FOREST GENETICS 4(1):43–50, 1997

# GENETIC VARIATION AMONG NATURAL *ABIES SACHALINENSIS* POPULATIONS IN RELATION TO ENVIRONMENTAL GRADIENTS IN HOKKAIDO, JAPAN

**Kazutoshi Nagasaka**[1], **Zhang M. Wang**[2] **& Kyoko Tanaka**

Forest Genetics Laboratory, Hokkaido Research Center, Forestry and Forest Products Research Institute, Sapporo, Japan
[1] Reprint request to present address: Genetic Analysis Laboratory, Forestry and Forest Products Research Institute, Matsunosato 1, Kukizaki, Ibaraki 305, Japan, and corresponding author
[2] Present address: Department of Biology, McGill University, 1205 Docteur Penfield avenue, Montreal, PQ, Canada, H3A 1B1

*Received October 28, 1996; accepted April 14, 1997*

## ABSTRACT

We studied genetic variation among 18 natural populations of *Abies sachalinensis* (Masters) in Hokkaido, Japan by polyacrylamide gel electrophoresis. Data from four polymorphic loci with 15 alleles in three enzyme systems were analyzed. Mean number ($A_p$) and mean effective number ($N_e$) of alleles per locus, percent polymorphic loci, and observed and expected heterozygosities were 3.04, 1.19, 77.78% (0.99 criterion), 0.143 and 0.157, respectively. Diversity among the populations was small with a mean $F_{st}$ 0.015 and genetic distance 0.0017. However, $x^2$-test showed that allele frequencies were different ($p$<0.05) at two of the four polymorphic loci. $H_e$, $N_e$, cluster and canonical discriminant analyses detected an east-west variation pattern among the populations with the same exceptional populations to this cline. These exceptions were considered population-specific genetic variation. Multiple regression analyses revealed that frequencies of nine of the 15 alleles were significantly related with some of the geographic and climatic parameters studied. Such variations related to population origins and environmental gradients are in congruence with previous studies in ecophysiological and morphological traits for the same species in the same area.

**Key words:** *Abies sachalinensis*, fir, conifer, allozyme variation, genetic diversity, geographical gradients

## INTRODUCTION

Isozyme eletrophoresis is a powerful tool for forest geneticists to characterize genetic structure of populations. Unlike quantitative traits which are subject to environmental effects, data from isozyme analyses provide direct measures of genetic variation within and among populations (BROWN & MORAN 1981). Such information is basic in developing strategies for genetic resource management, conservation and tree improvement programs (WICKNESWARI & NORWATI 1993).

*Abies sachalinensis* (Masters), which is also called Todo fir, Saghalin fir, Saghalien fir and Sakhalin fir, is the most important and commonly planted native tree species for timber production in Hokkaido, Japan. Here, it grows in all kinds of habitats from sea level up to 1650m in altitude. Outside Hokkaido, it grows in the southern Kuriles and Sakhalin island (SATO 1990). In recent years, tree improvement programs, seed orchards and genetic conservation stands have been established for it in Hokkaido. Variation patterns in the species have been found to be associated with geographic and environmental gradients based on ecophysiological and morphological traits (HATAKEYAMA 1983A,B; KURAHASHI & HAMAYA 1983; OKADA 1983; EIGA 1984). However, nobody has studied such patterns using genetic markers, such as isozymes. Therefore, we examined the allozyme variation within and among 18 natural *A. sachalinensis* populations in Hokkaido.

## MATERIALS AND METHODS

Current year needles were collected from mature trees in 18 natural *A. sachalinensis* stands throughout Hokkaido in the late autumn, early winter and early spring between autumn 1995 and spring 1996 (Fig. 1, Table 1). Longitudes and latitudes of the populations are not given in Table 1, but they can be easily read out in Fig. 1. The populations were numbered from 1 to 18 according to their longitudes from the west to the east. Effort was made to collect needles from dominant and widely-spaced trees in the stands. Samples were put in plastic bags, kept in ice containers, taken to the labora

**Table 1. Stand information for *Abies sachalinensis* populations. Alt: altitude; WI: warmth index; Feb, Aug, Mean, Min, and Max: monthly mean temperature for February and August, annual mean temperature, annual mean minimum and maximum temperatures, respectively; Rain and Snow: mean annual precipitation and maximum snow depth; and N: number of trees analysed per population.**

