Allozyme Variation in Natural Populations of *Picea asperata*

Jianxun Luo, Yuhua Wang, Helena Korpelainen and Chunyang Li

Luo, J., Wang, Y., Korpelainen, H. & Li, C. 2005. Allozyme variation in natural populations of *Picea asperata*. Silva Fennica 39(2): 167–176.

A survey of allozymic alleles and genetic diversity was conducted for ten natural populations of *Picea asperata* Mast. originating from the mountains of Southwest China. A total of twenty-seven alleles at seventeen loci were observed. Ten of the loci were found monomorphic. Our results showed that the populations sampled were characterized by low genetic diversity (mean *H*e = 0.096) and a low level of inbreeding (mean *F*is = 0.005). The UPGMA tree of genetic relationships indicated that there was significant differentiation among populations. The coefficient of genetic differentiation among populations, based on *F*st, equaled 0.311. Such extensive inter-populational differentiation detected in *P. asperata* could have resulted from allele frequency divergence among populations, particularly, in one population. Introgression from another species, variation in environmental conditions, and differing selection pressures could be some of the factors attributing to significant differences among populations. In addition, our results showed that the geographic and genetic distances were not correlated in the populations of *P. asperata*. Based on the genetic information obtained, we concluded that monitoring appropriate genetic markers may be an effective means of identifying potential genetic changes occurring during forest tree evolution.

Keywords allozymes, genetic variability, *Picea asperata*
Authors' addresses *Luo*, Sichuan Academy of Forestry, Chengdu 610081, P. R. China; *Wang*, Chengdu Institute of Biology, Chinese Academy of Sciences, P.O. Box 416, Chengdu 610041, P. R. China and Graduate School of the Chinese Academy of Sciences, Beijing 100039, P. R. China; *Li*, Chengdu Institute of Biology, Chinese Academy of Sciences, P.O. Box 416, Chengdu 610041, P. R. China; *Korpelainen*, Department of Applied Biology, P.O. Box 27, FI-00014 University of Helsinki, Finland **E-mail** licy@cib.ac.cn
Received 5 August 2004 **Revised** 3 February 2005 **Accepted** 30 March 2005

1 Introduction

Genetic diversity provides evolutionary potential for sustainable forest health in the face of a changing environment. Since trees are normally the keystone species of forest ecosystems, their genetic diversity is of a special significance. Genetic variability is also the basis for tree improvement. Thus, the genetic diversity of trees can be viewed as the foundation for forest sustainability and ecosystem stability. Allozymes and molecular markers based on DNA can assist in determining the genetic diversity and structure of populations, in estimating evolutionary genetic processes that maintain genetic diversity, and in the development of genetically sustainable forest management practices (Rajora 1999, Rajora and Mosseler 2001a, b, Mosseler et al. 2003).

A large number of papers investigating the pattern and distribution of genetic variation within forest tree species have been published following the fast progress in the development of marker techniques. Unlike in the case of species with wide distribution and outcrossing breeding systems, relatively low genetic diversities (Ledig and Conkle 1983, Dayanandan et al. 1999, White et al. 1999, Aguirre-Plantier et al. 2000) and considerable levels of inbreeding (Fuchs et al. 2003) have been observed in isolated populations of some tree species occupying a limited range of distribution. The presence of such low levels of genetic diversity have confirmed the view of Hamrick et al. (1992) that the genetic variation of endemic species may be reduced through the effects of genetic drift in small populations.

Picea asperata Mast. is one of the most important trees for the production of pulp wood and timber, and it is a prime reforestation species in Southwest China. *P. asperata* occurs in the alpine and canyon regions of northwestern Sichuan province and in the southeastern Gansu province (100°–105°E, 30°–35°N), both of which are important water self-restraint regions (Wu and Raven 1994). Fossil and pollen records have revealed that *P. asperata* is a tertiary relic, for which the Qinghai-Tibet Plateau has served as a refuge. During its evolutionary process, *P. asperata* has been exposed to long-term geographical isolation, and later also to artificial selection (Wu 1959). As a result of deforestation, large areas of the natural distribution range of *P. asperata* have been destroyed and only few remnant populations are preserved. Consequently, the genetic diversity of *P. asperata* may have decreased. For the development of effective strategies for sustainable ecosystem-based management and conservation of genetic diversity in *P. asperata*, it is essential to determine and benchmark the amount and pattern of inherent genetic diversity present in the populations. So far, very little attention has been given to the genetic implications resulting from the loss of late succession tree species and forest types.

In this paper, we report the level and pattern of genetic variability in ten populations of *P. asperata* in their natural range in Southwest China, as determined based on seventeen allozyme loci coding for eight enzymes. The objective is to provide valuable information for further conservation and breeding programs on *P. asperata*.

