Genetic Diversity and Differentiation of Natural Populations
of *Pinus koraiensis* (SIEB. et ZUCC.) in Russia

By V. V. POTENKO and A. V. VELIKOV

Department of Genetics and Breeding, Breeding and Seed Growing Forestry Center,
Zarechnaya 33, Sosnovka 682305, Khabarovsk Territory, Russia;
Department of Forest Genetics, Far East Forestry Research Institute,
Volochaevskaya 71, Khabarovsk 680020, Russia

Summary

Genetic diversity and genetic differentiation of nineteen Russian populations of Korean pine (*Pinus koraiensis* SIEB. et ZUCC.) were studied analyzing allozyme variation at 26 loci. On average, the mean number of alleles per locus was 1.93, the proportion of polymorphic loci ($P_{0.99}$) was 58.7%, the observed heterozygosity was 0.182 and the expected heterozygosity was 0.183. Only 1.5% of the total genetic variability was due to variation among populations. The mean value of NEI's genetic distance (0.003) confirmed that interpopulational differentiation is low. The resulting values of genetic variation were lower in the population group of the Maliy Khingan – Kukan mountain ranges, than in the population group of the Sikhote Alin – Low Amur mountain ranges. The high gene diversity of the Sikhote Alin and Low Amur mountain range populations may provide some support to the hypothesis about longterm existence of Korean pine in these areas.

Key words: allozymes, *Pinus koraiensis,* genetic diversity, genetic differentiation, colonization.

FDC: 165.3; 165.5; 181.1; 174.7 *Pinus koraiensis;* (571).

Introduction

The Korean pine, *Pinus koraiensis* SIEB. et ZUCC., occurs in natural and artificial stands of Russia, China, Korea, and Japan. In Russia, *P. koraiensis* is distributed in the Primorski Territory, in the southern part of Khabarovsk Territory, in the Jewish Autonomus Region and at the southeast end of the Amur Territory (*Figure 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 very different 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).

Intensive use and management of forests has led to development of strategies towards their conservation. Studying genetic variation of index species is the first step towards solving this problem (MILLAR and LIBBY, 1991). For the Korean pine-broadleaf forests *P. koraiensis* is one such index species. Recently, results of genetic variation in three *P. koraiensis* populations of Primorski and Khabarovsk Territories (KRUTOVSKII et al., 1990; POLITOV et al., 1992) and in eight populations from south Korea (KIM et al., 1994) have been reported. It had been supposed that genetic variation of *P. koraiensis* in south Korea would be much greater than in the Russian Far East. But differencies in electrophoretic procedures and isozyme loci studied in the two labs prevented an accurate comparison of genetic variation in the two regions (KIM et al., 1994).

Hence, to make a comparison of genetic data from different parts of Korean pine range it was the objectives of this work to estimate the level of genetic variability and differentiation of natural populations of *P. koraiensis* in Russia.

Materials and Methods

Seeds for electrophoresis were collected in 19 native populations from 44° to 51° latitude north (*Figure 2*). In 11 populations the collection of seeds was performed from individual trees, isolated each other more than 20 m. The remaining eight populations were represented by seed lots that were collected from native populations by state forest farms for artificial reforestation (*Table 2*).

Six megagametophytes per tree or 70 megagametophytes per seed lot were subjected to horizontal starch gel (12.5%) electrophoresis. Each megagametophyte was ground in 100 µl 5 mM tris-HCl (pH-8.0) extraction buffer containing 1 mM ascorbic acid, 1% PVP-40 and 1% Triton X-100. Enzymes in liquid extracts from individual seeds were separated simultaneously in three modified buffer systems:

A) Tris-EDTA-borate, pH 8.6: stock buffer – 900 mM tris/500 mM boric acid/20 mM EDTA; gel buffer – dilute 50 ml of stock buffer to 1 liter; electrode buffer – dilute 200 ml of stock buffer to 1 liter (GURIES and LEDIG, 1978);

B) Tris-citrate, pH 6.2: stock buffer – 220 mM tris/86 mM citric acid, adjust to pH 6.2 with 220 mM tris solution; gel buffer – dilute 35 ml of stock buffer to 1 liter; electrode buffer – stock buffer without dilution (ADAMS and JOLY, 1980);

C) Tris-HCl, pH 8.0/ tris citrate, pH 6.2: gel buffer – 220 mM tris/500 mM tris-HCl, pH 8.0 (dilute 33 ml of buffer to 1 liter); electrode buffer – stock buffer B without dilution (GONCHARENKO and POTENKO, 1990).