| Population | N | Alt (m) | WI | Feb (°C) | Aug (°C) | Mean (°C) | Min (°C) | Max (°C) | Rain (mm) | Snow (cm) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 Hiyama | 58 | 400 | 65 | −2 | 22 | 8 | 4 | 12.5 | 1400 | 80 |
| 2 Kikonai | 72 | 100 | 65 | −2.3 | 22 | 8.5 | 5.5 | 13 | 1600 | 60 |
| 3 Tomari | 89 | 500 | 60 | −2.5 | 21 | 8 | 4 | 12.5 | 1300 | 140 |
| 4 Kyogoku | 49 | 900 | 65 | −6 | 20 | 6 | 2 | 11 | 1400 | 130 |
| 5 Jozankei Oku | 61 | 750 | 50 | −6 | 20 | 6 | 2 | 11 | 1600 | 130 |
| 6 Jozankei Haide | 114 | 800 | 50 | −6 | 20 | 6 | 2 | 11 | 1400 | 120 |
| 7 Tomakomai | 105 | 300 | 50 | −6 | 20 | 6 | 2 | 11 | 1300 | 110 |
| 8 Nakatonbetsu | 100 | 200 | 50 | −10 | 18.5 | 5 | −0.5 | 10 | 1500 | 150 |
| 9 Ashibetsu | 86 | 300 | 65 | −7.5 | 20 | 6 | 1 | 12 | 1200 | 100 |
| 10 Furenai | 110 | 700 | 65 | −6.8 | 20 | 7 | 0.8 | 13 | 1200 | 60 |
| 11 Hidaka Nissho | 74 | 750 | 40 | −7 | 19.5 | 6 | 0 | 11 | 1200 | 100 |
| 12 Hidaka Uenzaru | 104 | 1100 | 35 | −7 | 19.5 | 6.5 | 0.5 | 12 | 1200 | 100 |
| 13 Shimokawa | 100 | 400 | 50 | −10 | 19 | 5 | 0 | 10 | 1000 | 100 |
| 14 Daisetsu | 112 | 1000 | 25 | −10 | 18.5 | 4 | −0.5 | 11 | 950 | 100 |
| 15 Saroma | 74 | 200 | 50 | −8.5 | 18 | 5 | 0 | 11 | 800 | 75 |
| 16 Shiranuka | 108 | 540 | 50 | −9.5 | 18 | 4.5 | −2 | 12 | 1000 | 100 |
| 17 Kiyosato | 102 | 220 | 40 | −9 | 18 | 4.5 | −0.5 | 12 | 1000 | 100 |
| 18 Nemuro | 115 | 20 | 50 | −6 | 17 | 5 | 2 | 10 | 1100 | 50 |

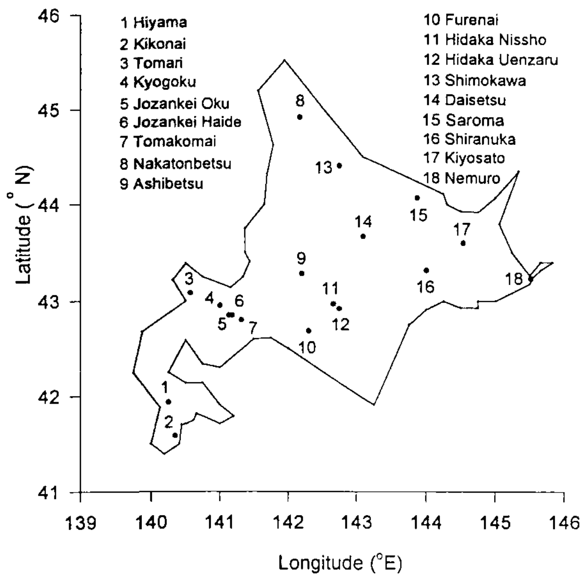

Figure 1. Population locations of *Abies sachalinensis* on a schematic map of Hokkaido, Japan.

tory and stored at −20°C until use. Polyacrylamide gel electrophoresis procedures and designations of the locus and alleles followed NAGASAKA and KOONO (1990) and TOMARU *et al.* (1994). We chose and assayed 3 enzyme systems (glutamate oxaloacetate transaminase, GOT; leucine aminopeptidase, LAP; and shikimate dehydrogenase, SHDH) because they were easy to stain. The genetic control of the isozymes has been demonstrated by NAGASAKA and KOONO (1990). If there was more than one zone stained for an enzyme, the slowest migrating one was numbered as '1', and the next as '2', etc. For a multiple allele locus, the slowest migrating one was designated as 'a', the next as 'b', and so on. Three loci were detected for GOT, but the slowest migrating isozyme zone (*Got–1*) was not stained consistently enough for all the samples and thus not scored.

We used BIOSYS-1 (SWOFFORD & SELANDER 1989) to obtain the mean number of alleles per locus ($A_p$), percent of polymorphic loci ($P_p$), $F$-statistics (WRIGHT 1965), $\chi^2$-test, genetic distance (NEI 1978), observed heterozygosity ($H_o$) and expected one which is based on Hardy-Weinberg expectation ($H_e$), and cluster analysis based on genetic distance (NEI 1978) with unweighted pair-group method. Effective number of alleles per locus ($N_e$) was also calculated. The genotypes of individual trees were transformed to allozyme profile data according to YEH *et al.* (1985). This data was analysed by PROC DISCRIM (discriminant procedure) with CAN (canonical) option in SAS (SAS 1990), and the scores of populations on the first two significant functions were plotted to detect possible variation patterns among the populations.

