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Allozyme variation in natural populations of *Picea glehnii* in Hokkaido, Japan

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Current-year needles from individual trees were used to study the genetic diversity in 10 natural populations of *Picea glehnii* (Masters) in Hokkaido, Japan, by polyacrylamide gel electrophoresis. Data from 12 polymorphic loci encoding 33 alleles identified by nine enzyme systems were analysed. Mean number of alleles per locus, percentage polymorphic loci and observed and expected heterozygosities were 1.98, 75 per cent, 0.08 and 0.088, respectively. These genetic parameters varied considerably among populations. Diversity among the populations was small with a mean F_{ST} of 0.022 and genetic distance of 0.0017. However, a χ^2-test showed that allele frequencies were different ($P<0.05$) among the populations at 10 of the 12 loci; cluster and canonical discriminant analyses indicated that some of the populations were very different from others; and correlation analyses revealed significant relationships between some of the allele frequencies and longitude, latitude and altitude. Results suggest that genetic variation in *P. glehnii* is both geographically clinal and population-specific.

Keywords: allozyme variation, discriminant analysis, genetic diversity, *Picea glehnii*, population genetics.

Introduction

Picea glehnii (Masters) is one of only two native spruce species in Hokkaido, Japan, where it grows in many types of habitats over a wide range of distribution. However, outside Hokkaido, only one very small population of the species has been found in the Hayachine mountains of northern Honshu, whereas outside Japan, it grows in the southern Kuriles to about 147°E and southern Sakhalin to about 47°N (Horikawa, 1972). Mature trees of *P. glehnii* may reach 40 m in height and 1.5 m in diameter (d.b.h.; Sato, 1990). Being a very important forest tree species, it has been planted in large areas of Hokkaido in recent years (Matsuda, 1989). Tree improvement programmes, seed orchards and genetic conservation stands have been established for the species in Hokkaido. To manage and utilize the genetic resources of *P. glehnii* more effectively, genetic information about the species is urgently needed. To encourage genetic studies of the species, the Hokkaido Regional Breeding Office, Forest Tree Breeding Institute, Japan, has been organizing meetings on the genetics of spruce species in Hokkaido since 1993. However, only three related studies have been reported. Okada (1975) studied geographical variation in seedling height and bud opening phenology from 12 seed sources of *P. glehnii* in a common garden experiment. Two other studies examined the inheritance and linkage of isozymes in seed megagametophytes (Kubota *et al.*, 1993; Wang *et al.*, 1996). Here, for the first time, we report allozyme variation in 10 natural *P. glehnii* populations in Hokkaido.

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Materials and methods

Current-year needles were collected from at least 59 mature trees in each of 10 natural *P. glehnii* stands throughout Hokkaido in the late autumn and early winter of 1994 (Fig. 1). All stands were widely separated from each other, except stands Jozankei A and B, which were only about 2 km apart (Fig. 1). In each stand, efforts were made to collect needles from widely spaced large trees. Samples were put in

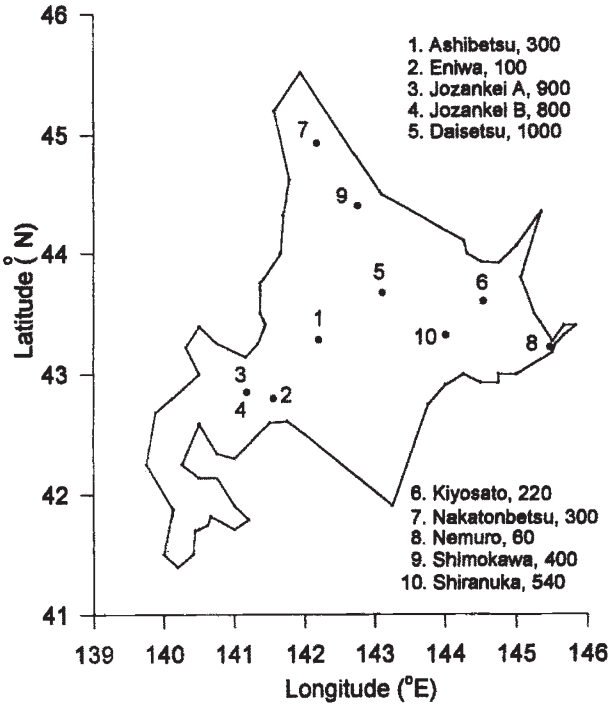

Fig. 1 Stand locations of *Picea glehnii* on a schematic map of Hokkaido. Numbers following stand names are altitudes (m).

