Genetic Diversity and Mating System of Korean Pine in Russia

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Abstract—Based on the analysis of 26 allozyme loci, levels of genetic variation were ascertained in 25 natural populations of Korean pine. On average, 58.0 percent of the loci were polymorphic; the number of alleles per locus was 1.92; the expected heterozygosity was 0.182, and the observed heterozygosity was 0.180. On average, the heterozygote deficiency was characteristic of Korean pine populations (F_{IS}=0.013). The most diversity was found within populations (F_{ST}=0.018). Genetic distances between populations were small (on average, D_N=0.003). Level of gene flow was 10.98 migrants per generation. Multilocus outcrossing estimates ranged from 0.751 to 1.031, indicating mating system differences. Results of this study lead to the assumption that the genetic structure of Korean pine populations is under the influence of a complex combination of microevolution factors, including genetic drift, gene flow and natural selection.

Key words: Korean pine, allozymes, genetic variation, differentiation, gene flow, mating system.

Introduction

The Korean pine, *Pinus koraiensis* Sieb. & Zucc., occurs in natural and artificial stands in Russia, China, Korea, and Japan. In the Russian Far East, *P. koraiensis* is distributed in the Primorski Territory, in the southern part of Khabarovsk Territory, in the Jewish Autonomous Region and at the southeast end of the Amur Territory (fig. 1). Usually, *P. koraiensis* grows in mixed stands with broadleaf tree species. The Korean pine-broadleaf forests occupy low and middle altitude zones growing in a wide range of relief and soil conditions. In the south Sikhote Alin mountain range they occur up to 900 m above sea level, while in the north, Korean pine reaches only to 500 m (Usenko 1969). 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 populations, 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 have been reported (Krutovskii and others 1995; Potenko and Velikov 1998) and South Korea (Kim and others 1994). Differences in levels of genetic variation were observed within and among the populations in different parts of Korean pine's natural range (Potenko and Velikov 1998). Greater variation was found in South Korean populations, with less variation occurring in the northwestern part of the natural range in Russia. Additionally, the measurements of mating systems showed a high proportion of outbred progeny in an earlier study of three Korean pine populations (Politov and Krutovskii 1994; Krutovskii and others 1995).

The primary objectives of this study were to analyze the genetic diversity and mating system of Korean pine throughout the natural range in Russia and to describe geographical patterns of genetic variation.

Materials and Methods

Seeds for electrophoresis were collected in 25 native populations from 43° to 51° latitude north (fig. 2). In 17 populations the collection of seeds was performed on individual trees. The remaining eight populations were represented by bulked seed lots that were collected from native populations by state forest farms for artificial reforestation (table 1). More details about seed samples and characteristics of the sampled populations can be found elsewhere (Potenko and Velikov 1998, 2001).

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). For mating system analysis, four loci (Aat-3, Dia-1, Pgm-1 and Skdh-1) were used.