For each population, values of the following climatic variables (Table 1) were estimated on contour maps:

warmth index (Society of Forest Environment 1972); mean monthly temperatures in February and August; mean annual, minimum and maximum temperatures; mean annual precipitation and maximum snow depth (METEOROLOGICAL SOCIETY OF JAPAN 1982). Warmth index was the annual summation of the monthly mean temperatures above 5°C. Correlation analysis showed that 54.5% of the 55 correlations among these climatic and geographic (latitude, longitude, and altitude) variables and 26.4% of the 121 correlations between these variables and allele frequencies were significant ($P<0.05$). Therefore, to avoid declaring significance for the same underlying ecological factors represented by the correlated variables, a multiple regression with SELECTION = STEPWISE and SLSTAY = 0.05 (SAS 1990) was used to examine whether allele frequencies, except the least common allele at each locus, were related to the geographic and climatic variables of the populations. This method has the potential to detect the most important independent variable(s) to account for variation in a given dependent variable.

## RESULTS

Four loci, *Got–2*, *Got–3*, *Lap* and *Shdh* with 2, 3, 7 and 3 alleles, respectively, were detected, scored and analysed. All the populations shared the most frequent allele at each locus (Table 2).

Genetic variability measures vary among the populations (Table 3). The Kikonai population has the smallest $A_p$ but the largest $H_e$ and $N_e$; the Hiyama population is distinct for its low $H_o$; and the populations Daisetsu and Tomakomai have $P_p$ 100%, and the rest, 75%. It is clear that the 18 populations can be divided into two groups: the western group (populations 1 – 9) had $H_e$ less than 0.17 except Kikonai, and the eastern group (populations 10 – 18) had $H_e$ greater than 0.17 except Shimokawa and Saroma. The $N_e$ shows the same pattern if $N_e$=1.2 is used to separate the populations (Table 3). $A_p$ ranged from 2.5 to 3.0 for western and 3.0 to 3.5 for the eastern populations. Genetic distances were very small, ranging from 0.000 to 0.007 with a mean of 0.0017 (A complete table for genetic distances is available upon request). The $F$-statistics indicated that only about 1.5% of the variation was due to differences among the populations, but the $x^2$-test showed that populations were different ($P<0.001$) in their allele frequencies at two (*Lap* and *Shdh*) of the four polymorphic loci (Table 4).

Multiple regression analyses revealed that allele frequencies of nine of the 15 alleles were significantly associated with some of the geographic and climatic parameters (Table 5). Latitude alone accounted for 38.3% of the variance in $Lap^C$ frequencies. For the other eight alleles, from 16.2% to 47.2% of the variances could be accounted for by an individual climatic parameter (Table 5), and on average, one parameter could account for more than 30% of the variance in frequencies of one allele. Because there were significant correlations among some of the geographic and climatic variables (complete results not shown), however, the STEPWISE multiple regression, taking correlation among independent variables into account, only selects the most important significant regressor(s) for a dependent variable – frequencies of an allele, other parameters not included in the reported models for some of the alleles might also have effects on the allele frequency variations (Table 5). For example, latitude was significantly correlated with annual mean and annual mean maximum temperatures, mean annual precipitation (Table 5), monthly mean temperatures of February ($R$ = –0.82, $p$ < 0.001) and August ($R$ = –0.72, $p$ < 0.001), and annual mean minimum temperature ($R$ = –0.72, $p$ < 0.001), therefore, the significant effects of latitude on $Lap^c$ may be partially associated with temperature and rainfall.

The 18 populations grouped into three clusters on the dendrogram based on genetic distances (Fig. 2). The Kikonai population is distinct as one cluster, and the other two clusters consist of six and 11 populations each. The latter two clusters indicate a geographic variation pattern related to longitudes: the western populations numbered from one to ten, except Kikonai, are in one cluster; and the eastern ones are in another. However, two eastern populations – Shimokawa and Saroma were exceptions since they are clustered in the western group.

In canonical discriminant analysis, the first three of the 11 possible functions could separate the populations significantly ($P<0.05$; Table 6). The first two accounted for about 59% of the total variance. A scatter plot (Fig. 3) based on them shows that function one separates the Kikonai population from the rest, and function two separates the rest. On function two, there is a general geographic variation pattern among the populations: discriminant function scores increase from the western to the eastern populations. The outstanding exception to this pattern is that a western population, Saroma, has the smallest score on this function, and therefore, it did not fit into the geographic variation pattern for most of the other populations.