plastic bags in ice containers, taken to the laboratory and then stored at −20°C until needed. Needle material preparation and polyacrylamide gel electrophoresis followed the methods of Tsumura *et al.* (1990). Designations of the loci and alleles followed those of Wang *et al.* (1996). Seed megagametophyte homogenates were loaded onto the same gels to verify that isozyme loci detected in the needles corresponded to those observed in the seed (Wang *et al.*, 1996). If the zones of activity overlapped, they were inferred to belong to the same loci, and the megagametophyte locus designations were used for the needles. The nine enzyme systems surveyed and the loci and alleles detected, scored and analysed are: 6-phosphogluconic dehydrogenase (*6pgd*^{a,b}), alanine aminopeptidase (*Aap-3*^{a,b}), diaphorase (*Dia-2*^{a,b}, *Dia-3*^{a,b}), esterase (*Est-1*^{a,b,c}, *Est-2*^{a,b,c}), fumarase (*Fm*^{a,b}), glycerate dehydrogenase (*G2dh*^{a,b,c}), leucine aminopeptidase (*Lap*^{a,b,c,d}), menadione reductase (*Mnr-2*^{a,b}) and shikimate dehydrogenase (*Shdh-1*^{a,b,c,d,e}, *Shdh-2*^{a,b,c}). Three enzymes (glucose-6-phosphate dehydrogenase, G6PD; glutamic-oxaloacetic transaminase, GOT; and sorbitol dehydrogenase, SODH) that stained well in seed gels were also stained in this study (Wang *et al.*, 1996). However, their stainings were too poor to interpret so that they were not scored.

Data were analysed using BIOSYS-1 (Swofford & Selander, 1989) to obtain the mean number of alleles per locus (A_p), percentage of polymorphic loci (P_p), *F*-statistics (Wright, 1965), genetic distance (Nei, 1978), observed and expected heterozygosities based on Hardy–Weinberg expectation (H_o, H_e), and cluster analysis based on genetic distance (Nei, 1978) using the unweighted pair-group method. Genetic diversity at the population level was measured by the means of A_p, P_p, H_o and H_e across all the populations. At the species level, these were obtained by analysing all the populations as one unit. Wang *et al.* (1996) used seeds from 38 trees to study the inheritance and linkage relationships of isozymes in *P. glehnii*. The genotypes of these 38 trees they observed were also analysed as one unit to obtain these four parameters.

Correlation analysis (SAS, 1988) was used to examine whether A_p, P_p, H_o, H_e and allele frequencies, except the least common allele at each locus, were related to geographical parameters (latitude, longitude and altitude) of the populations. PROC DISCRIM (discriminant procedure) with the CAN (canonical) option in SAS (SAS, 1988) was used to detect possible variation patterns of the populations. To do this, the genotypes of individual trees were transformed to allozyme profiles according to Yeh *et al.* (1985).

Results

Enzymes and their stainings

Except G2DH, the inheritance of all isozymes has been examined for *P. glehnii* using seed megagametophytes, and no significant linkages among the loci scored and analysed in this study were detected by Wang *et al.* (1996). However, the inheritance of G2DH has been demonstrated in other conifers (Cheliak & Pitel, 1984; Shiraishi, 1988; Na'iem *et al.*, 1989; Suyama *et al.*, 1992). All the loci, except *Est-2*, detected in the needles corresponded to those observed in seed megagametophytes (Wang *et al.*, 1996). However, *Aap-4* and *Dia-4*, detected in seed, did not show staining activities on needle gels. *Aap-1*, *Aap-2*, *Dia-1*, *Got-1*, *Got-2*, *Got-3* and *Mnr-1* did not stain consistently clearly enough for interpretation in needle tissue from all the populations and trees and were excluded from data analyses. G6PD, GOT and SODH were also stained too poorly to score in needles. Thus, in general, the number of enzymes and loci of some of the enzymes that stained well enough to score were fewer in needles than in seeds (Wang *et al.*, 1996). However,

the number of alleles observed at equivalent loci was very similar in both tissues of *P. glehnii*. Some of our poor stainings were caused by long storage time of the needles. Therefore, such storage should be avoided as much as possible for similar kinds of studies.

Allele frequencies and genetic variabilities

For the nine enzymes, 12 loci with 33 alleles could be scored consistently. At each locus, at least two alleles were observed in at least one population, and the most frequent allele was the same among all the populations. However, six private alleles were detected in four of the 10 populations (Table 1). A complete table of the allele frequencies is available on request.