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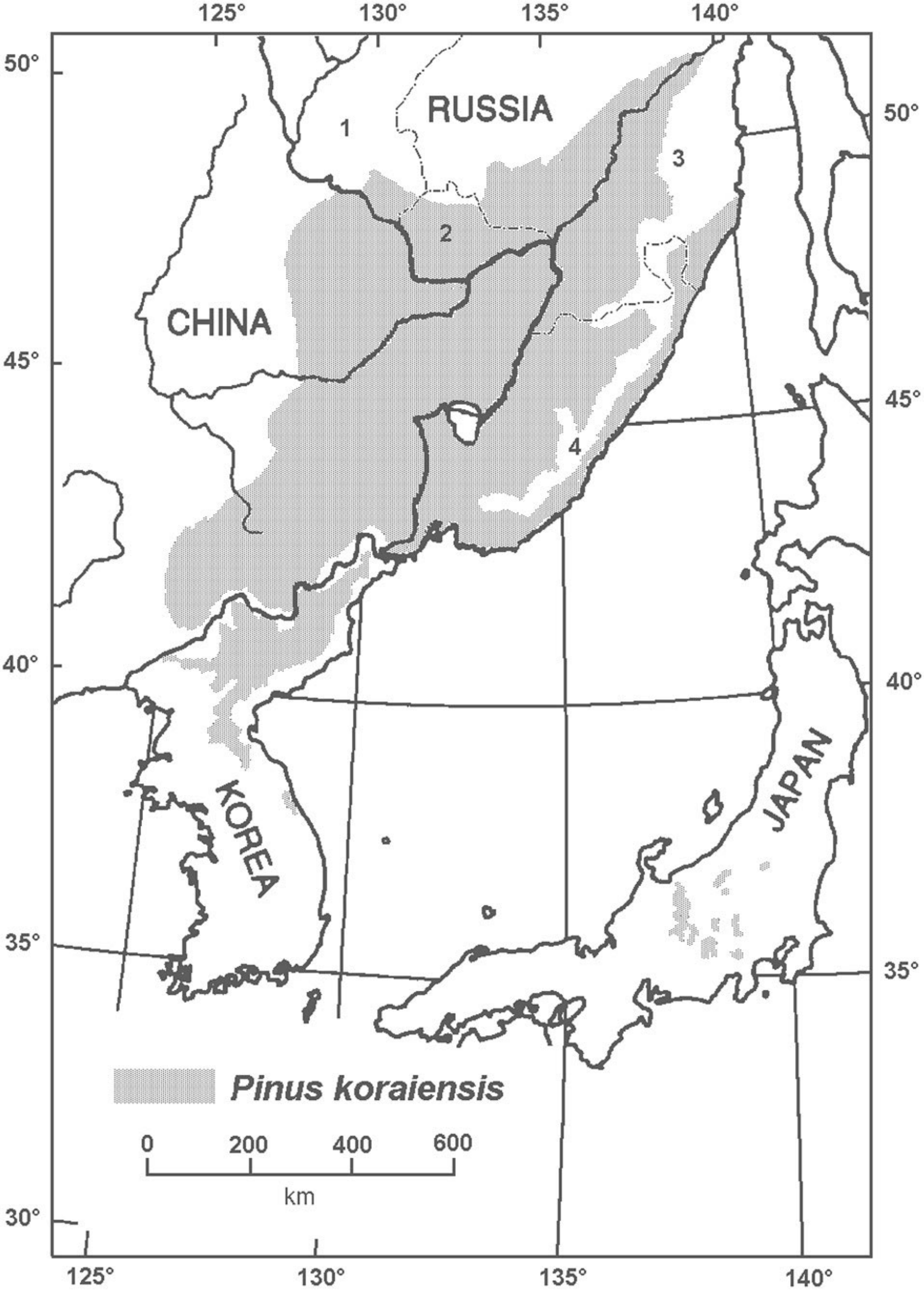

Figure 1—Distribution of Korean pine (modified from Schmidt 1994). Nos: 1 - Amur Territory; 2 - Jewish Autonomus Region; 3 - Khabarovsk Territory; 4 - Primorski Territory.