## DISCUSSION

The proportion of genetic diversity among the *A. sachalinensis* populations is 1.5%. However, as observed in many other conifers (EL-KASSABY 1991), the variation among the populations is small but signifi-

**Table 2. Allele frequencies in populations of *Abies sachalinensis* except the least common allele at each locus. See Table 1 for populations names.**

| Locus | Population | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| *Got-2* | | | | | | | | | |
| a | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.995 | 1.000 | 1.000 |
| *Got-3* | | | | | | | | | |
| b | 0.931 | 0.917 | 0.921 | 0.888 | 0.934 | 0.961 | 0.962 | 0.940 | 0.936 |
| c | 0.069 | 0.083 | 0.079 | 0.112 | 0.066 | 0.039 | 0.038 | 0.060 | 0.064 |
| *Lap* | | | | | | | | | |
| b | 0.000 | 0.000 | 0.000 | 0.000 | 0.008 | 0.000 | 0.000 | 0.000 | 0.000 |
| c | 0.052 | 0.035 | 0.096 | 0.031 | 0.066 | 0.057 | 0.029 | 0.115 | 0.087 |
| d | 0.052 | 0.035 | 0.107 | 0.102 | 0.066 | 0.145 | 0.133 | 0.065 | 0.105 |
| e | 0.784 | 0.701 | 0.753 | 0.837 | 0.820 | 0.776 | 0.729 | 0.720 | 0.762 |
| f | 0.095 | 0.132 | 0.017 | 0.020 | 0.008 | 0.013 | 0.038 | 0.030 | 0.017 |
| g | 0.017 | 0.097 | 0.028 | 0.010 | 0.033 | 0.009 | 0.071 | 0.060 | 0.029 |
| *Shdh* | | | | | | | | | |
| b | 0.983 | 0.917 | 0.966 | 0.939 | 0.959 | 0.961 | 0.981 | 0.975 | 0.983 |
| c | 0.009 | 0.000 | 0.028 | 0.031 | 0.033 | 0.022 | 0.014 | 0.025 | 0.012 |

| Locus | Population | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| Got-2 | | | | | | | | | |
| a | 1.000 | 1.000 | 1.000 | 1.000 | 0.991 | 1.000 | 1.000 | 1.000 | 1.000 |
| *Got-3* | | | | | | | | | |
| b | 0.850 | 0.932 | 0.923 | 0.915 | 0.924 | 0.938 | 0.944 | 0.936 | 0.917 |
| c | 0.141 | 0.061 | 0.072 | 0.075 | 0.063 | 0.063 | 0.051 | 0.059 | 0.078 |
| *Lap* | | | | | | | | | |
| b | 0.014 | 0.020 | 0.005 | 0.010 | 0.045 | 0.000 | 0.019 | 0.035 | 0.013 |
| c | 0.077 | 0.074 | 0.063 | 0.080 | 0.121 | 0.070 | 0.120 | 0.079 | 0.087 |
| d | 0.091 | 0.169 | 0.188 | 0.095 | 0.143 | 0.078 | 0.157 | 0.173 | 0.135 |
| e | 0.768 | 0.689 | 0.668 | 0.775 | 0.652 | 0.789 | 0.657 | 0.658 | 0.717 |
| f | 0.023 | 0.041 | 0.053 | 0.025 | 0.004 | 0.031 | 0.014 | 0.040 | 0.035 |
| g | 0.027 | 0.000 | 0.014 | 0.015 | 0.036 | 0.023 | 0.028 | 0.015 | 0.013 |
| *Shdh-b* | | | | | | | | | |
| b | 0.982 | 0.966 | 0.976 | 0.975 | 0.978 | 0.984 | 0.958 | 0.975 | 0.957 |
| c | 0.014 | 0.034 | 0.024 | 0.010 | 0.013 | 0.008 | 0.028 | 0.015 | 0.022 |

cant. It is comparable with that in *Abies Lasiocarpa* (1.3%, SHEA 1990), but it is lower than those observed in *Abies mariesii* (2.6%, SUYAMA *et al.* 1992), *Abies cephalonica* (4.8%, FADY & CONKLE 1993), and *Abies alba* (11%, VICARIO *et al.* 1995). On average, the genetic diversity among populations of *Abies* species has been found to be 6.3 % (HAMRICK *et al.* 1992).

Results in $H_e$, $N_e$, cluster and discriminant analyses all indicated that almost the same three populations (Kikonai, Shimokawa and Saroma) did not fit into the geographic variation patterns revealed by the other 15 populations. This suggests that there are population-specific variations in *A. sachalinensis*. Population Kikonai is at the southernmost limit in the distribution range of *A. sachalinensis*. It is small with only 0.9 ha, and the number of *A. sachalinensis* trees decreased from 343 in 1973 to 299 in 1991 due to natural mortality. Harsh environmental conditions in this marginal population might have imposed great selection pressure and resulted in the population being most different from others. The Difference between marginal and other populations has also been observed in *Abies balsamea* (NEALE & ADAMS 1985) and in *Pinus contorta* (YEH & LAYTON 1979). Shimokawa (20 ha) and Saroma (39.8 ha) populations are fairly large in size, and we cannot find any plausible ecological explanations as to why they were more different from others. Interestingly, Saroma stand grows extremely

**Table 3.** Genetic variability at 4 loci in 18 populations of *Abies sachalinensis*. $A_p$: mean number of alleles per locus. $N_e$: effective number of allele. $P_p$: percentage of loci polymorphic (0.99 criterion). $H_o$ and $H_e$: observed and Hardy-Weinberg expected mean heterozygosities.