Genetic variability measures varied greatly among the populations (Table 1). The Nemuro population had the lowest A_p, P_p and H_o values and the second smallest H_e value. The Eniwa population had much greater H_o and H_e than the rest and was the only population without a deficiency of heterozygotes. The χ^2-test indicated that the populations were different ($P<0.05$) in their allele frequencies at all the loci analysed, except at *Dia-2* ($P=0.477$) and at *Lap* ($P=0.062$). However, genetic distances were very small, ranging from 0.000 to 0.005 with a mean of 0.0017. F_{ST} values varied from 0.006 at *Dia-2* to 0.075 at *6pgd*, and overall, only 2.2 per cent of the variation was attributable to the differences among the populations. Complete tables for the χ^2-test, *F*-statistics and genetic distance are available on request.

The 10 populations are grouped into three clusters (Fig. 2). The Eniwa population is distinct, and the other populations form two clusters with four and five populations, respectively (Fig. 2). However, compared with the distributions of the populations (Fig. 1), these clusters do not indicate any clear patterns of geographical variation among the populations.

In canonical discriminant analysis, the first five of the nine possible discriminant functions are significant in separating the populations (Table 2). The first two of the five functions accounted for 57.6 per cent of the total variance, and 44 per cent (cumulative r^2) of this was caused by differences among the populations. This means that the first two functions account for more than 25 per cent (57.6 per cent × 44 per cent × 100 per cent) of the total intrapopulation variance. A scatter plot using the first two functions indicates that the 10 populations belong to three distinct groups: the Eniwa population; the Kiyosato population; and the rest (Fig. 3).

Table 1 Genetic variability in *Picea glehnii*

Population	A_p	P_p	H_o	H_e†	P_a
Ashibetsu	2.0	75.0	0.084	0.091	0
Eniwa	2.0	83.3	0.132	0.124	2
Jozankei A	2.0	83.3	0.078	0.080	1
Jozankei B	1.8	66.7	0.079	0.085	0
Daisetsu	2.3	83.3	0.078	0.083	2
Kiyosato	1.8	58.3	0.070	0.089	1
Nakatonbetsu	2.1	83.3	0.078	0.088	0
Nemuro	1.6	50.0	0.055	0.076	0
Shimokawa	2.3	100.0	0.087	0.092	0
Shiranuka	1.9	66.7	0.057	0.072	0
Mean‡	1.98	75.0	0.080	0.088	
Species§	2.8	100.0	0.081	0.089	
Seed¶	2.7	95.2	0.209	0.213	

A_p, number of alleles per locus; P_p, percentage polymorphic loci (criterion 0.99); H_o and H_e, observed and expected heterozygosity; P_a, private allele.
†Unbiased estimate (Nei, 1978).
‡Mean across all the populations.
§Analysis was performed by treating all the populations as one unit.
¶Genotypes were from seed megagametophytes of 38 trees (Wang *et al.*, 1996).

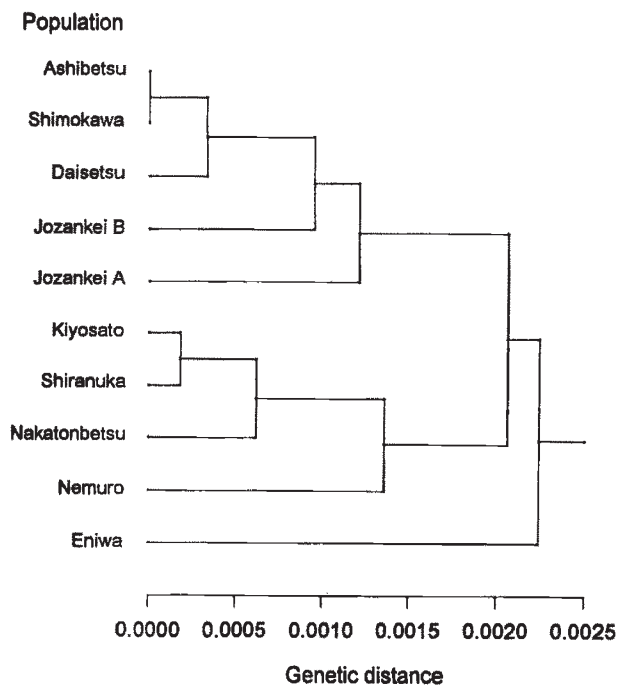

Fig. 2 Dendrogram for *Picea glehnii* populations based on genetic distance (Nei 1978).

