p_1 = vector of the uncorrelated random effects for block within site by clone interactions and p is N~(0, K). K is a square matrix ($s^2_{bsc} I_k$) and has dimensions equivalent to total number of combinations of block within site by clone (*bsc*) for trait 1, i.e. only present in the model for trait 1.

t = matrix of the uncorrelated random effects for block by clone interactions, and t is MVN~(0, T). T is a block-diagonal square matrix and has dimensions equal to the total number of blocks by clone combinations (*bc*) for traits 2 (n2) and 3 (n3), *i.e.* only present in the model for traits 2 and 3. The only non-zero elements for T are along the diagonal. Elements t_{11} through $t_{n2,n2}$ are s^2_{bc2} and elements $t_{n2+1,n2+1}$ through $t_{n2+n3,n2+n3}$ are s^2_{bc3}.

e = vector of random residual effects for traits i = 1, 2, and 3 and e is MVN~(0, R). R is a square matrix and has dimensions equal to the sum of the numbers of observations for traits 1 (n1), 2 (n3) and 3 (n3). R is block diagonal with the only non-zero elements occurring on the diagonal (no covariances among the error terms since the traits were not measured on the same observational units). The first n1 observations along the diagonal equal to s^2_{e1}, the next n2 observations equal to s^2_{e2}, and the final n3 observations equal to s^2_{e3}.

The variance for observations (**V**) is:

$$\mathrm{Var}\begin{bmatrix} y_1 \\ y_2 \\ y_3 \end{bmatrix} = \mathbf{ZCZ'} + \mathbf{NDN'} + \mathbf{QKQ'} + \mathbf{WTW'} + \mathrm{R} = \mathbf{V} \tag{12}$$

where:

X, Z, N, Q and **W** are incidence matrices relating records to the fixed, clonal and uncorrelated random effects, described in sub-matrix form in the Appendix, Equation 1. **X, Z, N, Q** and **W** have dimensions equivalent to (nxp) where n = the total number of observations corresponding to traits 1, 2 and 3 and p is the number of levels for the modeled effects.

Allozyme Variation and Mating System of Coastal Populations of *Pinus koraiensis* SIEB. et ZUCC. in Russia

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Summary

Based on the analysis of 26 allozyme loci, levels of gene variation were ascertained in coastal populations of Korean pine. On average, 55.4% loci were polymorphic, the number of alleles per locus was 1.84, the expected heterozygosity was 0.176, and the observed heterozygosity was 0.177. On average, the heterozygote deficiency was characteristic for Korean pine populations (F_{IS}=0.012). The most diversity was found within populations (F_{ST}=0.016). Genetic distances between populations were small (D_N=0.003). Level of gene flow was 16.96 migrants per generation. Multilocus outcrossing estimates ranged from 0.751 to 0.986 indicating mating system differences in coastal populations of Korean pine.

Key words: Pinus koraiensis, coastal populations, allozymes, genetic variation, mating system.

Introduction

The Korean pine, *Pinus koraiensis* SIEB. et ZUCC., with many species of deciduous broad-leaved trees and other conifers, form the broad-leaved Korean pine mixed forests of the Russian Far East. Selective harvesting and fires have repeatedly stressed most of the forests. At present, clear cuttings of broad-leaved Korean pine mixed forests are illegal. However, the harvest of the broad-leaved Korean pine forests is occurring without authorization because of demand for pine and hardwood timber. For this reason, the broad-leaved Korean pine forestlands are decreasing (KORYAKIN and ROMANOVA, 1996). Thus, there is a need to emphasize conservation of Korean pine genetic resources.