| Population | $N_e$ | $A_p$ | $P_p$ | $H_o$ | $H_e$ |
|---|---|---|---|---|---|
| 1 Hiyama | 1.15 | 2.8 | 75.0 | 0.082 | 0.133 |
| 2 Kikonai | 1.24 | 2.5 | 75.0 | 0.163 | 0.196 |
| 3 Tomari | 1.18 | 2.8 | 75.0 | 0.143 | 0.156 |
| 4 Kyogoku | 1.18 | 2.8 | 75.0 | 0.143 | 0.151 |
| 5 Jozankei Oku | 1.15 | 3.0 | 75.0 | 0.111 | 0.130 |
| 6 Jozankei Haide | 1.15 | 2.8 | 75.0 | 0.116 | 0.131 |
| 7 Tomakomai | 1.16 | 3.0 | 100.0 | 0.138 | 0.141 |
| 8 Nakatonbestu | 1.18 | 2.8 | 75.0 | 0.132 | 0.155 |
| 9 Ashibetsu | 1.16 | 2.8 | 75.0 | 0.134 | 0.139 |
| 10 Furenai | 1.21 | 3.3 | 75.0 | 0.166 | 0.172 |
| 11 Hidaka Nissho | 1.21 | 3.0 | 75.0 | 0.149 | 0.170 |
| 12 Hidaka Uenzaru | 1.21 | 3.3 | 75.0 | 0.168 | 0.175 |
| 13 Shimokawa | 1.17 | 3.3 | 75.0 | 0.147 | 0.147 |
| 14 Daisetsu | 1.23 | 3.5 | 100.0 | 0.181 | 0.185 |
| 15 Saroma | 1.15 | 3.0 | 75.0 | 0.117 | 0.128 |
| 16 Shiranuka | 1.22 | 3.5 | 75.0 | 0.160 | 0.178 |
| 17 Kiyosato | 1.21 | 3.3 | 75.0 | 0.183 | 0.174 |
| 18 Nemuro | 1.21 | 3.3 | 75.0 | 0.143 | 0.174 |
| **Mean** | **1.19** | **3.0** | **77.8** | **0.143** | **0.157** |

**Table 4.** Summary of *F*-statistics and $\chi^2$-test.

| Locus | Allele | F-statistics | | | $\chi^2$-test | | |
|---|---|---|---|---|---|---|---|
| | | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ | $\chi^2$ | d.f. | *P* |
| *Got-2* | 2 | -0.008 | -0.001 | 0.007 | 21.479 | 17 | 0.205 |
| *Got-3* | 3 | 0.006 | 0.015 | 0.009 | 46.149 | 34 | 0.079 |
| *Lap* | 7 | 0.127 | 0.141 | 0.017 | 302.243 | 102 | 0.000 |
| *Shdh* | 3 | 0.036 | 0.047 | 0.011 | 86.727 | 34 | 0.000 |
| **Mean** | | **0.091** | **0.105** | **0.015** | | | |
| **Total** | | | | | **456.59** | **187** | **0.000** |

well and has been chosen as the first grade seed stand and used seed collection by the Regional Forest Office. Nevertheless, such population-specific variation could be due to founder effects, mutation, genetic drift (ALLENDORF & PHELPS 1981), balancing selection for microgeographic differentiation (HAMRICK & ALLARD 1972), and/or reduced gene flow among the populations due to isolations by the mountains in the study area. No matter what the exact reasons may be, such variation might have local adaptation significance (WANG & NAGASAKA 1997).

On the other hand, multivariate analyses (cluster and discriminant analyses) detected a significant east-west geographic variation pattern among the *A. sachalinensis* populations, and univariate analyses showed that, in general, the western populations had lower genetic variabilities ($A_p$, $H_e$ and $N_e$) than the eastern populations. This east-west variation pattern may be due to the regional environmental variations. A central mountain range divides Hokkaido into east and west, resulting in regional climatic differences. In summer, tropical air masses result in warm climate in the southwest, while other parts of Hokkaido are under the influence of subpolar air masses (NAKAMURA *et al.* 1986). Phytogeographically, Hokkaido is divided into three major part – southwestern, central and eastern parts (TATEWAKI 1958).

Geographic allozyme variation patterns have also been found in many other conifers at small geographic scales (See WANG & NAGASAKA 1997), and such clines

**Table 5. Summary of multiple regressions which had independent variables with significant partial $r^2$. See Table 1 for abbreviations of the independent variables.**

| Dependent variable | Independent variable | Regression coefficient | $r^2$ | |
|---|---|---|---|---|
| | | | value | $p$ |
| G*ot–2*$^{a}$ | WI | 0.0001 | 0.280 | 0.024 |
| G*ot–3*$^{d}$ | WI | 0.0010 | 0.218 | 0.050 |
| *Lap*$^{b}$ | WI | –0.0008 | 0.472 | 0.002 |
| *Lap*$^{c}$ | Latitude | 0.0218 | 0.383 | 0.006 |
| *Lap*$^{d}$ | WI | –0.0026 | 0.446 | 0.002 |
| *Lap*$^{c}$ | WI | 0.0051 | 0.406 | 0.004 |
| | Max | –0.0335 | 0.172 | 0.026 |
| | Altitude | 0.0007 | 0.147 | 0.016 |
| *Lap*$^{f}$ | Mean | 0.0141 | 0.383 | 0.006 |
| | Snow | –0.0005 | 0.162 | 0.035 |
| S*hdh*$^{b}$ | Rain | –0.0037 | 0.242 | 0.038 |
| S*hdh*$^{c}$ | Snow | 0.0002 | 0.352 | 0.009 |