Recipes for enzyme staining follow standard methods (CHELIAK and PITEL, 1984) with non-significant modifications. The enzymes assayed, their abbreviations, the buffer systems used, and the number of consistently scorable loci are given in *table 1*.

Allele frequencies were analysed using the BIOSYS-1 (SWOFFORD and SELANDER, 1989) computer program. For each population, mean sample size per locus, 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. Chi-square tests of homogenity for allele frequency variation among populations and NEI's genetic distance (NEI, 1978) were calculated as well.

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. Calculation of the F-statistics and observed heterozygosity (H_o) were performed for the
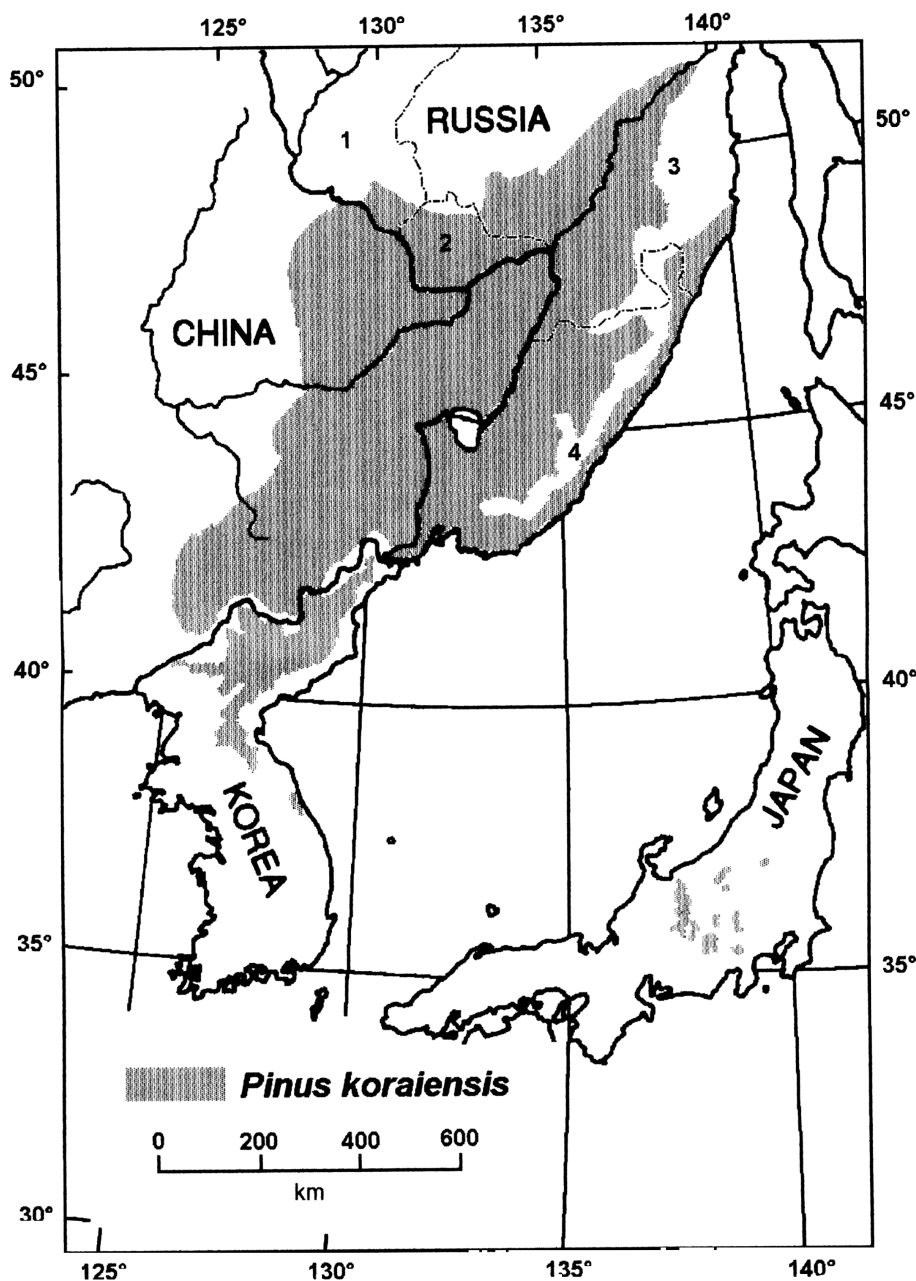
Figure 1. – Distribution of Korean pine (modified from SCHMIDT, 1994). Nos.: 1 – Amur Territory; 2 – Jewish Autonomus Region; 3 – Khabarovsk Territory; 4 – Primorski Territory.