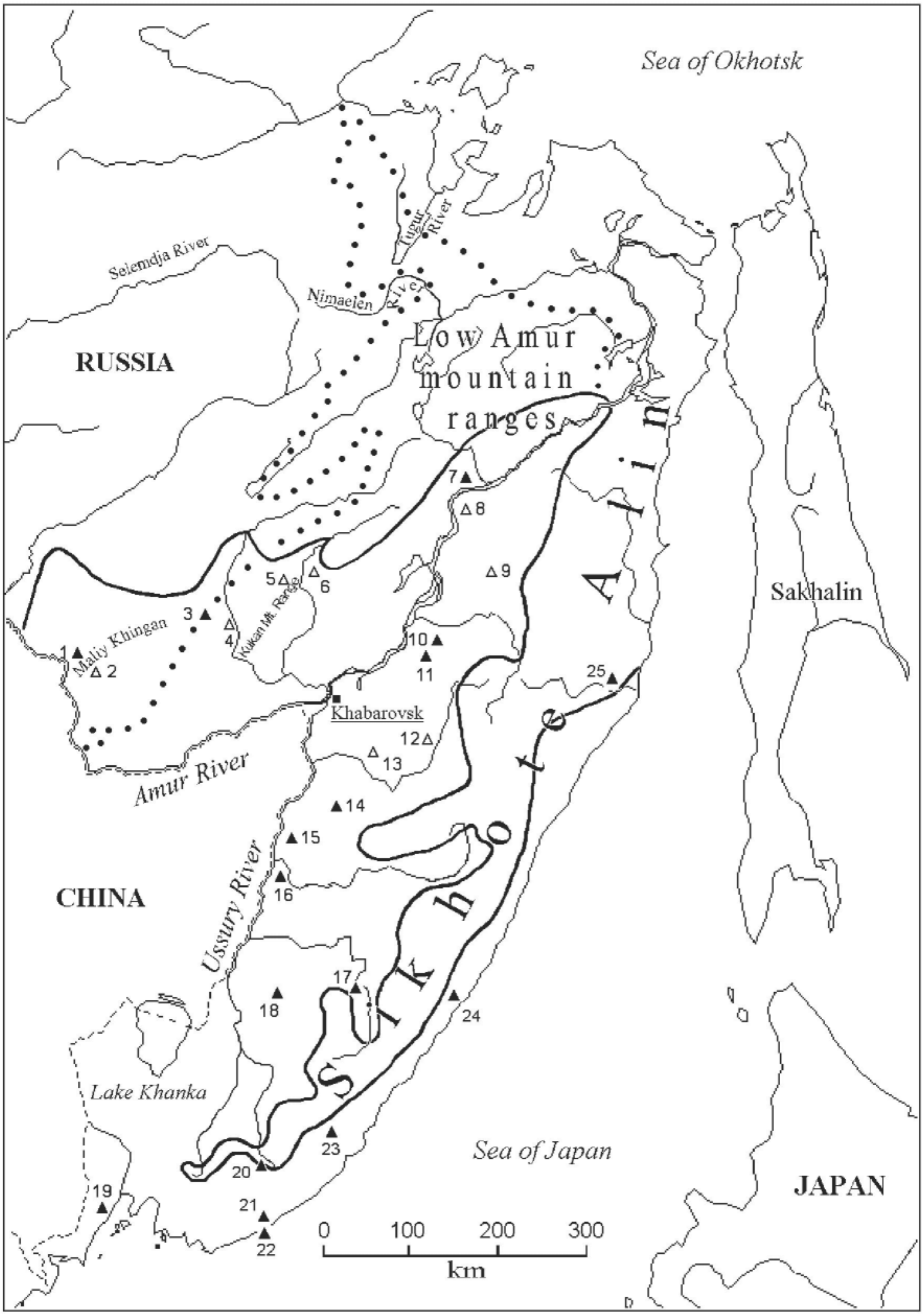

Figure 2—Location of the sampled populations: ▲ - seed collection was conducted from individual tree; Δ - seed lot was sampled. Nos: population numbers shown in Table 1. Solid line: limit of distribution of *P. koraiensis* in Russia. Dotted line: northern limit of the Korean pine-broadleaf and Korean pine-spruce-larch mixed forests in the Holocene climate optimum (modified from Korotkii and others 1997).

Table 1—Genetic variability at 26 loci in 25 populations of *P. koraiensis* (standard errors in parentheses).

Population	Number of trees	Mean No. of alleles per locus, A	Percentage of polymorphic loci		Mean heterozygosity	
			P_{95}	P_{99}	Observed, H_o	Expected, H_e
1. Obluchie	21	1.85	46.2	61.5	0.170	0.171
2. Sutara[a]	70[b]	1.69	42.3	57.7	-	0.169
3. Dogordon	52	1.92	50.0	53.8	0.164	0.171
4. Kukan[a]	70[b]	1.65	46.2	50.0	-	0.170
5. Niran[a]	70[b]	1.88	46.2	61.5	-	0.165
6. Selgon[a]	70[b]	1.96	42.3	53.8	-	0.154
7. Galichnoe	72	2.12	57.7	69.2	0.193	0.204
8. Pivan[a]	70[b]	1.85	53.8	53.8	-	0.194
9. Innokentievka[a]	70[b]	1.77	46.2	53.8	-	0.174
10. Burga	53	1.92	50.0	53.8	0.174	0.187
11. Mulcha	62	2.08	46.2	57.7	0.191	0.189
12. Sukpay[a]	70[b]	1.96	50.0	61.5	-	0.181
13. Obor[a]	70[b]	2.00	53.8	61.5	-	0.183
14. Medvezhy	49	1.88	53.8	57.7	0.180	0.194
15. Boicovo	24	1.81	46.2	53.8	0.164	0.181
16. Pokrovka	73	2.19	50.0	61.5	0.188	0.193
17. Mel'nichnoe	73	2.15	46.2	57.7	0.180	0.180
18. Malinovo	50	2.08	53.8	69.2	0.189	0.218
19. Kedrovaya Pad'	38	1.85	50.0	61.5	0.186	0.187
20. Arkhipovka	51	2.08	50.0	61.5	0.199	0.197
21. Kievka	68	1.85	50.0	53.9	0.172	0.178
22. Petrov's Island	21	1.73	42.3	50.0	0.177	0.161
23. Ustinovka	63	2.00	50.0	61.5	0.193	0.187
24. Ternei	61	1.85	46.2	53.9	0.192	0.190
25. Lesnoi	58	1.92	50.0	57.7	0.156	0.163
Mean for populations Nos. 1-6 Maliy Khingan - Kukan mountain ranges		1.83 (0.13)	45.5 (2.9)	56.4 (4.6)	0.167 (0.004)	0.167 (0.007)
Mean for populations Nos. 7-25 Sikhote Alin - Low Amur mountain ranges		1.95 (0.14)	49.8 (3.7)	58.5 (5.2)	0.182 (0.012)	0.186 (0.013)
Mean		1.92 (0.14)	48.8 (3.9)	58.0 (5.1)	0.180 (0.012)	0.182 (0.015)