[1/] Significant correlations among the independent variables maintained in the regressions: Mean with Max ($R = 0.66, p = 0.003$), with WI ($R = 0.645, p = 0.004$) and with Rain ($R = 0.629, p = 0.005$), and Latitude with Max ($R = -0.67, p = 0.002$), with Mean ($R = -0.755, p < 0.001$) and with Rain ($R = -0.49, p < 0.041$).

**Table 6. Statistics for the three significant canonical discriminant functions (CDF). $r^2$: squared canonical correlation. $cr^2$: cumulative $r^2$.**

| CDF | Variance | $r^2$ | $cr^2$ | d.f. | $P$>F |
|---|---|---|---|---|---|
| 1 | 41.02 | 0.098 | 0.098 | 187 | 0.0001 |
| 2 | 17.96 | 0.045 | 0.143 | 160 | 0.0001 |
| 3 | 11.42 | 0.029 | 0.172 | 135 | 0.0158 |

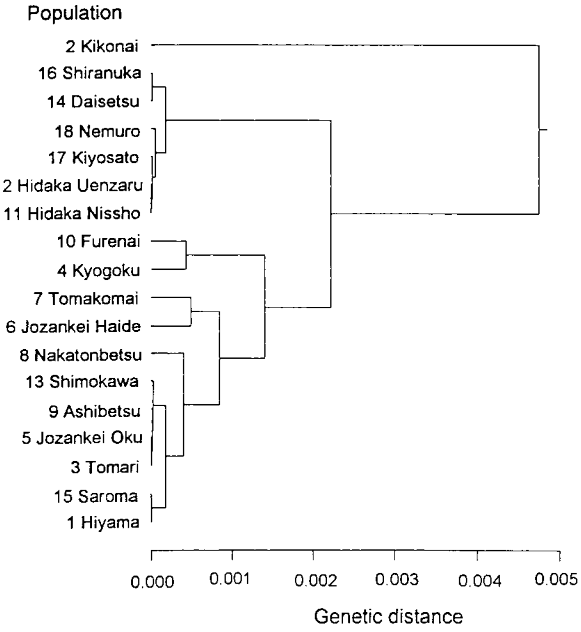

Figure 2. Dendrogram for *Abies sachalinensis* populations based on genetic distance.

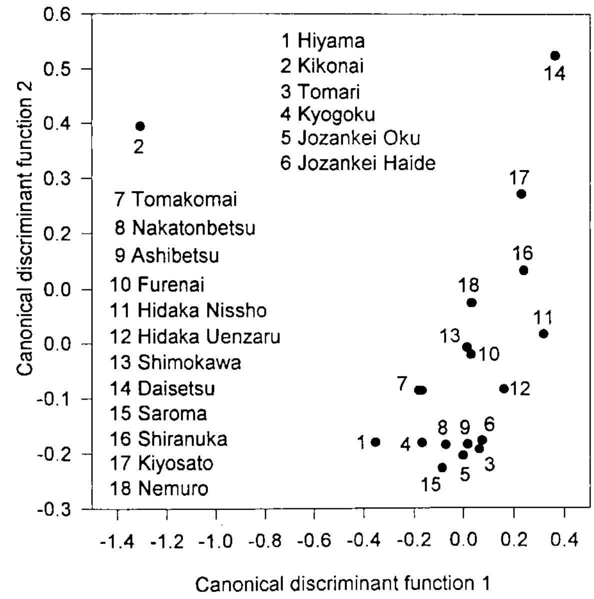

Figure 3. Scatter plot for *Abies sachalinensis* populations on the first two significant canonical discriminant functions.

has been demonstrated that such a clinal variation in *Abies alba* is due to refugia during the last glaciation and postglacial migration in Europe (KONNERT & BERGMANN 1995). In our study, the sample range is small geographically, and the multiple regression analyses showed that climatic, but not geographic, parameters were the most important independent variables for eight of the nine allele frequencies with significant regressions. Therefore, migration history is unlikely the main reason for the observed clinal variation in our study.