11 populations where individual tree seed collections were conducted (*Figure 2*).

Results

Staining of the 15 enzymes revealed gene products of 26 loci. Parameters of genetic variation were calculated on the basis of frequencies of 81 alleles (*Table 1*). The mean number of alleles per locus ranged from 1.65 to 2.19, and averaged 1.93. The proportion of polymorphic loci ($P_{0.99}$) ranged from 50.0% to 69.2%, with an average of 58.7%. The observed heterozygosity was from 0.164 to 0.199, with an average of 0.182. The mean value of expected heterozygosity was higher and amounted to 0.183, with variation from 0.154 to 0.218. The resulting values of genetic variation were lower in the population group of the Maliy Khingan – Kukan mountain ranges (population nos. 1 to

6), than in the Sikhote Alin – Low Amur mountain ranges (population nos. 7 to 19) and on average they were respectively: mean number of allele per locus – 1.83 and 1.98, number of polymorphic loci ($P_{0.99}$) – 56.4 and 59.7, observed heterozygosity – 0.167 and 0.186, expected heterozygosity – 0.167 and 0.191 (*Table 2*).

Among the 11 populations, for which values H_o were calculated, in eight the average proportion of observed heterozygotes was lower than expected under the HARDY-WEINBERG equilibrium. Only in populations Mulcha (11), Arkhipovka (18) and Ustinovka (19), H_o values were higher than H_e, i.e. there was an excess of heterozygotes in these populations (*Table 2*).

F_{IS} values ranged from –0.084 for *Adh-2* to 0.159 for *Mdh-4*, with an overall mean of 0.016. F_{IT} values at loci *Aat-3, Adh-2* and *Mdh-2* were negative and at 20 loci were positive, reaching

0.177 at *Mdh-4*. On average F_{IT} was 0.031. Positive F_{IS} and F_{IT} values indicate, that relative to the HARDY-WEINBERG equilibrium a deficiency of heterozygotes is typical for *P. koraiensis* populations and the whole species. F_{ST} values at 26 loci was 0.015. Thus, about 98% of the total genetic variability resided within populations and only 1.5% – among populations (*Table 3*).

Chi-square tests of homogeneity for allele frequences in populations showed that there are no significant differencies at the *Adh-2, Pgm-2, Mdh-2, 6-Pgd-1, 6-Pgd-2, Idh, Sdh* and *Aco* loci. Among populations most heterogenous allele frequences were at *Aat-3, Adh-1, Gdh, Lap-1, Lap-2, Skdh-2, Mdh-3, Dia-1* and *Gpt* (*Table 3*).

Unbiased NEI's genetic distance values (D) were low between the 19 populations of *P. koraiensis* and averaged 0.003. The largest value (D = 0.011) was detected between Obluchie and Sukpay (1 to 12) and also between Kukan and Sukpay (4 to 12) populations that pertain to different population groups (*Table 4*).

Discussion

A comparison of genetic variation for Korean pine with that for other stone pines in the same subsection Cembrae and for other pines in general was conducted by KIM et al. (1994). Here a comparison can be made of the genetic variation between different geographic regions of Korean pine range.