[a] Bulked seed lot analyzed. Total weight of any seed lot was 500 kg.
[b] Number of analyzed seeds per seed lot.

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 population 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 and others 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 three alleles per locus. Both t_s and t_m estimates are based on the mixed mating model, which assumes (1) that each viable offspring is the result of a random outcross (with probability t) or a self-fertilization (with probability s=1-t), (2) that the probability of an offspring being an outcross is independent of the genotype of the maternal parent, (3) that outcross pollen pool allele frequencies are homogeneous over space and over time, and (4) that there is no selection between pollination and the time that seeds or seedlings are sampled (Shaw and others 1981). Multilocus estimations require the additional assumption of independence among loci in the outcross pollen pool. Conkle (1981), Politov and others (1989) and Goncharenko and others (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 and Discussion

Genetic Diversity and Differentiation

Parameters of genetic variation (table 1) were calculated on the basis of allele frequencies of 26 loci. In Korean pine populations, the mean number of alleles per locus ranged from 1.65 to 2.19, with an average of 1.92. The proportion of polymorphic loci ($P_{0.99}$) ranged from 50.0 to 69.2 percent, with an average of 58.0 percent. The observed heterozygosity was from 0.156 to 0.199, with an average of 0.180. The expected heterozygosity ranged from 0.154 to 0.218, with an average of 0.182. The genetic variation was lower than in Korean pine populations of South Korea (on average, P_{99}=69.0, A=2.0, H_0=0.200, H_e=0.208; Kim and others 1994). The results seem to support the hypothesis that Korean pine expanded to the far eastern region of Russia from the south in the Holocene. The studies of fossil conifer pollen (Korotkii and others 1997) indicate that 18,000 to 20,000 years ago the vegetation of Sikhote Alin was similar to that of the contemporary northwest coast of the Sea of Okhotsk. After climatic cooling, the Korean pine appeared among mountain vegetation approximately 9,500 years ago, in the Holocene period, and in the middle Holocene the northern border of its area had spread to the Selemdja, Tugur and Nimaelen rivers. As can be seen, the range of *P. koraiensis* was previously much wider than at present (fig. 2). Southward decline of Korean pine occurred because of the cooler climate periods in the middle and late Holocene, resulting in the expansion of taiga boreal forests with *Picea*, *Abies*, and *Larix* species.

Natural populations of Korean pine in Russia contain an appreciable amount of genetic variation comparable to the mean value for the genus *Pinus* (on average, P_{99}=52.0, H_0=0.159, H_e=0.159; Goncharenko and others 1989). The average values for genetic variation of P. *koraiensis* are intermediate among pine species of subsection *Cembrae*. In particular, the values of expected heterozygosity for these species are: for *P. cembra* – 0.109 and 0.118, *P. sibirica* – 0.158 and 0.169, and *P. pumila* – 0.249 and 0.271. These values are in agreement with those of Politov and Krutovskii (1994) and Goncharenko and Silin (1997). A higher heterozygosity level (H_e=0.204) was also found in the only population of *P. albicaulis* studied (Politov and Krutovskii 1994).

Geographical patterns of the distribution of expected heterozygosity (fig. 3) and mean number of alleles per locus (fig. 4) show that Korean pine has a small number of centers of genetic variation. The largest of them is situated in the south of Sikhote Alin. Two small centers are located at the northwestern limit of natural range of Korean pine and the middle part of Sikhote Alin. As the Sikhote Alin and Low Amur mountain range populations have appreciable levels of genetic variation, this may serve as confirmation of the hypothesis of the long-term existence of Korean pine within these areas. In that region, the mean expected heterozygosity was higher than in populations of the Maliy Khingan - Kukan mountain ranges (table 1).