Knowledge of the level and distribution of genetic variation, both within and among population, facilitates the conservation of gene resources (BROWN, 1978; MILLAR and LIBBY, 1991). Recently, the results of genetic variation studies of Korean pine populations in the Russian Far East (KRUTOVSKII et al., 1995; POTENKO and VELIKOV, 1998) and South Korea (KIM et al., 1994) have been reported. Differences in levels of genetic variation, within and among the population in different parts of Korean pine's natural range, were observed (POTENKO and VELIKOV, 1998). Greater variation was found in South Korean populations, with less occurring in the north-west part of natural range in Russia. Additionally, the measurements of mating systems showed a high proportion of outbreed progeny in three earlier studied Korean pine populations (POLITOV and KRUTOVSKII, 1994; KRUTOVSKII et al., 1995). All previously analyzed populations in Russia represented the internal range of naturally occurring Korean pine.

Marginal populations of some conifer species tend toward to lower heterozygosity than central population (BERGMANN and GREGORIUS, 1979; YEH and LAYTON, 1979; GURIES and LEDIG, 1982; HAWLEY and DEHAYES, 1994), which might be expected because of isolation and restricted gene flow, and because marginal populations frequently owe their origin to colonizing events and suffer from the bottleneck of the founder effect (LEDIG, 1986).

In this study, the primary objectives were to analyze the genetic variation and mating system of native coastal populations of *P. koraiensis* and to make a comparison of genetic data from internal and marginal parts of Korean pine range in Russia.

Materials and Methods

In 1998, a year of middle seed production, collections of open-pollinated seeds were taken from individual trees in five native stands: in the Kedrovaya Pad' Natural Reserve, on Petrov's Island and adjacent to the village of Kievka in the Lazov Natural Reserve, near the village of Ternei, and near the former village of Lesnoi (*Figure 1*). In the Kedrovaya Pad' Natural Reserve, cones were collected from 38 trees within 10 km to 12 km of the Amur Bay coast. In the Lazov Natural Reserve, cones were collected from 21 trees on Petrov's Island and 68 trees located 1 km along the coast of the Sea of Japan. Cones from 61 trees were collected from the Korean pine stand near the village of Ternei; trees situated 10 km from the coast of the Sea of Japan. Finally, near the former village of Lesnoi, 25 km from the Tatar Strait coast, cones were collected from 58 trees. All five stands are located near the seacoast in the Korean pine's natural range and contained scattered Korean pine trees in mixture with various hardwood species. Within each stand cones were collected along transect with a minimum distance of 30 m between individual trees so as to decrease the risk of relatedness.