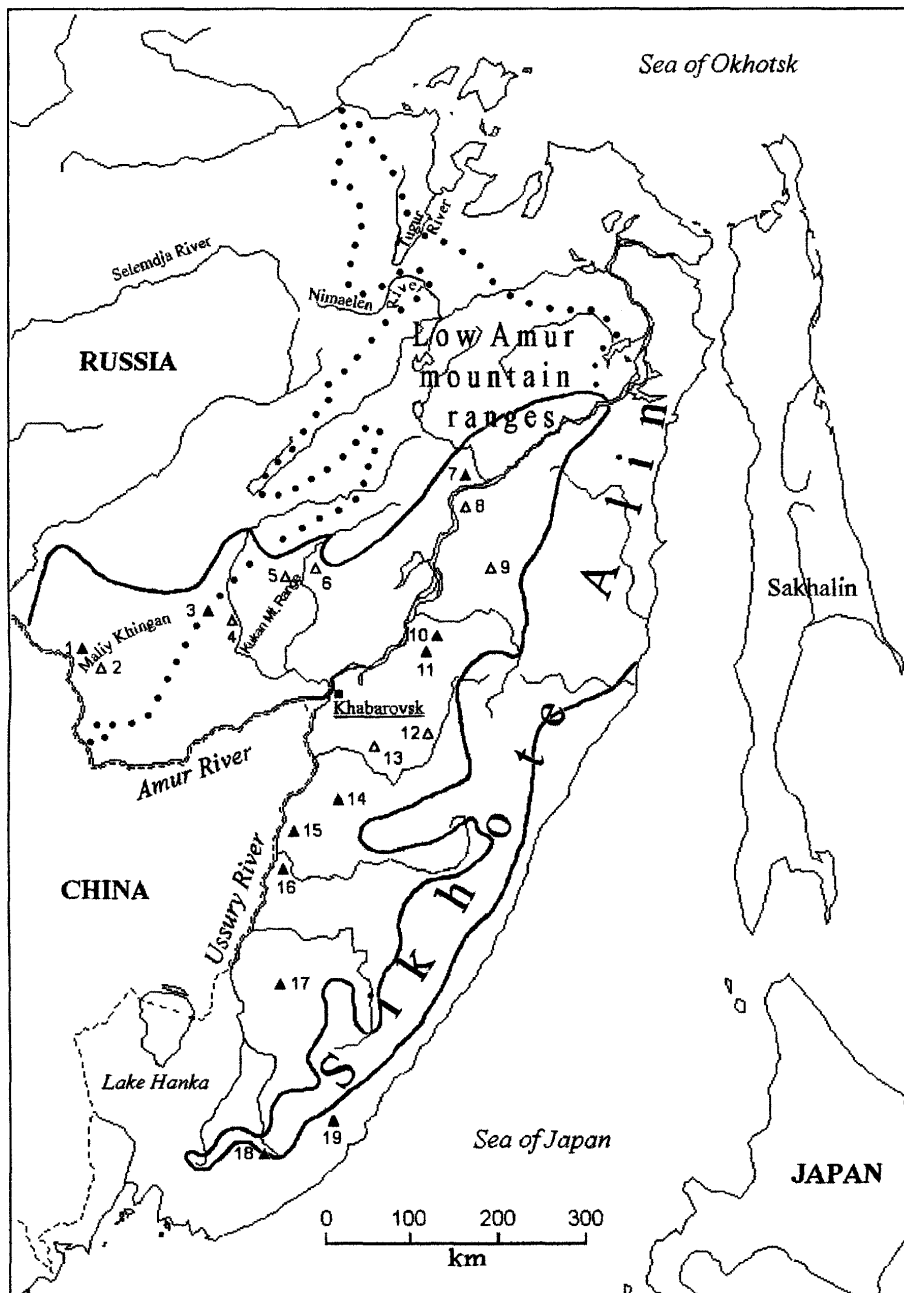*Figure 2.* – Location of the sampled populations: ▲ – seed collection was conducted from individual tree; △ – seed lot was sampled. Nos.: population numbers shown in *table 2*. Solid line: limit of distribution of *P. koraiensis* in Russia. Dotted line: northern limit of the Korean pine-broadleaf and Korean pine-spruce-larch mixed forests in the Holocene climate optimum – 6880 ± 270 years ago (modified from KOROTKII et al., 1997).