In the coastal region, the peripheral population Lesnoi possesses a lower heterozygosity. This can be explained by genetic drift due to the founder effect of populating a territory by a small number of individuals in recent history, possibly the result of the northward migration of the Korean pine during the Holocene along the narrow coastline (fig. 1, 2). Lower estimates of genetic variation in the Petrov's Island population can also be attributed to a founder effect of Korean pine colonizing the island. Apparently the population was established 9,500 to 9,800 years ago, during the Holocene period, when Korean pine appeared as a member of the mountain vegetation complex of Sikhote-Alin (Golubeva and Karaulova 1983; Korotkii and others 1997). At present, the Petrov's Island area encompasses 36 hectares, on which grow a few hundred Korean pine trees. Heterozygosity decrease has also been found in peripheral populations of other conifers, including *Pinus contorta* Dougl. ex Loud. (Yeh and Layton 1979), *Pinus rigida* Mill. (Guries and Ledig 1982), *Picea abies* (L.) Karst. (Bergmann and Gregorius 1979) and *Picea rubens* Sarg. (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 several studies (Yeh and Layton 1979; Guries and Ledig 1982; Hawley and DeHayes 1994).

Positive F_{IS} and F_{IT} values indicate that a deficiency of heterozygotes is typical for *P. koraiensis* populations and for the whole species (table 2). The deficiency of heterozygotes was also found in the south Korean populations, where the F_{IS} and F_{IT} values were 0.007 and 0.066, respectively (Kim and others 1994). 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 and others 1994; Politov and Krutovskii 1994; Changtragoon and Finkeldey 1995; Lee and others 1998).

The mean F_{ST} value (F_{ST}=0.018) was lower than the mean G_{ST} estimate for genus *Pinus* (G_{ST}=0.065; Hamrick and others 1992). The value indicates that 1.8 percent of the genetic variation is distributed among the Korean pine populations; in other words, the majority of the variation resides within populations and any prominent differentiation processes are absent between populations.

The estimates of Nm, averaged over all populations per locus, were well above 1.0 (ranged from 5.00 at Skdh-2 and Mdh-3 to 26.51 at Pgm-2 and 6-Pgd-2) with a mean of 10.98 migrants per generation (table 2). The gene exchange exceeded those of *P. koraiensis* in South Korea (Nm=3.987; Kim and others 1994) and most of the coniferous tree species (Govindaraju 1989; Goncharenko and Silin 1997). Animal dispersing of Korean pine seeds may explain the large values of Nm. Tomback and Schuster (1994) noted that dispersal of pine seeds by nutcrackers, *Nucifraga* (Corvidae), which occurs routinely over large distances, might result in higher