Our results indicate that the allele frequencies with significant variations among the populations were strongly influenced by some climatic and the underlying environmental conditions of the geographic parameters. Furthermore, some of the same climatic factors had different effects on the frequencies of different alleles. For example, warmth index is negatively related to frequencies of $Lap^b$ but positively related to frequencies of $Got\text{–}3^d$. On the other hand, the same alleles responded differently to different climatic factors, such as frequencies of $Lap^f$ were related to mean annual temperature positively and snowfall negatively. Together, these results signify that the ecological gradients related with these environmental factors might have selected specific allozymes or genes linked to them in *A. sachalinensis*. A large number of studies have also indicated natural selection at the allozyme level (NEVO *et al.* 1988; BUSH & SMOUSE 1992).

Although only four loci with 15 alleles were analysed for populations in a small geographic range, our results clearly demonstrate that both geographically clinal and population-specific allozyme variations exist in *A. sachalinensis*, and climatic gradients played a significant role in such a genetic structure. Similar variations in *A. sachalinensis* in Hokkaido have also been well documented in seedling growth, resistance to snow-damage, tolerance to freezing damage and to winter-desiccation injuries (OKADA *et al.* 1973; HATAKEYAMA 1983a,b; OKADA 1983; KURAHASHI & HAMAYA 1983; EIGA 1984). Therefore, we believe that our results are in concordance with these found in ecophysiological and morphological traits in *A. sachalinensis*. Similar congruence has also been observed in other conifers (XIE *et al.* 1992; LAGERCRANTZ & RYMAN 1990; YEH *et al.* 1985; GRANT & MITTON 1977). Such concordant variation patterns between allozymes and other traits provide further evidence that allozymes are related to natural selection.

The results of our study contribute to the understanding of the genetic structure of *A. sachalinensis* and may have practical applications. Tree improvement programs and seed orchards have been established by plus tree selection by the Regional Forest Management Offices and the Regional Breeding Offices of the Forestry Agency of the ministry of Agriculture, Forestry and Fisheries of Japan on a regional basis in Hokkaido. Considering the small variation among populations, the clinal and population-specific variations in this species, the existing programs by sampling individuals from different stands within individual regions seem a good strategy for tree improvement. So far, more than 24 *ex situ* and 21 *in situ* conservation stands have been established in different regions in Hokkaido. The *in situ* stands were those looking healthy and showing good growth, and each *ex situ* stand was established by seeds from about 30 trees from the *in situ* stand in the same region. We suggest that in the future more studies should be done to investigate genetic variations among populations within regions, and conservation stands should be chosen on the basis of genetic information and principles with special attention to those particularly different populations. For germplasm collection, effort should be made to sample from as many populations as possible in all parts of Hokkaido.

## ACKNOWLEDGEMENTS

This project was conducted while ZMW was supported by a STA fellowship from the Government of Japan. We are grateful to Y. Sakamoto who helped in tissue preparation and gel electrophoresis, to T. Matsuzaki and K. Ishida who helped to collect the needles, and to the Regional Forest Management offices in Hokkaido who permitted and/or led us to their forests to collect the needles. General support from K. Nakamura in our lab is greatly appreciated.

## REFERENCES

ALLENDORF, F. W. & PHELPS, S. R. 1981: Use of allelic frequencies to describe population structure. *Can. J. Fish. Aquat. Sci.* **38**:1507–1514.

BROWN, A. D. H. & MORAN, G. 1981: Isozymes and genetic resources of forest trees. *In*: Proceedings of a symposium on isozymes of North American forest trees and insects. USDA For. Serv. Gen. Tech. Rep. PSW–48, pp. 1–10.

BUSH, R. M. & SMOUSE, P. E. 1992: Evidence for the adaptive significance of allozyme in forest trees. *New Forests* **6**: 179–196.

EIGA, S. 1984: Ecogenetical study on the freezing resistance of Saghalin fir (*Abies sachalinensis* Mast.) in Hokkaido (in Japanese with English summary). *Bull. For. Tree Bree. Inst. Japan* **2**:61–107.

EL-KASSABY, Y. A. 1991: Genetic variation within and among conifer populations: review and evaluation of methods. *In*: Biochemical markers in the population genetics of forest trees. (ed. S. Fineschi, M. E. Malvolti, F. Cannata, & H. Hattemer H.). pp. 61–76. H. SPB Academic Publishing bv, The Hague.

FADY, B. & CONKLE, M. T. 1993: Allozyme variation and possible phylogenetic implications in *Abies cephalonica* Loudon and some related eastern Mediterranean firs. *Silvae Genet.* **42**:351–359.

Grant, M. C. & Mitton, J. B. 1977: Genetic differentiation among growth forms of Engelmann spruce and subalpine fir at tree line. *Arct. Alp. Res.* **9**:259–263.

Hamrick, J. L. & Allard, R. W. 1972: Microgeographical variation in allozyme frequencies in *Avena barbata*. *Proc. Natl. Acad. Sci. USA* **69**:2100–2104.

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

Hatakeyama, S. 1983a: Studies on the interprovenance variation of resistance to snow-damage in Sakhalin fir (in Japanese with English summary). *In*: Ecological-genetic studies in natural forests and their practical applications. Hoppo-Ringyu Kai, Japan, pp. 173–189.