2 Materials and Methods

2.1 Plant Materials

Seed samples were collected from ten natural populations of *P. asperata* distributed throughout their native range in Southwest China (Table 1). From each population, 30 mother trees, each separated by a distance of 50 m, were sampled.

2.2 Allozyme Analysis

Seeds were germinated until the emerging radicle was 2–5 mm in length. Diploid embryo tissues were excised, and enzyme extracts were made from the mixture of two haploid megagametophytes from each mother tree in 0.2 M phosphate buffer (pH 7.5). Extracts were absorbed onto wicks made from Whatmann filter paper and stored at –70 °C until used for enzyme electrophoresis. Two discontinuous buffer systems and horizontal starch-gel electrophoresis (12%) were employed to resolve allozymes for eight enzyme systems (Table 2). The gels were sliced and stained using the methods of Soltis et al. (1983) and Weeden and Wendel (1989) with some

Table 1. *Picea asperata* populations surveyed, and their ecological and geographical parameters.

Population	Landform feature	Water system	Longitude (E)	Latitude (N)	Altitude (m)	Annual rainfall (ml)	Annual average temperature (°)	January average temperature (°)	July average temperature (°)
XJ	Alpine and canyon	Minjang River	102°27′	31°30′	3300	614	12.0	2.2	19.9
CP	Alpine and canyon	MinjiangRiver	103°37′	32°53′	3100	730	5.7	–4.3	14.5
TB	Alpine and canyon	Dadu River	103°08′	34°07′	2450	570	7.0	–10.5	10.7
AB	Alpine and canyon	Dadu River	101°27′	32°33′	3100	712	3.3	–7.9	12.5
HS	Alpine and canyon	Minjiang River	103°19′	32°25′	2900	833	9.0	–0.9	17.5
ZN	Alpine and canyon	Bailong River	103°32′	34°20′	2800	564	4.3	–2.9	13.7
LP	Alpine and canyon	Minjiang River	103°38′	32°45′	3200	730	5.7	–4.3	14.5
DL	Alpine and canyon	Dadu River	103°40′	33°28′	2800	553	12.7	1.7	22.2
BX	Plateau and hilly	Bailong River	103°13′	33°36′	2980	647	0.7	–10.5	10.7
RWG	Plateau and mountain	Minjiang River	103°27′	31°24′	2850	730	5.7	–4.3	14.5

Table 2. Enzyme and buffer system used in the analysis.

Histidine pH7.0/7.0	Tris-Glysin pH8.3/8.3
MDH (E.C.1.1.1.37)	AAT (E.C.2.6.1.1)
PGD (E.C.1.1.1.44)	GDH (E.C.1.4.1.2)
ADH (E.C.1.1.1.1)	FDH (E.C.1.2.1.2)
SKD (E.C.1.1.1.25)	IDH (E.C.1.1.1.42)

modification. The genotypes of individual trees were inferred from the banding patterns (Weeden and Wendel 1989).

For enzymes encoded by multiple loci, the loci were numbered from the anodal to cathodal direction. Multiple alleles within a locus were numbered in a similar fashion. A total of seventeen allozyme loci were scored coding for eight enzymes: MDH (Malate dehydrogenase, 3 loci); PGD (Phosphogluconate dehydrogenase, 3 loci); ADH (Alcohol dehydrogenase, 1 locus); SKD (Shikimate dehydrogenase, 1 locus); AAT (Aspartate aminotransferase, 4 loci); GDH (Glutamate dehydrogenase, 1 locus); FDH (Formate dehydrogenase, 2 loci); IDH (Isocitrate dehydrogenase, 2 loci).

2.3 Data Analysis

For each population, genetic diversity parameters were assessed in terms of mean number of alleles per locus (*A*), effective number of alleles per locus (*A*e), percentage of polymorphic loci (99% criterion) (*P*), expected heterozygosity (*H*e) (Nei 1978), and observed heterozygosity (*H*o). Within each population, single-locus genetic structure was investigated by testing for deviations from the Hardy-Weinberg (H-W) equilibrium. The extent and direction of the deviation from the H-W equilibrium within each locus was quantified by calculating the weighted mean of *F*is (inbreeding coefficient) across all populations and by testing it for the significance of deviation from zero (Wright 1978).

Genetic differentiation among all ten populations was determined by analyzing the heterogeneity of the allele frequencies, by calculating genetic distances (Nei 1978), and by conducting the hierarchical *F*-statistics analysis (Wright 1978). The heterogeneity of the allele frequencies over all ten populations was examined using a contingency chi-square test (Workman and Niswander 1970). Gene flow (*N*m) was estimated from $Nm = 0.25(1 - Fst)/Fst$. The genetic relationships among all populations were assessed by estimating Nei's genetic distances for all population pairs (Nei 1978). The distances were used to construct a phylogenetic tree (UPGMA). All of the above calculations were performed using the program POPGENE ver 1.32 (Yeh et al. 1997). To reveal the potential correlation between genetic and geographic distances between populations, Mantel's test was performed on original data.