Table 1. – List of enzyme systems tested.

Enzyme	Abbreviation	E.C. No.	Buffer	No. of scored loci (No. of detected alleles)
Aspartate aminotransferase	AAT	2.6.1.1	A,C	1 (1) 2 (1) 3 (3)
Alcohol dehydrogenase	ADH	1.1.1.1	A	1 (4) 2 (3)
Aconitase	ACO	4.2.1.3	C	1 (3)
Glutamate dehydrogenase	GDH	1.4.1.2	A	1 (5)
Diaphorase	DIA	1.6.4.3	C	1 (3) 2 (3)
Isocitrate dehydrogenase	IDH	1.1.1.42	B,C	1 (3)
Leucine aminopeptidase	LAP	3.4.11.1	A	1 (4) 2 (12)
Malate dehydrogenase	MDH	1.1.1.37	C	1 (1) 2 (3) 3 (2) 4 (3)
Glutamate pyruvate transaminase	GPT	2.6.1.2	C	1 (3)
Sorbitol dehydrogenase	SDH	1.1.1.14	A	1 (2)
Fluorescent esterase	FL-EST	3.1.1.2	A	1 (4)
Formiate dehydrogenase	FDH	1.2.1.2	A	1 (2)
Phosphoglucomutase	PGM	2.7.5.1	A,C	1 (3) 2 (2)
6-Phosphogluconate dehydrogenase	6-PGD	1.1.1.44	B	1 (2) 2 (2)
Shikimate dehydrogenase	SKDH	1.1.1.25	C	1 (4) 2 (3)

Table 2. – Genetic variability at 26 loci in 19 populations of *P. koraiensis* (standard errors in parentheses).

Population	Mean No. of trees	Mean No. of alleles per locus (A)	Percentage of polymorphic loci (P_{95})	Percentage of polymorphic loci (P_{99})	Mean heterozygosity Observed (H_o)	Mean heterozygosity Expected (H_e)[1]
1. Obluchie	20.3 (.5)	1.85	46.2	61.5	0.170	0.171
2. Sutara	70[2]	1.69	42.3	57.7	-	0.169
3. Dogordon	51.5 (.3)	1.92	50.0	53.8	0.164	0.171
4. Kukan	70[2]	1.65	46.2	50.0	-	0.170
5. Niran	70[2]	1.88	46.2	61.5	-	0.165
6. Selgon	70[2]	1.96	42.3	53.8	-	0.154
7. Galichnoe	71.7 (.1)	2.12	57.7	69.2	0.193	0.204
8. Pivan	70[2]	1.85	53.8	53.8	-	0.194
9. Innokentievka	70[2]	1.77	46.2	53.8	-	0.174
10. Burga	52.2 (.3)	1.92	50.0	53.8	0.174	0.187
11. Mulcha	61.4 (.5)	2.08	46.2	57.7	0.191	0.189
12. Sukpay	70[2]	1.96	50.0	61.5	-	0.181
13. Obor	70[2]	2.00	53.8	61.5	-	0.183
14. Medvezhy	48.6 (.2)	1.88	53.8	57.7	0.180	0.194
15. Boicovo	23.6 (.3)	1.81	46.2	53.8	0.164	0.181
16. Pokrovka	69.2 (2.1)	2.19	50.0	61.5	0.188	0.193
17. Malinovo	48.3 (.8)	2.08	53.8	69.2	0.189	0.218
18. Arkhipovka	49.2 (1.0)	2.08	50.0	61.5	0.199	0.197
19. Ustinovka	59.2 (1.9)	2.00	50.0	61.5	0.193	0.187
Mean		1.93 (0.14)	49.2 (4.1)	58.7 (5.3)	0.182 (0.012)	0.183 (0.015)
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. 7-19 Sikhote Alin - Low Amur mountain ranges		1.98 (0.13)	50.9 (3.5)	59.7 (5.3)	0.186 (0.011)	0.191 (0.011)