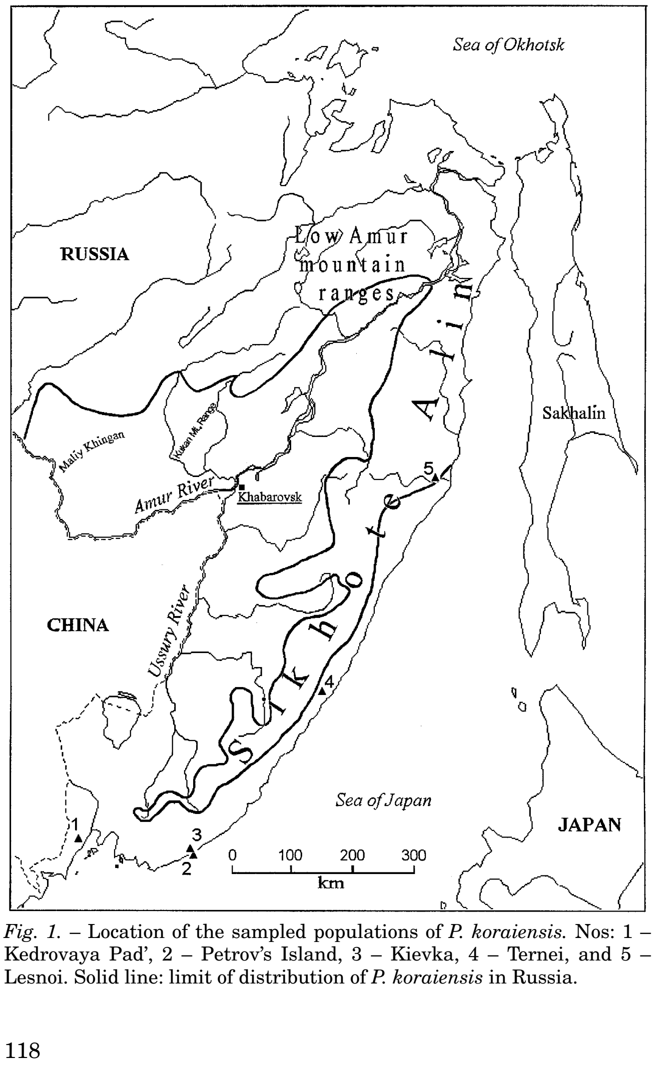

Fig. 1. – Location of the sampled populations of *P. koraiensis*. Nos: 1 – Kedrovaya Pad', 2 – Petrov's Island, 3 – Kievka, 4 – Ternei, and 5 – Lesnoi. Solid line: limit of distribution of *P. koraiensis* in Russia.

Six megagametophytes and ten embryos per tree were subjected to horizontal starch gel electrophoresis. Details of laboratory procedures are described in POTENKO and VELIKOV (1998). Seed tissues were analyzed for 15 enzyme systems: aspartate aminotransferase (*AAT*), alcohol dehydrogenase (*ADH*), aconitase (*ACO*), diaphorase (*DIA*), fluorescent esterase (*Fl-EST*), formate dehydrogenase (*FDH*), glutamate dehydrogenase (*GDH*), glutamate pyruvate transaminase (*GPT*), isocitrate dehydrogenase (*IDH*), leucine aminopeptidase (*LAP*), malate dehydrogenase (*MDH*), phosphoglucomutase (*PGM*), 6-phosphogluconate dehydrogenase (*6-PGD*), shikimate dehydrogenase (*SkDH*) and sorbitol dehydrogenase (*SDH*). In total, 26 loci were scored for genetic variation analysis (*Aat-1, Aat-2, Aat-3, Adh-1, Adh-2, Aco, Gdh, Dia-1, Dia-3, Idh, Lap-1, Lap-2, Mdh-1, Mdh-2, Mdh-3, Mdh-4, Gpt, Sdh, Fl-Est, Fdh, Pgm-1, Pgm-2, 6-Pgd-1, 6-Pgd-2, Skdh-1* and *Skdh-2*). To accurate comparison of the genetic variation between different parts of the Korean pine natural range, the loci were same as in POTENKO and VELIKOV (1998). For mating system analysis, four loci (*Aat-3, Dia-1, Pgm-1* and *Skdh-1*) were used (*Figure 2*).