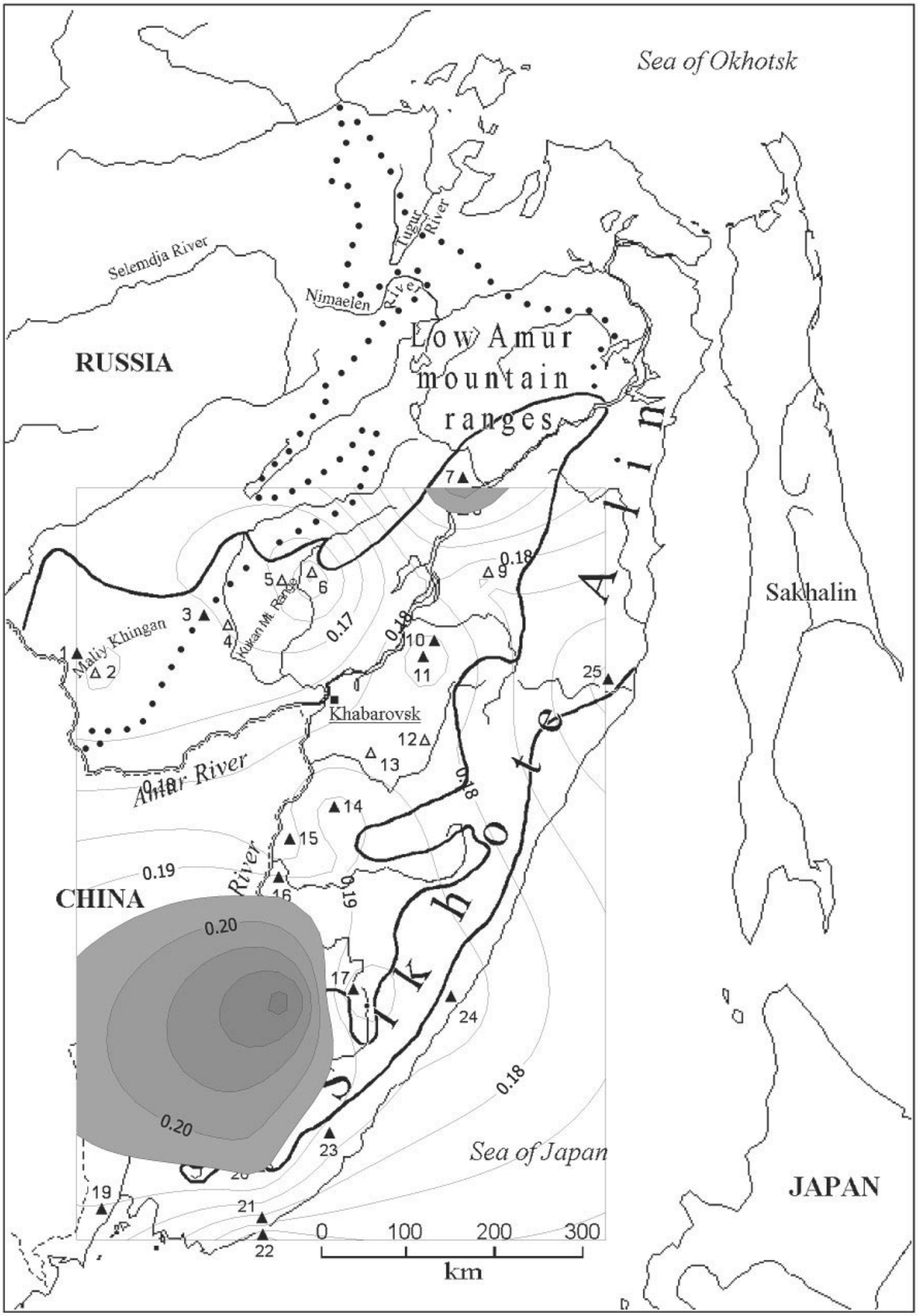

Figure 3—Geographical patterns of the distribution of expected heterozygosity. Grey scale gradient show the most variable parts of Korean pine natural range. Five levels of expected heterozygosity (0.195 to 0.200, 0.200 to 0.205, 0.205 to 0.210, 0.210 to 0.215, and above 0.215) are indicated.

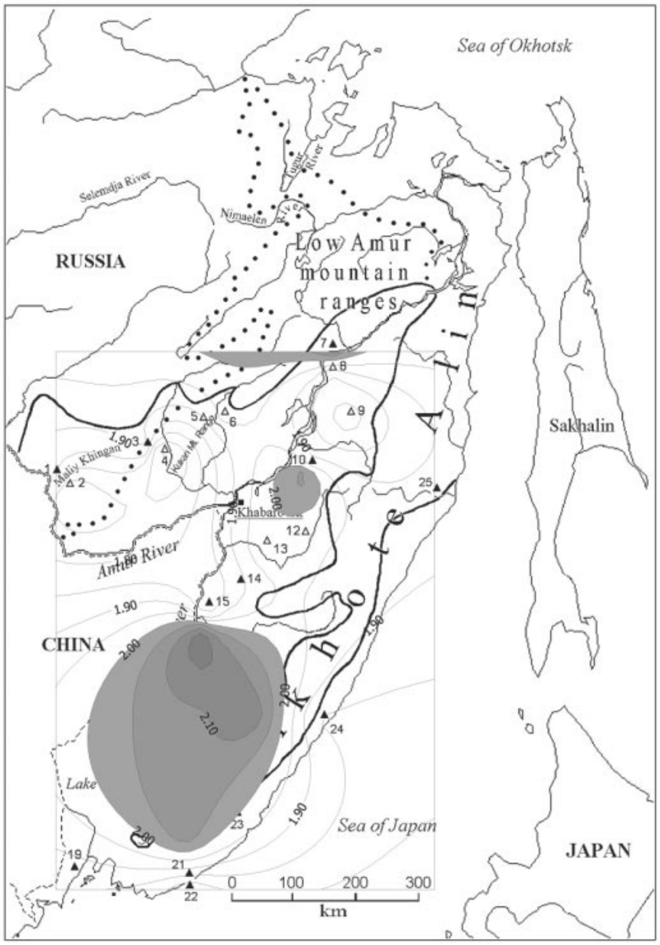

Figure 4—Geographical patterns of the distribution of mean number of alleles per locus. Grey scale gradient shows the most variable parts of Korean pine natural range. Four levels of mean number of alleles per locus (2.00 to 2.05, 2.05 to 2.10, 2.10 to 2.15, and above 2.15) are indicated.