Table 2 Statistics for the five significant canonical discriminant functions (CDFs)

CDF	Percentage of variance	r^2	Cr^2	d.f.	$P>F$
1	34.71	0.2558	0.2558	189	0.0001
2	22.90	0.1849	0.4407	160	0.0001
3	11.19	0.0997	0.5404	133	0.0001
4	8.95	0.0814	0.6218	108	0.0018
5	8.28	0.0758	0.6976	85	0.0284

r^2, squared canonical correlation; Cr^2, cumulative r^2.

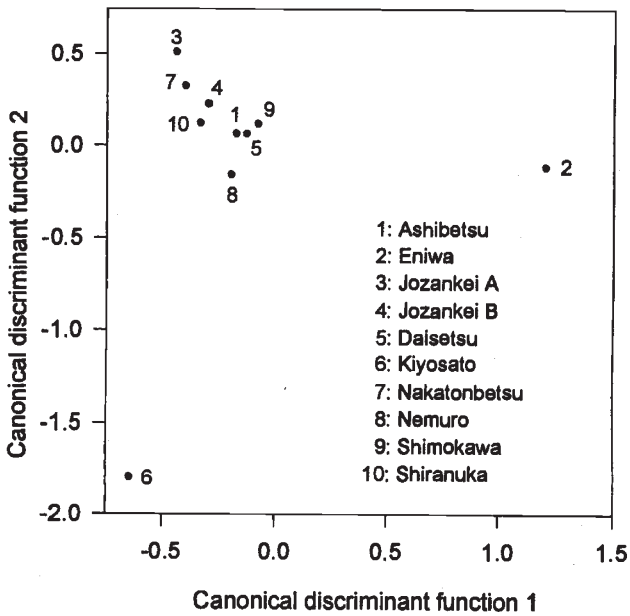

Fig. 3 Scatter plot of *Picea glehnii* populations on the axes of the first two canonical discriminant functions.

As in the cluster analysis, these groups do not indicate any clear patterns of geographical variation among the 10 populations.

Correlation analysis indicated that longitude, latitude and altitude were not significantly ($P<0.05$) correlated among themselves or with A_p, P_p, H_o and H_e. However, six (50 per cent) of the 12 loci had significant correlations with the geographical variables. Specifically, they were allele frequencies at *Lap*^{a} ($r=0.698$, $P=0.025$), *Shdh-1*^{b} ($r=0.861$, $P=0.001$) and *Shdh-2*^{a} ($r=0.708$, $P=0.021$) with longitude, at *Fm*^{a} ($r=0.644$, $P=0.044$), *G2dh*^{a} ($r=0.648$, $P=0.042$), *Shdh-1*^{a} ($r=-0.686$, $P=0.028$) and *Shdh-1*^{c} ($r=0.634$, $P=0.048$) with latitude, and at *Dia-3*^{a} ($r=0.802$, $P=0.005$) and *Shdh-1*^{a} ($r=0.698$, $P=0.024$) with altitude. The total number of pairwise correlations was 63 for allele frequencies, and the number of significant correlations found was nine, which is about three times as many as would be expected at the 0.05 significance level and therefore cannot be a result of chance alone. A complete table of the correlation coefficients is available on request.

Discussion

Heterozygosities observed in needles and in seeds

A_p and P_p observed in seeds are very similar to those at the species level in needles (Table 1). However, the H_o and H_e at either the population or species levels obtained in needles are much lower than those in seeds (Table 1). Even when only the loci in common between both data sets are compared, H_o and H_e are 0.156 and 0.174, respectively, from seeds, which are still much greater than from needle tissue (0.07 and 0.079 at the species level, and 0.07 and 0.077 at the population level). Because both seed megagametophytes and needles represent the genetic make-up of maternal trees, they should give similar results, but they did not. The possible reason for the lower heterozygosities observed in the needles compared with the megagametophytes might be because the seeds used by Wang *et al.* (1996) were obtained from plus trees that might have higher heterozygosities than others. Allozyme heterozygosity has been found to be positively correlated with growth (Bush & Smouse, 1992) and fitness (Hertel & Kohlstock, 1994) in many tree species. Clearly, this hypothesis deserves further studies.