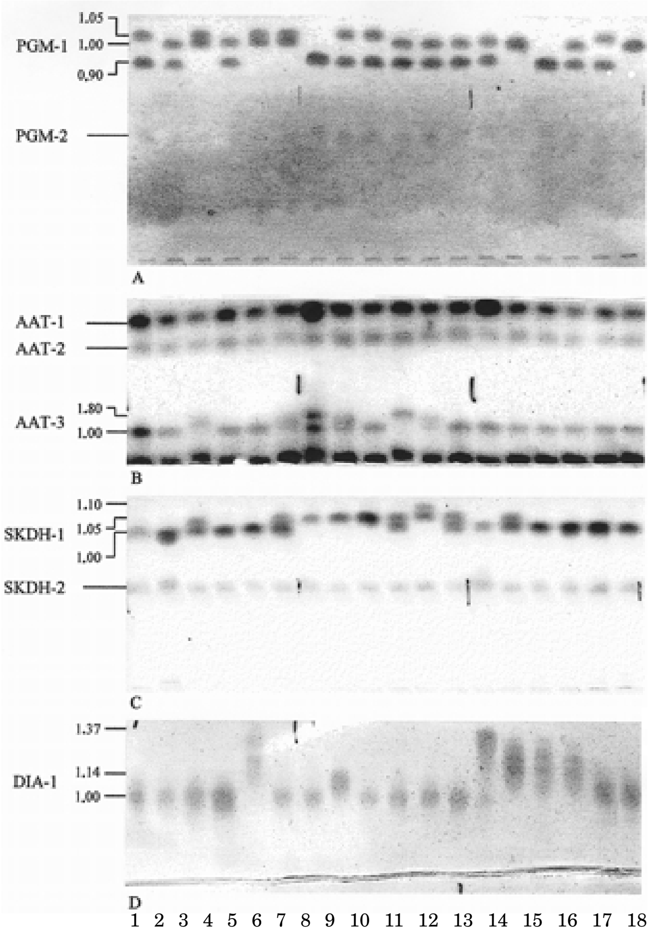

Fig. 2. – Zymograms of phosphoglucomutase (A), aspartate aminotransferase (B), shikimate dehydrogenase (C), and diaphorase (D) of megagametophyte mix (slots 1, 7, 13) and embryos of three Korean pine trees (slots 1 to 6, 7 to 12, and 13 to 18). A: 1, 8, 9, 17 – PGM-$1^{1.05/0.90}$; 2, 4, 10 to 13, 16 – PGM-$1^{1.00/0.90}$; 3, 5, 6 – PGM-$1^{1.05/1.00}$; 7, 15 – PGM-$1^{0.90/0.90}$; 14, 18 – PGM-$1^{1.00/1.00}$. B: 1, 2, 4, 5, 9, 12 to 18 – AAT-$3^{1.00/1.00}$; 3, 6 to 8, 11 – AAT-$3^{1.80/1.00}$; 10 – AAT-$3^{1.80/1.80}$. C: 1, 2, 4, 5, 13, 15 to 18 – SKDH-$1^{1.00/1.00}$; 3, 6, 10, 12, 14 – SKDH-$1^{1.05/1.00}$; 7 to 9 – SKDH-$1^{1.05/1.05}$; 11 – SKDH-$1^{1.10/1.05}$. D: 1 to 4, 6, 7, 9 to 12 – DIA-$1^{1.00/1.00}$; 5, 13 to 16 – DIA-$1^{1.37/1.00}$; 8, 17, 18 – DIA-$1^{1.14/1.00}$.

Table 1. – Genetic variability at 26 loci in 5 coastal populations of *P. koraiensis* (standard errors in parentheses).

Population	Mean No. of alleles per locus, A	Percentage of polymorphic loci		Mean heterozygosity	
		P_{95}	P_{99}	Observed, H_o	Expected, H_e 1)
1. Kedrovaya Pad'	1.85	50.0	61.5	0.186	0.187
2. Petrov's Island	1.73	42.3	50.0	0.177	0.161
3. Kievka	1.85	50.0	53.9	0.172	0.178
4. Ternei	1.85	46.2	53.9	0.192	0.190
5. Lesnoi	1.92	50.0	57.7	0.156	0.163
Mean	1.84 (0.07)	47.7 (3.4)	55.4 (4.4)	0.177 (0.014)	0.176 (0.013)

1) Unbiased estimate (see Nei, 1978)

Allele frequencies were analyzed using the BIOSYS-1 computer program (Swofford and Selander, 1989). For each population, mean number of alleles per locus (A), percentage of polymorphic loci ($P_{0.95}$ and $P_{0.99}$) and expected heterozygosity (H_e) were computed. In addition, Nei's genetic distances (D_N) were calculated (Nei, 1978).

For assaying the populations genetic structure, the fixation indices (F_{IS}, F_{IT} and F_{ST}) were used (Nei, 1977). F_{IS} and F_{IT} measure the deviation of genotype frequencies from Hardy-Weinberg proportions in the populations and in the total population respectively, whereas F_{ST} measures the degree of genetic differentiation among populations. The F_{ST} values were used to calculate interpopulational gene flow (Nm) as follows: $F_{ST}=1/[4Nma+1]$, where $a=[n/n-1]^2$, and n is the number of populations (Govindaraju, 1989).

The expected fixation index at inbreeding equilibrium was computed as $F_e=(1-t_m)/(1+t_m)$, where t_m is the multilocus outcrossing rate (Allard et al., 1968).