3 Results

3.1 Allele Frequencies and Allelic Differentiation

Eight enzyme systems were analyzed with seventeen loci showing clear banding patterns: ten loci were monomorphic and seven loci were polymorphic. The numbers of alleles observed at each polymorphic locus ranged from two (Idh-1, Mdh-1, Mdh-3 and Pgd-1) to three (Aat-2, Fdh-2 and Idh-2). A total of 27 alleles were identified among the ten *P. asperata* populations sampled. The distribution frequencies of the alleles are presented in Table 3. Most of the alleles were well-spread over the populations: nineteen alleles were common to all populations while seven alleles were common to the majority of the populations (at least seven populations). Only the allele Idh-2-C was found to be unique and present in population TB only.

3.2 Genetic Variation within Populations

The numbers of alleles per population ranged from 23 in populations XJ, HS, LP to 27 in population TB. In individual populations, the effective numbers of alleles per locus (A_e) varied from 1.08 to 1.22, with an average of 1.15. The percentage of polymorphic loci (P) ranged from 29.4% to 41.2%, with an average of 37.1%. The expected heterozygosities (He) and observed heterozygosities (Ho) were relatively low and varied from 0.066 to 0.131, and from 0.059 to 0.141, with an average of 0.096 and 0.094, respectively. For the whole species, the He and Ho values equaled 0.138 and 0.094, with the effective number of alleles per locus (A_e) and the percentage of polymorphic loci (P) equaling 1.22 and 41.2. Genetic variability among the populations is presented in Table 4. Large differences among populations existed with respect to the effective numbers of alleles, levels of poly-

Table 3. Distribution of allele frequencies in the populations of *P. asperata*.

Loci	Allele	XJ	CP	TB	AB	HS	ZN	LP	DL	BX	RWG
Aat-1	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Aat-2	A	0.050	0.017	0.117	0.000	0.000	0.267	0.083	0.100	0.067	0.000
	B	0.950	0.967	0.833	0.933	0.917	0.667	0.917	0.817	0.917	0.883
	C	0.000	0.017	0.050	0.067	0.083	0.067	0.000	0.083	0.017	0.117
Aat-3	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Aat-4	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Adh-1	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Fdh-1	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Fdh-2	A	0.183	0.517	0.233	0.033	0.000	0.167	0.250	0.300	0.467	0.017
	B	0.700	0.417	0.717	0.600	1.000	0.733	0.683	0.700	0.450	0.917
	C	0.117	0.067	0.050	0.367	0.000	0.100	0.067	0.000	0.083	0.067
Gdh	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh-1	A	1.000	0.833	0.900	0.700	0.900	0.083	1.000	0.983	0.933	0.600
	B	0.000	0.167	0.100	0.300	0.100	0.917	0.000	0.017	0.067	0.400
Idh-2	A	0.000	0.150	0.067	0.017	0.083	0.833	0.000	0.033	0.050	0.000
	B	1.000	0.850	0.917	0.983	0.917	0.167	1.000	0.967	0.950	1.000
	C	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-1	A	0.383	0.800	0.950	0.900	0.883	0.150	0.950	0.883	0.917	0.917
	B	0.617	0.200	0.050	0.100	0.117	0.850	0.050	0.117	0.083	0.083
Mdh-2	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-3	A	0.250	0.133	0.217	0.000	0.200	0.883	0.283	0.183	0.017	0.183
	B	0.750	0.867	0.783	1.000	0.800	0.167	0.717	0.817	0.983	0.817
Pgd-1	A	0.067	0.600	0.800	0.933	0.950	0.933	0.550	0.667	0.900	0.950
	B	0.933	0.400	0.200	0.067	0.050	0.067	0.450	0.333	0.100	0.050
Pgd-2	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgd-3	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Skd	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table 4. Genetic variation within population based on seventeen allozyme loci.