Table 2—Deviation of genotype frequencies from Hardy-Weinberg proportions in individual populations (F_{IS}) and the total population (F_{IT}), degree of genetic differentiation among populations (F_{ST}), and degree of interpopulation gene flow (Nm).

Locus	F_{IS}	F_{IT}	F_{ST}	Nm
Aat-3	–0.019	0.004	0.023	6.80
Adh-1	–0.005	0.015	0.020	7.84
Adh-2	–0.009	0.012	0.022	7.11
Gdh	0.052	0.076	0.025	6.24
Lap-1	–0.051	–0.027	0.023	6.80
Lap-2	0.069	0.085	0.017	9.25
Pgm-1	0.025	0.039	0.014	11.27
Pgm-2	0.001	0.008	0.006	26.51
Skdh-1	0.009	0.025	0.016	9.84
Skdh-2	0.051	0.080	0.031	5.00
Mdh-2	–0.015	–0.004	0.011	14.39
Mdh-3	0.008	0.039	0.031	5.00
Mdh-4	0.115	0.133	0.020	7.84
6-Pgd-1	0.050	0.067	0.018	8.73
6-Pgd-2	0.001	0.008	0.006	26.51
Dia-1	0.002	0.022	0.020	7.84
Dia-3	0.018	0.043	0.026	5.99
Fl-Est	–0.008	0.007	0.015	10.51
Idh	0.002	0.011	0.009	17.62
Sdh	–0.001	0.009	0.009	17.62
Fdh	0.016	0.030	0.014	11.27
Aco	–0.002	0.011	0.013	12.15
Gpt	–0.013	0.002	0.015	10.51
Mean for 23 loci	0.013	0.030	0.018	10.98

Table 3—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
Obluchie	0.885 (0.044)	0.901 (0.041)	0.118	0.052
Galichnoe	1.018 (0.044)	1.001 (0.048)	0.069	0.000
Boicovo	1.042 (0.050)	1.031 (0.049)	0.029	-0.015
Malinovo	0.896 (0.043)	0.906 (0.038)	0.084	0.049
Ustinovka	0.863 (0.053)	0.861 (0.046)	-0.031	0.075
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

levels of gene flow between pine populations than from seed dispersal by wind.

Unbiased Nei's genetic distance values between the 25 populations of *P. koraiensis* were low, averaging 0.003. Low estimates of Nei's genetic distances confirm the close genetic relationship between investigated populations and indicate a widespread gene flow between populations.

Mating System Analysis

Single locus estimates of outcrossing ranged from 0.763 to 1.042, and multilocus estimates were from 0.751 to 1.031 (table 3). The lowest value t_m was found in the Petrov's Island population and the highest in the Boicovo population. Negligible differences were found between single locus and multilocus estimates of outcrossing in any population.

The mean multi-locus value of outcrossing in this study (t_m=0.909) was lower than that for the three populations (t_m=0.974) studied earlier 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 Ustinovka, Ternei and Lesnoi 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 a Hardy-Weinberg equilibrium. Any one of these estimates is much lower than those expected under inbreeding equilibrium, given the levels of t_m (table 3). The relationship between multilocus estimates of outcrossing (t_m) and fixation index (F_{IS}) shown in figure 5 suggests that the excess of heterozygotes in Korean pine populations is due to “pseudo-overdominance” as result of inbreeding depression (Ledig 1986), rather than selection in favor of heterozygotes (overdominance) as concluded by Politov and Krutovskii (1994) and Krutovskii and others (1995). These results showed that in Korean pine populations, the selection against inbred progeny appears when outcrossing rate is below 0.9 (fig. 5).