Single locus (t_s) and multilocus (t_m) estimates of the proportion of progeny resulting from outcrossing in a population were determined using the MLT computer program (Ritland, 1990). Maternal genotypes, assessed from megagametophyte segregations, were taken into account. The confidence intervals of the outcrossing rates were estimated after 100 boot-straps. At *Dia-1,* the 4th allele *Dia-1*$^{0.60}$ with the lowest frequency was combined with the allele *Dia-1*$^{1.37}$ having the nearest frequency because the computer program can only process a maximum of 3 alleles per locus. Both t_s and t_m estimates are based on the mixed mating model, which assumes that each viable offspring is the result of a random outcross (with probability t) or a self-fertilization (with probability s=1-t), that the probability of an offspring being an outcross is independent of the genotype of the maternal parent, that outcross pollen pool allele frequencies are homogeneous over space and over time, and that there is no selection between pollination and the time that seeds or seedlings are sampled (Shaw et al., 1981). Multilocus estimations require the additional assumption of independence among loci in the outcross pollen pool. Conkle (1981), Politov et al. (1989) and Goncharenko et al. (1998) showed that the gene arrangement is highly conservative in the pines and found no linkage among loci *Aat-3, Dia-1, Pgm-1* and *Skdh-1.*

Results

Parameters of genetic variation (*Table 1*) were calculated on the basis of allele frequencies of 26 loci. In coastal Korean pine populations, the mean number of alleles per locus ranged from 1.73 to 1.92, with an average of 1.84. The proportion of polymorphic loci ($P_{0.99}$) ranged from 50.0% to 61.5%, with an average of 55.4%. The observed heterozygosity was from 0.156 to 0.192, with an average of 0.177. The expected heterozygosity ranged from 0.161 to 0.190, with an average 0.176. Low level of genetic variation was found in the Lazov Nature Reserve on Petrov's Island. The northernmost population near Lesnoi also had low values of observed and expected heterozygosity, however estimates of mean number of alleles per locus and proportion of polymorphic loci were high and exceed the mean of all populations.

F_{IS} values ranged from -0.158 for *Lap-1* to 0.240 for *Gdh*, with an overall mean of 0.012. F_{IT} values at loci *Adh-1, Lap-1, Mdh-2, Dia-3, Fl-Est* and *Gpt* were negative and at 11 loci were positive, reaching 0.249 at *Gdh.* The average F_{IT} was 0.028. Positive mean values of fixation indices indicate that, relative to the Hardy-Weinberg equilibrium, a deficiency of heterozygotes is typical for the majority of *P. koraiensis* populations (F_{IS}) and for the species (F_{IT}) in the coastal part of natural range. An F_{ST} value at 17 polymorphic loci was 0.016. Thus, approximately 98% of the total genetic variability resided within populations and only 1.6% among populations (*Table 2*).

Table 2. – F-statistics and Nm values for 17 polymorphic loci.

Locus	F_{IS}	F_{IT}	F_{ST}	Nm
Aat-3	0.029	0.045	0.017	9.25
Adh-1	-0.053	-0.037	0.015	10.51
Adh-2	0.091	0.108	0.019	8.26
Gdh	0.240	0.249	0.012	13.17
Lap-1	-0.158	-0.151	0.007	22.70
Lap-2	0.044	0.061	0.018	8.73
Pgm-1	-0.018	0.000	0.017	9.25
Skdh-1	0.015	0.018	0.002	79.84
Skdh-2	0.079	0.097	0.019	8.26
Mdh-2	-0.026	-0.023	0.003	53.17
Mdh-4	0.015	0.027	0.013	12.15
6-Pgd-1	-0.009	0.036	0.044	3.48
Dia-1	0.068	0.092	0.026	5.99
Dia-3	-0.034	-0.014	0.020	7.84
Fl-Est	-0.022	-0.005	0.017	9.25
Fdh	-0.007	0.006	0.013	12.15
Gpt	-0.047	-0.036	0.011	14.39
Mean for 17 loci	0.012	0.028	0.016	16.96

The estimates of Nm, averaged over all populations per locus, were well above 1.0 (ranged from 3.48 at *6-Pgd-1* to 79.84 at *Skdh-1*) with a mean of 16.96 migrants per generation (*Table 2*).