Population	N	M	*P*	*A*	*Ae*	*Ho*	*He*	No. and (%)
XJ	60	23	29.4	1.35	1.15	0.0706 ± 0.1734	0.0915 ± 0.1734	5 (71.4)
CP	60	26	41.2	1.53	1.22	0.1412 ± 0.2009	0.1306 ± 0.1903	7 (100.0)
TB	60	27	41.2	1.59	1.16	0.1137 ± 0.1663	0.1082 ± 0.1523	7 (100.0)
AB	60	24	35.3	1.41	1.13	0.0941 ± 0.1927	0.0829 ± 0.1572	6 (85.7)
HS	60	23	35.3	1.35	1.08	0.0706 ± 0.1166	0.0662 ± 0.1020	6 (85.7)
ZN	60	26	41.2	1.53	1.17	0.1176 ± 0.1708	0.1149 ± 0.1624	7 (100.0)
LP	60	23	29.4	1.35	1.17	0.0941 ± 0.1879	0.0966 ± 0.1809	5 (71.4)
DL	60	25	41.2	1.47	1.16	0.0745 ± 0.1288	0.1067 ± 0.1656	7 (100.0)
BX	60	26	41.2	1.53	1.13	0.0588 ± 0.0939	0.0785 ± 0.1465	7 (100.0)
RWG	60	24	35.3	1.41	1.13	0.1078 ± 0.2152	0.0876 ± 0.1491	6 (85.7)
Species	600	27	41.2	1.59	1.22	0.0943 ± 0.1271	0.1378 ± 0.1785	7 (100.0)
Mean	60	24.7	37.1	1.45	1.15	0.0943	0.0964	6 (85.7)

N, sample size, 2 megagametophytes from each tree analysed; M, number of alleles per population; *A*, number of alleles per locus; *P*, percentage of polymorphic loci; *Ae*, effective number of alleles per locus (Kimura and Crow 1964); *Ho*, observed heterozygosity; *He*, expected heterozygosity; No. and (%), allozyme loci showing heterogeneity of allele frequencies.

morphism and heterozygosity. The order of the genetic diversity among populations was found to be CP>ZN>TB>DL>LP>XJ>RWG>AB>BX >HS. The highest values of these three variability parameters were found in population CP and the lowest values in population HS.

3.3 Population Genetic Structure

Significant ($P < 0.05$) allele frequency heterogeneity was observed at six of the seven polymorphic loci among the ten populations. Only the locus Aat-2 did not possess significant allele frequency heterogeneity over the populations. The number (and %) of loci showing significant allele frequency heterogeneity within individual populations ranged from 5 (71.4%) to 7 (100.0%) (Table 4). The genetic analyses revealed a high level of differentiation among populations. The coefficient of hierarchical *F*st (Table 5), estimated according to Wright (1978), ranged from 0.067 for locus Aat-2 to 0.529 for locus Idh-2, with the average value equaling 0.311. This showed that 31.1% of the total genetic diversity existed among populations. The overall gene flow (*N*m) among populations equaled 0.554, which gives an estimate of the average number of migrants between all studied populations per generation. The observed value indicated that gene exchange between populations is limited.

Table 5. Summary of F-statistics at seven loci for ten populations of *P. asperata*.

Locus	*F*is	*F*it	*F*st	*N*m
*A*at-2	0.051	0.114	0.067	3.503
Fdh-2	0.064	0.199	0.144	1.483
Idh-1	–0.263	0.288	0.437	0.323
Idh-2	–0.130	0.468	0.529	0.223
Mdh-1	0.001	0.391	0.391	0.400
Mdh-3	–0.148	0.191	0.295	0.598
Pgd-1	0.288	0.546	0.363	0.439
Mean	0.005	0.314	0.311	0.554

*F*is, inbreeding coefficient at the population level; *F*it, inbreeding coefficient at the total sample level; *F*st, proportion of differentiation among populations; *N*m, gene flow.

3.4 Genotypic Structure and Departure from Hardy-Weinberg Equilibrium

In individual populations, tests for departures from the Hardy-Weinberg equilibrium showed significant deviations for at least one locus in other populations, except in populations TB and DL. Most of the loci (76%) followed the Hardy-Weinberg equilibrium in the majority of the populations. However, significant departures from the Hardy-Weinberg equilibrium were observed in a number of cases (24%), primarily due to a deficit of heterozygotes. Over the whole sample of *P. asperata*, a mean *F*it value of 0.314 was

Table 6. Genetic distances and genetic identities among populations of *P. asperata* (Nei's unbiased measurements, Nei 1978).

Population	ZN	TB	CP	AB	RWG	DL	BX	HS	LP	XJ
ZN	****	0.8432	0.8452	0.8363	0.8669	0.8291	0.8172	0.8482	0.8203	0.8162
TB	0.1706	****	0.9881	0.9871	0.9887	0.9976	0.9922	0.9934	0.9942	0.9420
CP	0.1682	0.0120	****	0.9770	0.9683	0.9912	0.9911	0.9711	0.9888	0.9593
AB	0.1788	0.0130	0.0233	****	0.9890	0.9803	0.9868	0.9852	0.9744	0.9214
RWG	0.1429	0.0114	0.0322	0.0110	****	0.9801	0.9751	0.9932	0.9749	0.9178
DL	0.1874	0.0024	0.0088	0.0199	0.0201	****	0.9909	0.9880	0.9973	0.9581
BX	0.2018	0.0078	0.0089	0.0133	0.0252	0.0091	****	0.9805	0.9838	0.9286
HS	0.1647	0.0066	0.0293	0.0149	0.0068	0.0121	0.0196	****	0.9823	0.9284
LP	0.1980	0.0058	0.0113	0.0260	0.0254	0.0027	0.0164	0.0179	****	0.9637
XJ	0.2031	0.0597	0.0416	0.0819	0.0857	0.0428	0.0741	0.0743	0.0370	****