Hatakeyama, S. 1983b: Studies on the interprovenance variation of resistance to desiccation damage in winter of Sakhalin fir (Japanese with English summary). *In*: Ecological-genetic studies in natural forests and their practical applications. Hoppo-Ringyu Kai, Japan, pp. 161–171.

Konnert, M. & Bergmann, F. 1995: The geographical distribution of genetic variation of silver fir (*Abies alba*, Pinaceae) in relation to its migration histroy. *Pl. Syst. Evol.* **196**:19–30.

Kurahashi, A. & Hamaya, T. 1983: Variation in characters and growth response of Sakhalin fir (*Abies sachalinensis*) associated with altitudinal gradients (in Japanese with English summary). *In*: Ecological-genetic studies in natural forests and their practical applications. Hoppo-Ringyu Kai, Japan, pp. 217–235

Lagercrantz, U. & Ryman, N. 1990: Genetic structure of Norway spruce (*Picea abies*): concordance of morphological and allozyme variation. *Evolution* **44**:38–53.

Meteorological Society Of Japan 1982: Climate of Hokkaido. Hokkaido Branch, Meteorological Society of Japan. Fuji Print Company, Sapporo, Japan.

Nagasaka, K. & Koono, K. 1990: Inheritance of isozymes in cross- and open-pollinated seeds of Saghalin fir (*Abies sachalinensis* Mast.) (in Japanese with English summary). *Bull. For. Tree Bree. Inst. Japan* **8**:169–179.

Nakamura, K., Kimura, R. & Uchijima, Z. 1986: Climate of Japan. Iwanami Shoten, Japan (in Japanese).

Neale, D. B. & Adams, W. T. 1985: Allozyme and mating-system variation in balsam fir (*Abies balsamea*) across a continuous elevational transect. *Can. J. Bot.* **63**:2448–2453.

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

Nevo, E., Beiles, A., & Krugman, T. 1988: Natural selection of allozyme polymorphisms: a microgeographical differentiation by edaphic, topographical, and temporal factors in wild emmer wheat (*Triticum dicoccoides*). *Theor. Appl. Genet.* **76**:737–752.

Okada, S. 1983: On the variation in Sakhalin fir (*Abies sachalinensis* Mast.) from different areas of Hokkaido (in Japanese with English summary). *Bull. For. Tree Bree. Inst. Japan* **1**:15–92.

Okada, S., Mukaide, H. & Sakai, A. 1973: Genetic variation in Sakhalien fir from different areas of Hokkaido. *Silvae Genet.* **22**:24–29.

SAS. 1990: SAS/STAT user's guide, version 6. SAS Institute Inc., Cary, N.C.

SATO, T. 1990: Trees and shrubs of Hokkaido. Arisu, Japan (in Japanese).

Shea, K. L. 1990: Genetic variation between and within populations of Engelmann spruce and subalpine fir. *Genome* **33**:1–8.

Society of Forest Environment. 1972: Forest environment maps of Japan. Society of Forest Environment, Tokyo, Japan.

Suyama, Y., Tsumura, Y. & Ohba, K. 1992: Inheritance of isozyme variants and allozyme diversity of *Abies mariesi* in three isolated natural forests. *J. Jpn. For. Soc.* **74**:65–73.

Swofford, D. L. & Selander, R. B. 1989: BIOSYS-1. A computer program for the analysis of allelic variation in population genetics and biochemical systematics. Release 7. University of Illinois, Urbana, USA.

Tatewaki, M. 1958. Forest ecology of the islands of the north Pacific ocean. *J. Facul. Agr., Hokkaido Univ., Sapporo* 50(4):371–475.

Tomaru, N., Tsumura, Y. & Ohba, K. 1994: Genetic variation and population differentiation in natural populations of *Cryptomeria japonica*. *Plant Species Biology* **9**: 191–199.

Vicario, F., Vendramin, G. G., Rossi, P., Lio, P. & Giannini, R. 1995: Allozyme, chloroplast DNA and RAPD markers for determining genetic relationships between *Abies alba* and the relic population of *Abies nebrodensis*. *Theor. Appl. Genet.* **90**:1012–1018.

Wang, Z.M. & Nagasaka, K. 1997: Allozyme variation in natural populations of *Picea glehnii* in Hokkaido, Japan. *Heredity* **78**: (in print).

Wickneswari, R. & Norwati, M. 1993: Genetic diversity of natural populations of *Acacia auriculiformis*. *Aust. J. Bot.* **41**:65–77.

Wright, S. 1965: The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution* 19:395–420.

Xie, C. Y., Bruce, P. D. & Yeh, F. C. 1992: Genetic structure of *Thuja orientalis*. *Biochem. Syst. Ecol.* **5**:433–441.

Yeh, F. C., 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. *Can. J. Genet. Cytol.* **27**:210–218.

Yeh, F. C. & Layton, C. 1979: The organization of genetic variability in central and marginal popultions of lodgepole pine *Pinus contorta* spp. *latifolia*. *Can. J. Genet. Cytol.* **21**:487–503.