It is suggested that a contrary direction of selection occurs in populations with a deficit of heterozygotes and high outcrossing rates; that is, selection against heterozygotes. High outcrossing is probably maintained by a high migration rate between Korean pine populations (Nm=10.98). However, the validity of the suggested selection against heterozygotes in Korean pine needs to be field-proven by making biparental crosses. Selection against hybrid forms of plants due to outbreeding depression is found in crosses between distant plants of *Delphinium nelsoni* (Price and Waser 1979) and *Lotus scoparius* (Montalvo and Ellstrand 2001).

Possible microsite differentiation of allele frequencies that would upwardly bias the fixation index cannot be excluded as an explanation of a deficit of heterozygotes in some Korean pine populations. If different subpopulations sustain different alleles, the allele frequencies will be maintained at a high level in the whole population (Brown 1979). This phenomenon explains the high level of heterozygosity in populations with a positive fixation index, although reliable conclusions can only be made after an investigation of the genetic parameters of subpopulations.

Thus the coastal Korean pine populations we sampled exhibit different levels of genetic variation and outcrossing. Results of this study lead to the conclusion that the genetic structure of the Korean pine populations is under the influence of a complex combination of microevolution factors, genetic drift, gene flow and natural selection.

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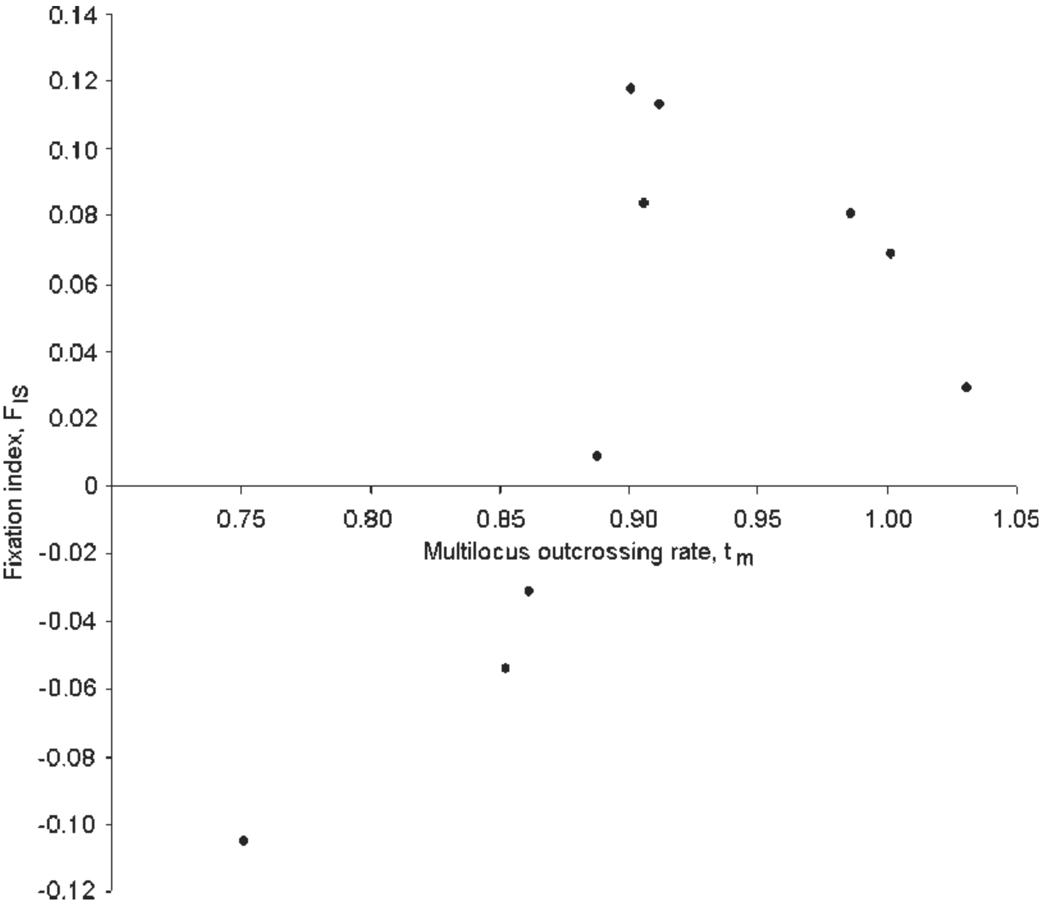

Figure 5—The relationship between multilocus estimates of outcrossing (t_m) and fixation index (F_{IS}).

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Part IV: White Pine Blister Rust Resistance

***Pinus albicaulis* (whitebark pine)**

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