathayana* (Lu et al., 2006; Peng et al., 2005). Habitat fragmentation in the southeast of the Qinghai-Tibet Plateau may account for the relatively high levels of F_{ST} values. On one hand, fragmented habitats in regions with complex topography may limit pollen exchange and seed dispersal among populations, while on the other hand, variable climate with different temperature regimes among the discontinuous and patchy *P. balfouriana* populations can postpone or advance flower development and, further, cause asynchronous flowering periods among populations. Therefore, the phenological gap may also contribute to the observed differentiation.

Bayesian analyses can be used to both assess the genetic structure of populations and infer possible populations of provenance for the studied populations or individuals (Bertorelle and Excoffier, 1998; Meloni, 2007; Pritchard et al., 2000). In this study, Bayesian assignment analysis assigned individuals of the same sampling site mostly to the same cluster, especially based on SSR markers, although the presence of gene flow was evident among some populations. The results of the structure analysis also proved that the majority of *P. balfouriana* populations in the southeast of the Qinghai-Tibet Plateau show a mosaic-like pattern and a high degree of differentiation among populations.

The correlation between Nei's (1972) pairwise standard genetic distances between populations based on the two types of markers was significant ($r = 0.40$, $P < 0.05$). The UPGMA clustering analyses and PCA plot similarly showed
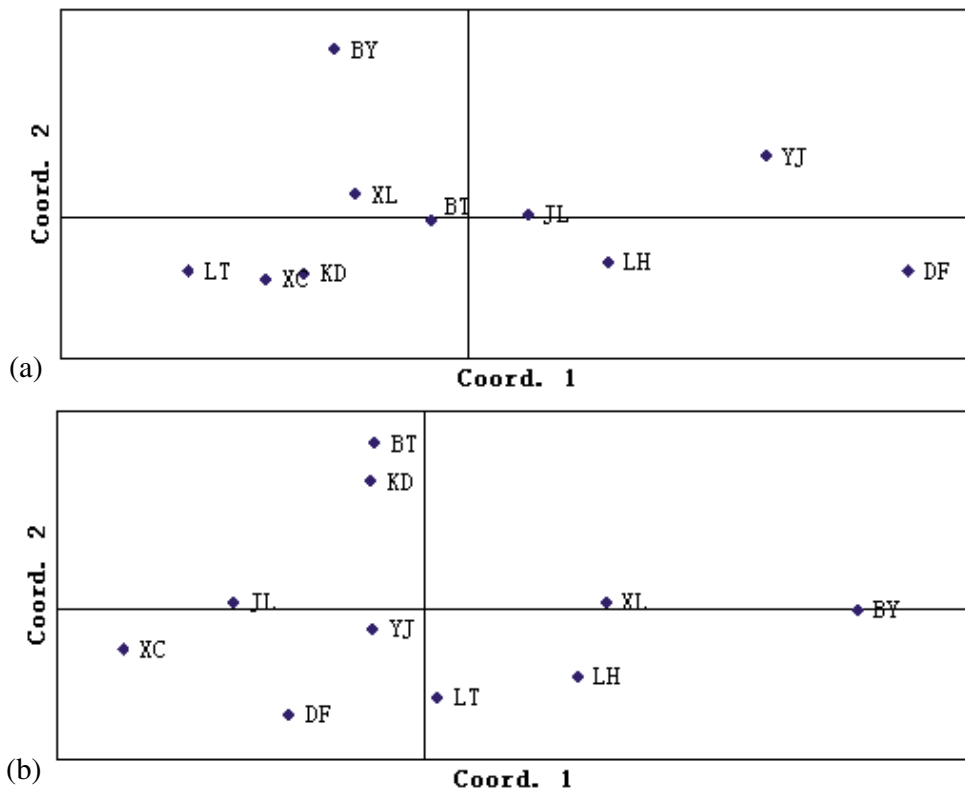
Figure 3. The PCA plot of coordinates via a covariance matrix with data standardization based on pairwise Nei genetic distances. PCA plot of coordinates: (a) based on nine SSR markers; (b) based on six STS markers.

that population BY differed from other populations based on both kinds of markers. Population BY, located in the north-western section of the Hengduan Mountains, is embraced by the Saluli Mountain in the eastern, Magongga Mountain in the southern and Jinsha River in the western direction, and thus it is isolated from other *P. balfouriana* populations. Mountains form barriers and play an important role in preventing gene exchange between population BY and other populations,which consequently leads to great divergence.

In summary, the results obtained in this study revealed that: (1) a moderate degree of genetic variation is present in *P. balfouriana* in the southeast of the Qinghai-Tibet Plateau. (2) Considerable population differentiation exists among the ten *P. balfouriana* populations based on both SSR and STS markers, possibly caused by habitat fragmentation and heterogeneous environments. (3) The result of the Bayesian assignment analyses demonstrated that individuals from the same sampling site mostly belong to the same cluster, but they also showed the presence of gene flow among some populations. (4) The UPGMA clustering and PCA analyses congruously separated population BY from other populations, which is likely due to the presence of mountain barriers. A priority in conservation biology is to preserve genetic variation in order to promote species survival (Allendorf and Leary, 1986). Thus, enhancing gene exchange should be taken into account in the conservation management strategies of *P. balfouriana* in the southeast of the Qinghai-Tibet Plateau, e.g., crossings,

seed exchange and transplantation between populations. So far, although *P. balfouriana* is used as a prime reforestation species in its region of distribution, the available knowledge of its ecological, physiological and genetic features has been limited. Further advanced research on *P. balfouriana*, especially investigations of eco-physiological parameters, and of demographic and genetic population structures, will facilitate advanced conservation actions and improvement strategies in *P. balfouriana*.

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