Unbiased Nei's genetic distance values were low between the 5 populations of *P. koraiensis* averaging 0.003. The largest genetic differences were detected between Petrov's Island and Kedrovaya Pad'; Kedrovaya Pad' and Lesnoi; and Lesnoi and Ternei populations (*Table 3*). The lowest level of differentiation was detected between Kedrovaya Pad' and Kievka (D_N=0.001), thus indicating the similarity of the populations gene frequencies.

Table 3. – Estimates of NEI's (1978) genetic distance based on data from 26 loci.

Population	1	2	3	4	5
1. Kedrovaya Pad'	****	0.005	0.001	0.003	0.005
2. Petrov's Island		****	0.002	0.003	0.004
3. Kievka			****	0.003	0.003
4. Ternei				****	0.005
5. Lesnoi					****

Table 4. – Estimations of single locus (t_s) and multilocus (t_m) outcrossing rates, fixation index (F_{IS}) and expected inbreeding coefficient (F_e) based on data from 4 polymorphic loci (standard errors in parentheses).

Population	t_s	t_m	F_{IS}	F_e
Kedrovaya Pad'	0.958 (0.050)	0.986 (0.043)	0.081	0.007
Petrov's Island	0.763 (0.061)	0.751 (0.057)	-0.105	0.142
Kievka	0.923 (0.053)	0.912 (0.053)	0.113	0.046
Ternei	0.851 (0.061)	0.852 (0.057)	-0.054	0.080
Lesnoi	0.884 (0.046)	0.888 (0.042)	0.009	0.059

Single locus estimates of outcrossing ranged from 0.763 to 0.958, and multilocus estimates were from 0.751 to 0.986 (*Table 4*). The lowest value t_m was found in the Lazov Nature Reserve on Petrov's Island and highest in the Kedrovaya Pad' Nature Reserve. No significant differences were found between single locus and multilocus estimates of outcrossing in any population.

The values of fixation index F_{IS} in populations at Petrov's Island, Ternei, and Lesnoi are much lower then those expected under inbreeding equilibrium given the levels of t_m (*Table 4*). However, F_{IS} estimates in Kedrovaya Pad' and Kievka are higher then values F_e and reached 0.081 and 0.113 respectively.

Discussion

According to our data, coastal populations of Korean pine in Russia contain an appreciable amount of genetic variation comparably to the mean value for genus *Pinus* (on average, P_{99}=52.0, H_o=0.159, H_e=0.159; GONCHARENKO et al., 1989). The genetic variation of coastal populations was lower than in Korean pine populations of South Korea (on average, P_{99}=69.0, A=2.0, H_o=0.200, H_e=0.208; KIM et al., 1994) as well as lower than in the interior natural range in Russia (on average, P_{99}=58.7, A=1.93, H_o=0.182, H_e=0.183; POTENKO and VELIKOV, 1998).

In the coastal, the peripheral population Lesnoi possesses a lower heterozygosity that can be explained by gene drift due to founder effect of populating a territory by a small number of individuals in recent history. This may be as a result of the northward migration of the Korean pine during the Holocene along the narrow coastline (*Figure 1*). Lower estimates of genetic variation in the Petrov's Island population can also be attributed to founder effect of Korean pine colonizing the island. Apparently the populating was 9800-9500 years ago, during the Holocene period when Korean pine appeared as a member of the mountain vegetation of Sikhote-Alin (GOLUBEVA and KARAULOVA, 1983; KOROTKII et al., 1997). At present, the Petrov's Island area encompasses 36 hectares on which grow a few hundred Korean pine trees. Also, heterozygosity decrease has been found in peripheral populations of *Pinus contorta* (YEH and LAYTON, 1979), *Pinus rigida* (GURIES and LEDIG, 1982), *Picea abies* (BERGMANN and GREGORIUS, 1979) and *Picea rubens* (HAWLEY and DEHAYES, 1994). More intense selection in marginal environments, genetic drift, greater inbreeding in small populations, or migration from different glacial refugia explained the heterozygosity differences between central and peripheral populations in the studies (YEH and LAYTON, 1979; GURIES and LEDIG, 1982; HAWLEY and DEHAYES, 1994).