Above diagonal: Genetic identities
Below diagonal: Genetic distances

observed, meaning that *P. asperata* was inbred overall. Three polymorphic loci, Aat-2, Fdh-2, and Mdh-1, possessed observed heterozygosities similar to those expected under Hardy-Weinberg, while other loci showed a surplus of heterozygotes (*F*is ranging from –0.130 to –0.263) or a deficiency of heterozygotes (Pgd-1, *F*is = 0.288), with an average *F*is value equaling 0.005, which is a value not significantly different from zero (Table 5). These *F*is values show that there was no consistent tendency toward heterozygote excess or deficiency within each sampled population, and therefore no indication of inbreeding within populations.

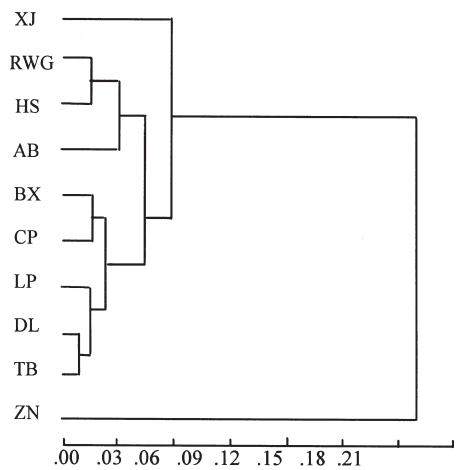

Fig. 1. UPGMA dendrogram of *P. asperata* populations based on Nei's (1978) unbiased genetic distances.

3.5 Genetic Relationships

The genetic distances, based on the allele frequencies of the allozyme markers, were calculated for each pair of populations to estimate the extent of their divergence (Table 6). The average genetic distance between populations equaled 0.056. The lowest genetic distance (0.002) was found between populations TB and DL, and the greatest genetic distance (0.203) was found between populations XJ and ZN. Mantel's test showed no significant correlation between genetic and geographical distances among the ten populations of *P. asperata*.

The UPGMA cluster analyses based on Nei's (1978) unbiased genetic distances was performed to further reveal the genetic relationships among the populations (Fig. 1). The cluster analysis indicated clear differentiation among populations. Three main clusters were evident. The first group consisted of the populations TB, DL, LP, CP, BX, AB, RWG and HS. Within the first group, the populations RWG, HS, and AB were distinct from the others. The populations XJ and ZN differed considerably from the other populations and they were clustered alone as a second and third group, respectively.

4 Discussion

Many previous studies have revealed that narrowly restricted species tend to have a lower level of genetic diversity than widespread congeners (Karron 1987, Gitzendanner and Soltis 2000). This result was further confirmed in our study. The number of loci analyzed (17) enabled us to obtain a clear picture of genetic variation in natural populations of *P. asperata* by means of allozyme markers. The populations of *P. asperata* possessed a low level of polymorphism and heterozygosity ($P = 41.2\%$ and $He = 0.096$) when compared to *Pinus pinaster* ($He = 0.136$, Hamrick et al. 1992; $He = 0.158$, Salvador et al. 2000). Genetic diversity is generally the result of long-term evolution. The low level of genetic variation present in *P. asperata* populations may be due to severe contractions in the distribution area and population size during the last glacial period, as it has occurred in a number of conifers (Ledig and Conkle 1983, Hawkins and Sweet 1989, Billington 1991, Shapcott 1991, Gibson et al. 1995).

The evidence for a severe contraction in the distribution of *P. asperata* is mainly reckoned on the analysis of pollen and fossil records. Fossilized fragments have been found in Tertiary deposits from western Sichuan and northern Yunnan (Fang 1996). This confirms that in the past *P. asperata* was probably much more widely distributed in the southwestern region in China when compared to its present area of occurrence. The results of pollen analysis suggest that north-temperate conifer forests were restricted to a small refuge at the southeastern edge of the Qinghai-Tibet Plateau during the last ice age. Following the retreat of the glacier and climate warming during postglacial times, *P. asperata* extended from the glacial refuge toward the north and migrated into the mountainous area (Wang 1992, Liu et al. 2002). As a result of low latitude, the forest areas were probably limited to higher altitudinal areas of the mountains, which isolated those populations from each other.