Genetic diversity at population and species levels

The average A_p, P_p and H_e were found to be 2.38, 71.1 per cent and 0.169, respectively, at the species level and 1.83, 53.4 per cent and 0.151, respectively, at the population level in gymnosperms (Hamrick *et al.*, 1992). Thus, at both levels observed in needles, *P. glehnii* had greater A_p and P_p but smaller H_e (Table 1) than the above. However, all these diversity measures obtained in seeds (Table 1) were higher than the above. Overall, the genetic diversity of *P. glehnii* is compatible with other conifers.

Genetic diversity among populations

The genetic diversity among the *P. glehnii* populations (mean $F_{ST}=2.2$ per cent) is lower than the mean (7.3 per cent) of other gymnosperms (Hamrick *et al.*, 1992). The low genetic diversity

among populations in *P. glehnii* might be caused by long life span, wide distribution, outcrossing mating system and widely dispersed seeds (Hamrick *et al.*, 1992). Nevertheless, four of the 10 populations had private alleles; the χ^2-test indicated that the populations were significantly different in allele frequencies at 10 of the 12 loci analysed; and five canonical discriminant functions could separate them significantly. Therefore, a small but significant component of the diversity resides among the populations of *P. glehnii*, as observed in many other conifers (El-Kassaby, 1991).

The Nemuro population has the smallest A_p, P_p, H_o and the smallest number of variable loci among the 10 populations. It seems to have the lowest within-population genetic diversity. This population is the only one growing in a lowland habitat. Compared with other highland habitats, the harsh environmental conditions (high water level, cold soil and low nutrient content) in such a habitat (Payandeh, 1973) might have imposed strong selection pressure, resulting in reduced genetic diversity in the Nemuro population. However, according to the H_o and H_e values (Table 1), the Eniwa population seems to be the most different from the others. This is also confirmed by the results from cluster and discriminant analyses (Figs 2 and 3). Moreover, the latter analysis also indicates that the Kiyosato population is very different from the others and from Eniwa (Fig. 3). However, there are no plausible ecological explanations as to why these two populations could be more different from each other and from the other populations.

Nevertheless, the above results indicate that there are population-specific variations in *P. glehnii*. Such variation could be caused by founder effects, mutation, genetic drift (Allendorf & Phelps, 1981) and/or balancing selection for microgeographical differentiation (Hamrick & Allard, 1972), together with limited gene exchange among *P. glehnii* populations owing to isolation by the mountains. No matter what the exact reasons may be, such a population-specific variation might have local adaptive significance.

On the other hand, Hokkaido is mountainous with a total area of 78 510 km^2. A central mountain range divides it into two parts: east and west. In winter, the north-west is characterized by severe cold and heavy snowfall. In summer, tropical air masses result in a warm climate in the south-west, while other parts are under the influence of subpolar air masses (Nakamura *et al.*, 1986). Regionally, mean monthly temperature varies from -2 to -8°C in January and 14 to 19°C in July; and annual precipitation ranges from 600 to 2000 mm (Igarashi, 1994). In such a variable climate, therefore, it is not surprising that there was significant geographical variation in allele frequencies at some loci among the *P. glehnii* populations. Generally, different alleles are correlated with different geographical parameters except *Shdh-1*^{a}. However, the frequency of *Shdh-1*^{a} is negatively associated with latitude, but positively associated with altitude. Therefore, it is possible that these geographical parameters represent different ecological gradients that have selected different alleles or the same alleles in different ways in *P. glehnii* populations. Even if allozymes are neutral, natural selection can affect them indirectly if they are linked to genes associated with fitness. In other conifers, allozyme variations have also been found to be associated with ecological gradients on regional or small geographical scales (Mitton *et al.*, 1977, 1980; Bergmann, 1978; Yeh & O'Malley, 1980; El-Kassaby & Sziklai, 1982; Hamrick *et al.*, 1989; Schuster *et al.*, 1989; Aguinagalde & Bueno, 1994). Differences in bud opening phenology between east and west seed sources and among populations within each source have been observed in *P. glehnii* (Okada, 1975).

Overall, the results of this study indicate that there is geographical variation among *P. glehnii* populations. Within such a general pattern, there is also population-specific variation. This genetic structure has also been described for *Thuja orientalis* (Xie *et al.*, 1992). Therefore, it is suggested that genetic conservation, tree improvement programmes and seed use in reafforestations should emphasize local sources. However, germplasm collections should cover the whole range to assure adequate sampling of the genome of *P. glehnii*.

In this study, it is clear that different genetic parameters and analysis methods can give similar and/or different variation patterns among populations. Results obtained from them complement for each other. Therefore, they should be used together to examine genetic diversities among populations in similar kinds of studies.

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