The deficiency of heterozygotes observed for the majority of investigated populations (F_{IS}=0.012) and for coastal part of *P. koraiensis* natural range (F_{IT}=0.028) was also found in the south Korea and in Russia for interior part of natural range (KIM et al., 1994; POTENKO and VELIKOV, 1998). Thus, deficiency of heterozygotes is typical for Korean pine populations and the whole species. For pines, this deficiency was attributed to mating among closely adjacent individuals within a stand, partial self-pollination, pooling of individuals (during sampling) from different family groups within populations, and selection against heterozygotes (GURIES and LEDIG, 1982; DANCIK and YEH, 1983; KIM et al., 1994; POLITOV and KRUTOVSKII, 1994; CHANGTRAGOON and FINKELDEY, 1995; LEE et al., 1998).

The mean F_{ST} value (F_{ST}=0.016) was lower than the mean G_{ST} estimate for genus *Pinus* (G_{ST}=0.065; HAMRICK et al., 1992). The value indicates that 1,6% of the genetic variation is distributed among the five coastal populations. The estimate is close to that for interior part of the natural range of *P. koraiensis* in Russia (POTENKO and VELIKOV, 1998) and slightly lower than in South Korea (KIM et al., 1994). That is to say, the majority of the variation resides within Korean pine populations and absent of any prominent differentiation processes between populations.

Low estimates of NEI's genetic distances confirm the close genetic relationship between the five coastal populations. The mean value of D_N is similar to that of the interior population in Russia (D_N=0.003). The overall differentiation among different regions of the Korean pine's natural range was not significantly higher than the differentiation within regions (*Table 5*). This indicates some restriction of gene exchanges between regions due to the main geographic barriers in the Russian Far East, including the Sikhote-Alin mountain range and the Amur valley. Conversly, the low values of D_N and F_{ST} can indicate a widespread gene flow between populations inside a region and/or similar types of natural selection (BUSH and SMOUSE, 1992).

The widespread gene exchange hypothesis can be supported by the average Nm value (Nm=16.96) that exceed to those of *P. koraiensis* in South Korea (Nm=3.987; KIM et al., 1994) and most of the coniferous tree species (GOVINDARAJU, 1989; GONCHARENKO and SILIN, 1997).

Table 5. – Estimates of Nei's (1978) genetic distance within and among regions.

Region	No. of populations.	1	2	3
1. Maliy Khingan-Kukan mountain ranges1)	6	0.002 (0.000-0.004)	0.004 (0.000-0.011)	0.006 (0.001-0.012)
2. Sikhote Alin -Low Amur mountain ranges1)	13	****	0.002 (0.000-0.008)	0.004 (0.001-0.009)
3. Coastal populations	5	****	****	0.003 (0.001-0.005)

1) Data from Potenko and Velikov (1998)

The mean multi-locus value of outcrossing in this study (t_m=0.878) was lower than that for the three populations (t_m=0.974) studied earler by Politov and Krutovskii (1994), but typical for most coniferous forest tree species (Muona, 1990; Adams and Birkes, 1991; Mitton, 1992). The lowest value t_m on Petrov's Island can be attributed to both selfing and mating among related individuals supporting the hypothesis that the populating of the island was by a limited number of migrants. The low estimates of the Lesnoi and Ternei populations can be attributed to partial mating among related individuals due to the founder effect. Although t_m was low in these populations, the estimates of F_{IS} were either negative or slightly positive, thus indicating an excess of heterozygotes or practically Hardy-Weinberg equilibrium. Any one is much lower then those expected under inbreeding equilibrium given the levels of t_m (*Table 4*).