Data from fossil and pollen analyses indicated that *P. asperata* may have suffered a severe population bottleneck during the glacial time (Fang 1996). As summarized by Luikart and Cornuet (1998), populations, which have experienced a long period of bottleneck, should have a low level of genetic variation and a high rate of inbreeding. Traditional measures of genetic variation, e.g., heterozygosity and allelic diversity, can be used to identify past bottlenecks. In principle, it is possible to detect bottlenecks by testing for transient heterozygosity excess that recently bottlenecked populations exhibit at marker loci (Cornuet and Luikart 1996, Luikart et al. 1998). However, in our study on *P. asperata*, there was no detectable effect of a bottleneck on the extent of variation because of a lack of a reference sample either from the time before the event or from another population known to be non-bottlenecked. Thus, there is no powerful support for the presumption that a genetic bottleneck had accompanied isolation in the case of populations sampled in our study. The populations of *P. asperata* investigated here may have gone through a genetic bottleneck but not recently enough to be detected. Also, it is possible that the variation pattern of allozymes may not be effective enough to identify a bottleneck, as the same method has failed to detect potentially significant bottleneck in other cases, e.g., in *Triturus carnifex* (Arntzen 2001).

The genetic variation pattern detected in the present study is relatively consistent with the general observation that woody species, especially conifers, maintain most of their variation within populations (Hamrick et al. 1992, Müller-Starck 1995, Collignon and Favre 2000, Collignon et al. 2002, Kraj 2002). However, the degree of population differentiation ($Fst = 0.311$) observed in *P. asperata* was quite large when compared with other conifer species (Petit et al. 1995, Zheng and Ennos 1999, Salvador et al. 2000, Gomez et al. 2003, Prus-Glowacki et al. 2003). However, the significant differences mainly resulted from allele frequency divergence among populations, particularly in population ZN. The UPGMA dendrogram, based on genetic distances, showed that population ZN differed considerably from the other populations and was clustered alone as a group. In fact, the *F*st value equaled only 0.178 (instead of 0.311) when population ZN was removed from the analysis. All morphological traits showed that the sampled populations belonged to the same variety. Therefore, the reason for the genetical distinctiveness of population ZN may be introgression from another species, as a result of hybridization or pollen contamination. Hybridi-

zation occurs commonly between many *Picea* species, and that explains differentiation patterns in some other Chinese conifer species as well (Szmidt and Wang 1993). On the other hand, the large genetic variability among populations in Southwest China may be due to a high ecological variability, for example, the topography of the area and the variety of climate, which can create differing selection pressures in the populations and a low level of gene flow.

Slatkin (1987) has suggested that about one individual or more exchanged between two populations will prevent different neutral alleles at the same locus from being nearly fixed in the populations. The estimates of gene flow among all sampled populations and among the populations excluding population ZN, $Nm = 0.554$ and $Nm = 1.156$ (number of migrants exchanged between populations per generation; Wright 1931), respectively, indicated a quite low rate of gene exchange between population ZN and the other nine populations. The private allele found in population TB with a low frequency might be an exotic gene resulting from pollen contamination. In addition, according to Thomas et al. (1994), disjunctive habitats, and population isolation in general, affect a species' ability to survive and disperse. In the circumstances, the isolated habitats of *P. asperata* have played an important role in preventing sufficient gene flow, which has consequently lead to a considerable genetic heterogeneity among populations (Wright 1943, Kimura and Weiss 1964, Endler 1977).

The lack of a correlation between genetic and geographic distances detected here is consistent with previous isozyme studies that showed significant geographic variation only over a much wider range of the species distribution (Yeh and Layton 1979, Yeh et al. 1985). Yeh et al. (1985) suggested that altitudinal variation may be important at a small scale, but our sampling was not intensive enough to test this aspect. Our results indicated that geographical distance as such was not an important factor influencing the genetic relatedness of populations in *P. asperata*, and there were other factors which are likely to affect genetic differentiation, e.g., introgression from another species, the existence of mountain barriers and the presence of rivers, such as Min River, Jialing River and Yellow River.

In conclusion, the data presented here demonstrate the level and structure of the genetic variability in the distribution area of *P. asperata* and, consequently, it could supply information for the management of its genetic resources and contribute to the conservation of the species. Our results demonstrated that allozymes are genetic markers that could be practical in monitoring a defined but a limited set of genetic changes that may have occurred during the process of evolution in tree species. Although isozyme loci are relatively easy to screen and they have a particular value for estimating gene frequencies and heterozygosities, their limited numbers and levels of variation lower their marker value. Therefore, the on-going research on *P. asperata* based on a wide range of markers covering non-coding regions, including SSR, AFLPs, RAPDs and DNA sequence data, will enable us to obtain a more complete picture of the existing genetic structure of *P. asperata,* and to create specific conservation strategies for the restoration of the degraded populations.