Excess heterozygotes (F_{IS}=–0.148) and a low proportion of outcrossed progeny (t_m=0.78) were also found in one of four populations of *Abies balsamea* (Neale and Adams, 1985). The authors explained the excess of heterozygotes by selection against inbred progeny and the advantage of heterozygotes. Possibly the selection against inbred progeny or both types of selection are responsible for excess fixation index observed (F_{IS}) over expected (F_e) in three Korean pine populations. It is suggested that a contrary direction of selection occurs in populations with a deficit of heterozygotes and high outcrossing rates (Kedrovaya Pad' and Kievka), i.e. selection against heterozygotes. Selection against hybrid forms of plants probably due to the outbreed depression is found in crosses between distant plants of *Delphinium nelsoni* (Price and Waser, 1979). However, this suggestion for Korean pine needs to be field-proven in bi-parental crosses.

Possible explanation may be microsite differentiation of allele frequencies that will be biased up the fixation index. If different subpopulations hold up different alleles, the allele frequencies will support on high level in whole population (Brown, 1979). This phenomenon explains the high level heterozygosity in populations with positive fixation index though reliable conclusion can be made after investigation of genetic parameters of subpopulations.

Thus the coastal Korean pine populations we sampled exhibit different levels of genetic variation and outcrossing. We assume that the genetic structure of the populations is under influencing of the complex combination of microevolution factors: gene drift, gene flow and natural selection.

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Genetic Variation in *Pinus banksiana* Populations From the Sudbury (Ontario, Canada) Region

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Summary

Genetic variation within and among jack pine (*Pinus banksiana*) populations from heavy metal – contaminated and uncontaminated areas in the Sudbury (Ontario, Canada) region was investigated using random amplified polymorphic markers (RAPDs). DNA samples from individual trees were analyzed using 15 oligonucleotides of random sequence. Eight of these primers allowed amplifications of random polymorphic (RAPD) loci. Close genetic relationships among jack pine populations from contaminated and uncontaminated sites were observed. Overall 26% of the scored loci were polymorphic. The analysis of molecular variance revealed that the within -group (among individuals) variations accounted for 63.3% of the total molecular variance. The difference among populations which explained 36.7% of total variation was statistically significant and higher than RAPD variations reported in *P. resinosa*. The jack pine trees were easily distinguished from red pine trees using RAPD markers. RAPD markers specific to *P. banksiana* were also identified.

Key words: RAPD, *Pinus*, DNA polymorphism, genetic diversity, heavy metals.

Introduction

The genus *Pinus* is among the most widely distributed and prominent genera of trees in the world and includes many of the most economically valuable species of forest trees (Critchfield and Little, 1966; Strauss and Doerksen, 1990). A number of investigators have been studying range-wide, and regional variation, differences between mature, regenerating and seed source plantations as well as relationships to environmental variables in jack and lodgepole pine (Dancik and Yeh, 1983; Yeatman, 1984; Knowles, 1985; Gordon, 1994). Allelic heterogeneity tests have indicated no differences between mature, natural young stands and seed zone plantations. Several allozyme studies have reported existence of genetic variability in ponderosa pine (*Pinus ponderosa*), lodgepole pine (*Pinus contorta* var *latifolia*), and jack pine (*P. banksiana*) populations (O'Malley et. al., 1979; Yeh and Layton, 1979). Similar analyses revealed that the levels of genetic variability were lower in jack and red pines compared to other pine species (Dancik and Yeh, 1983; Mosseler et al., 1991). RAPD analysis of red pine populations from Newfoundland confirmed the low levels of genetic variability (Mosseler et al., 1992).

On the other hand, there is great concern that air pollution and its conversion products alter the genetic structure of forest tree populations by processes which are assumed to have selective effects (Scholz, 1986). Preliminary investigations indicate that environment markedly influences the relationship between biochemical variation and quantitative characters as well as the degree of expression of these characters. Several authors have reported various responses to air pollution in trees growing in contaminated areas. Enzymatic studies of Norway spruce revealed genetic differences between groups of sensitive trees in polluted areas (Bergmann and Scholz, 1987).

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