Acknowledgements

The research was supported by the Program of "100 Distinguished Young Scientists" and "Knowledge Innovation Engineering" of the Chinese Academy of Sciences (No. KSCX2-SW-115), and by the Academy of Finland (No. 206577).

References

Aguirre-Plantier, E., Furnier, G.R. & Eguiarte, L.E. 2000. Low levels of genetic variation within and high levels of genetic differentiation among populations of species of Abies from southern Mexico and Guatemala. American Journal of Botany 87: 365–371.

Arntzen, J.W. 2001. Genetic variation in the Italian crested newt, Triturus carnifex, and the origin of a non-native population north of the Alps. Biodiversity Conservation 10: 971–987.

Billington, H.L. 1991. Effect of population size on genetic variation in a dioecious conifer. Conserva-

tion Biology 5: 115–119.

Collignon, A.M. & Favre, J.M. 2000. Contribution to the Postglacial History at the Western Margin of Picea abies Natural Area Using RAPD Markers. Annals Botany 85: 713–722.

— , Hervé Van de Sype & Favre, J.M. 2002. Geographical variation in random amplified polymorphic DNA and quantitative traits in Norway spruce. Canadian Journal of Forest Research 32: 266–282.

Cornuet, J.M. & Luikart, G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144: 2001–2014.

Dayanandan, S., Dole, J., Bawa, K. & Kesseli, R. 1999. Population structure delineated with microsatellite markers in fragmented populations of a tropical tree, Carapa guianensis (Meliaceae). Molecular Ecology 8: 1585–1592.

Endler, J. 1977. Geographic variation, speciation, and clines. Princeton University Press, Princeton.

Fang, J. 1996. The distribution pattern of Chinese natural vegetation and its climatologic and topographic interpretations. In: Fang, J. (ed.). Researches on hotspots of modern ecology. China Science and Technology Press, Beijing. p. 369–380.

Fuchs, E.J., Lobo, J.A. & Quesada, M. 2003. Effects of forest fragmentation and flowering phenology on the reproductive success and mating patterns of the tropical dry forest tree Pachira quinata. Conservation Biology 17: 149–157.

Gibson, N., Barker, P.C.J., Cullen, P.J. & Shapcott, A. 1995. Conifers of southern Australia. In: Enright, N.J. & Hill, R.S. (eds.). Ecology of the Southern Conifers. Melbourne University Press, Australia. p. 223–251.

Gitzendanner, M.A. & Soltis, P.S. 2000. Patterns of genetic variation in rare and widespread plant congeners. American Journal of Botany 87: 782–792.

Gomez, A., Gonzalez-Martinez, S.C., Collada, C., Climent, J. & Gil, L. 2003. Complex population genetic structure in the endemic Canary Island pine revealed using chloroplast microsatellite markers. Theoretical and Applied Genetics 107: 1123–1131.

Hamrick, J.L., Godt, M.J. & Sherman-Broyles, S.L. 1992. Factors influencing levels of genetic diversity in woody plant species. New Forest 6: 95–124.

Hawkins, B.J. & Sweet, G.B. 1989. Genetic variation in Rimu: an investigation using isozyme analysis. New Zealand Journal of Botany 27: 83–90.

Karron, J.D. 1987. A comparison of levels of genetic polymorphism and self-compatibility in geographically restricted and widespread plant congeners. Evolutionary Ecology 1: 47–58.

Kimura, M. & Crow, J. 1964. The number of alleles that can be maintained in a finite population. Genetics 49: 725–738.

— & Weiss, G.W. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics 49: 561–576.

Kraj, M. 2002. The estimation of genetic variation within and between Polish provenances of Norway spruce (Picea abies (L.) Karst.) on the basis of RAPD polymorphism. Electronic Journal of Polish Agricultural University, Forestry 5(2). [Online journal]. Available from: http://www.ejpau.media.pl/series/volume5/issue2/forestry/art-02.html.

Ledig, F.T. & Conkle, M.T. 1983. Gene diversity and genetic structure in a narrow endemic, Torrey pine (Pinus torreyana Parry ex Carr). Evolution 37: 79–85.

Liu, Z., Fang, J. & Piao, S. 2002. Geographical distribution of species in genera Abies, Picea, and Larix in China. Acta Geographica Sinica 57: 577–586.

Luikart, G. & Cornuet, J.M. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conservation Biology 12: 228–237.

— , Allendorf, F.W., Cornuet, J.M. & Sherwin, W.B. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. Journal of Heredity 89: 238–247.

Mosseler, A., Major, J.E. & Rajora, O.P. 2003. Old-growth red spruce forests as reservoirs of genetic diversity and reproductive fitness. Theoretical and Applied Genetics 106: 931–937.

Müller-Starck, G. 1995. Genetic variation in high elevated populations of Norway spruce (Picea abies (L.) Karst.) in Switzerland. Silvae Genetica 44: 356–362.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590.

Petit, R.J., Bahrman, N. & Baradat, P.H. 1995. Comparison of genetic differentiation in maritime pine (Pinus pinaster Ait.) estimated using isozyme, total protein and terpenic loci. Heredity 75: 382–389.

Prus-Glowacki, W., Stephan, B.R., Bujas, E., Alia,

R. & Marciniak, A. 2003. Genetic differentiation of autochthonous populations of Pinus sylvestris (Pinaceae) from the Iberian peninsula. Plant Systematics and Evolution 239: 55–66.

Rajora, O.P. 1999. Molecular biology in sustainable forest management. In: Espinel, S. & Ritter, E. (eds.). Proc Appl of Biotechnology to Forest Genetics, Biofor 99 Conference, 22–25 September, 1999, Vitoria-Gasteiz, Spain. p. 29–40.

— & Mosseler, A. 2001a. Molecular markers in sustainable management, conservation, and restoration of forest genetic resources. In: Müller-Starck, G. & Schubert, R. (eds.). Genetic response of forest systems to changing environmental conditions. Kluwer Academic Publishers, Volume 70. p. 187–201.

— & Mosseler, A. 2001b. Challenges and opportunities for conservation of forest genetic resources. Euphytica 118: 197–212.

Salvador, L., Alía, R., Agúndez, D. & Gil, L. 2000. Genetic variation and migration pathways of maritime pine (Pinus pinaster Ait) in the Iberian peninsula. Theoretical and Applied Genetics 100: 89–95.

Shapcott, A. 1991. Dispersal and establishment of Huon pine (Lagarostrobus franklinii). Papers and Proceedings of the Royal Society of Tasmania 125: 17–26.

Slatkin, M. 1987. Gene flow and geographic structure of natural populations. Science 236: 787–792.

Soltis, D.E., Haufler, C.H., Darrow, D.C. & Gastony, G.J. 1983. Starch Gel electrophoresis of ferns: a compilation of grinding buffers, Gel and electrode buffer, and staining schedules. American Fern Journal 73: 9–27.

Szmidt, A.Z. & Wang, X.R. 1993. Molecular systematics and genetic differentiation of Pinus sylvestris (L.) and P. densiflora (Sieb. et Zucc.). Theoretical and Applied Genetics 86: 159–165.

Thomas, P.E., Blinn, D.W. & Keim, P. 1994. A test of an allopatric speciation model for congeneric amphipods in an isolated aquatic ecosystem. Journal of the North American Benthological Society 13: 100–109.

Wang, H.S. 1992. Floristic geography. Science Press, Beijing, China.

Weeden, N.F. & Wendel, J.F. 1989. Genetics of plant Isozymes. In: Soltis, D.E. & Soltis, P.S. (eds.). Isozymes in plant biology. Dioscorides Press, Portland, Oregon. p. 5–72.

White, G.M., Boshier, D.H. & Powell, W. 1999. Genetic variation within a fragmented population of Swietenia humilis Zucc. Molecular Ecology 8: 1899–1909.

Workman, P.L. & Niswander, J.D. 1970. Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. American Journal of Human Genetics 22: 24–49.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16: 195–197.

— 1943. Isolation by distance. Genetics 28: 114–138.

— 1978. Evolution and genetics of populations. Vol. 4.Variability within and among populations. University of Chicago Press, Chicago.

Wu, Z. 1959. The distribution of major tree species in alpine forest area of western Sichuan and some proposals for regeneration report and planning of tree species for plantation. Scientia Silvae Sinicae 5: 465–478.

Wu, Z.Y. & Raven, P.H. 1994. Flora of China. Vol. 4. Science Press (Beijing, China) & Missouri Botanical Garden (St. Louis, USA).

Yeh, F.C. & Layton, C. 1979. The organization of genetic variability in central and marginal populations of lodgepole pine Pinus contorta ssp. latifolia. Canadian Journal of Genetics and Cytology 21: 487–503.

— , Cheliak, W.M., Dancik, B.P., Illingworth, K., Trust, D.C. & Pryhitka, B.A. 1985. Population differentiation in lodgepole pine, Pinus contorta spp. latifolia: a discriminant analysis of allozyme variation. Canadian Journal of Genetics and Cytology 27: 210–218.

— , Yang, R.C. & Boyle, T. 1997. POPGENE, version 1.32 edition. Software Microsoft window-based freeware for population genetic analysis. University of Alberta, Edmonton, Alta.

Zheng, Y.Q. & Ennos, R.A. 1999. Genetic variability and structure of natural and domesticated populations of Caribbean pine (Pinus caribaea Morelet). Theoretical and Applied Genetics 98: 765–771.

Total of 50 references