[1] unbiased estimate (see NEI, 1978)
[2] number of analysed seeds per seed lot

According to our data Korean pine has a significant amount of genetic diversity: our measures were significantly higher than those obtained by KRUTOVSKII et al. (1990) at three Sikhote Alin and Sikhote Alin spur's populations. In our work the mean number of alleles per locus was 1.98 against 1.69 obtained by KRUTOVSKII et al. (1990), the number of polymorphic loci ($P_{0.99}$) was 59.7 against 47.9, and expected heterozygosity – 0.191 against 0.123. In that study *Lap-2, Lap-3* and *Dia-1* loci having highest levels of heterozygosity were excluded. Basing on the description given by KRUTOVSKII et al. (1987)

they seems to be analogous to *Lap-1, Lap-2* and *Dia-1* in our study. Besides this KRUTOVSKII et al. (1990) sampled about 19 trees per population, explored 16 loci, and analyzed embryos at 12 loci. Thus, the observed differences in values of diversity were more likely caused by differences in measurements, than by differences in genetic variation of the populations.

It turned out that levels of genetic variation were different between samples from different geographical regions of the Russian Far East. The populations of the Sikhote Alin – Low Amur mountain ranges were more variable. In that region the

Table 3. – F-statistics for 23 polymorphic loci and test of homogeneity of allele frequency.

Locus	F_{IS}	F_{IT}	F_{ST}	χ^2	df	P
Aat-3	-0.024	-0.002	0.022	82.814	28	***
Adh-1	0.028	0.043	0.015	102.917	42	***
Adh-2	-0.084	-0.068	0.015	32.061	28	ns
Gdh	-0.023	0.005	0.028	116.213	56	***
Lap-1	0.034	0.056	0.023	87.183	42	***
Lap-2	0.122	0.133	0.013	193.391	112	***
Pgm-1	0.048	0.058	0.010	44.774	28	*
Pgm-2	0.002	0.008	0.006	8.705	14	ns
Skdh-1	0.000	0.017	0.017	68.417	42	**
Skdh-2	0.044	0.090	0.049	72.669	14	***
Mdh-2	-0.021	-0.007	0.014	37.737	28	ns
Mdh-3	0.012	0.053	0.042	59.173	14	***
Mdh-4	0.159	0.177	0.022	51.748	28	**
6-Pgd-1	0.049	0.056	0.007	11.820	14	ns
6-Pgd-2	0.002	0.008	0.006	8.143	14	ns
Dia-1	0.010	0.027	0.017	65.589	28	***
Dia-3	0.042	0.060	0.019	44.522	28	*
Fl-Est	-0.005	0.008	0.013	58.628	42	*
Idh	0.003	0.012	0.009	12.663	14	ns
Sdh	-0.001	0.008	0.009	13.209	14	ns
Fdh	0.050	0.062	0.012	24.270	14	*
Aco	-0.003	0.009	0.012	30.884	28	ns
Gpt	-0.019	0.004	0.023	61.591	28	***
Mean for 23 loci	0.016	0.031	0.015			

*) $p < 0.05$; **) $p < 0.01$; ***) $p < 0.001$

Table 4. – Estimates of NEI's (1978) genetic distance based on data from 26 loci among 19 populations of *P. koraiensis*.

	Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	Obluchie	****	0.002	0.004	0.004	0.000	0.003	0.004	0.004	0.007	0.006	0.006	0.011	0.007	0.004	0.004	0.005	0.003	0.003	0.004
2	Sutara		****	0.001	0.001	0.000	0.001	0.001	0.000	0.003	0.000	0.001	0.005	0.002	0.001	0.000	0.000	0.001	0.001	0.001
3	Dogordon			****	0.003	0.002	0.004	0.004	0.002	0.006	0.006	0.003	0.008	0.004	0.007	0.002	0.005	0.004	0.003	0.004
4	Kukan				****	0.004	0.004	0.003	0.002	0.008	0.006	0.004	0.011	0.002	0.001	0.002	0.004	0.005	0.005	0.005
5	Niran					****	0.002	0.003	0.002	0.006	0.005	0.005	0.009	0.003	0.004	0.003	0.005	0.004	0.005	0.005
6	Selgon						****	0.001	0.001	0.001	0.002	0.002	0.003	0.002	0.002	0.000	0.001	0.003	0.003	0.003
7	Galichnoe							****	0.002	0.002	0.002	0.002	0.004	0.002	0.001	0.000	0.001	0.002	0.002	0.003
8	Pivan								****	0.001	0.000	0.001	0.002	0.001	0.000	0.000	0.000	0.000	0.002	0.002
9	Innokentievka									****	0.000	0.001	0.001	0.002	0.001	0.000	0.000	0.002	0.002	0.004
10	Burga										****	0.001	0.002	0.002	0.000	0.000	0.000	0.000	0.003	0.003
11	Mulcha											****	0.004	0.001	0.003	0.001	0.001	0.003	0.002	0.003
12	Sukpay												****	0.005	0.006	0.003	0.002	0.006	0.006	0.006
13	Obor													****	0.000	0.001	0.002	0.003	0.006	0.008
14	Medvezhy														****	0.000	0.001	0.000	0.004	0.004
15	Boicovo															****	0.000	0.000	0.000	0.001
16	Pokrovka																****	0.000	0.001	0.001
17	Malinovo																	****	0.001	0.002
18	Arkhipovka																		****	0.000
19	Ustinovka																			****

Region	No. of populations	1	2
1. Maliy Khingan-Kukan mountain range	6	0.002 (0.000- 0.004)	0.004 (0.000- 0.011)
2. Sikhote Alin -Low Amur mountain range	13	****	0.002 (0.000- 0.008)

mean expected heterozygosity was 14% higher than in populations of the Maliy Khingan – Kukan mountain ranges (*Table 2*).

In spite of the obtained high values of variation, the value of mean number of alleles per locus was lower than in the south Korean populations which had 2.0 (KIM et al., 1994), against 1.93 in the Russian populations. Other values of variation were also lower in our study: the number of polymorphic loci ($P_{0.99}$) was 69.0% in the Korean populations, against 58.7% in Russian; observed heterozygosity – 0.200 against 0.182; expected heterozygosity – 0.208 against 0.183. Thus, the difference between south Korean and Russian populations in values of genetic variation is not as significant as was reported in the comparison of KRUTOVSKII et al. (1990) data with that of KIM et al. (1994). It is also interesting to note that the obtained values of variation at 16 common loci [*Aco, Fl-Est, Gdh, Aat-1, Aat-2, Aat-3, Idh, Lap-1, Mdh-1, Mdh-2, Mdh-3, Mdh-4, Dia-1* (analogous to *Mnr-A* of KIM et al., 1994), *Pgm-1, Skdh-1* and *Skdh-2*] were also lower in Russian Far East populations than in the Korean ones. This points to comparability of study results of genetic variation at 23 loci in south Korea with that at 26 loci in Russia.

Positive F_{IS} and F_{IT} values indicate that deficiency of heterozygotes is typical for *P. koraiensis* populations and the whole species. 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 et al., 1994). Unfortunately, without a study of the mating system of this species it is now dificult to answer the question as to the reasons for the deficiency of heterozygotes in south Korean and Russian populations?

The obtained data are not in agreement with the earlier results for three *P. koraiensis* populations in Russia (POLITOV et al., 1992). For these populations an excess of heterozygotes is reported (F_{IS} = –0.077, F_{IT} = –0.033).

The mean F_{ST} value was 0.015; much lower than values found for south Korean populations (KIM et al., 1994) and for three populations in Russia (POLITOV et al., 1992), where the values were 0.059 and 0.040, respectively.

On the average NEI's genetic distances were also lower among Russian (D = 0.003) than among south Korean populations (D = 0.010). Genetic distances turned out to be the lowest for conifers (GONCHARENKO et al., 1989).

These major differences between diversity values among populations and NEI's distances in south Korea and Russia may be caused by the influence of genetic drift in south Korean populations which are distinguished by scattered distribution.

Despite differences in variation indices between populations from different geographic regions of Russia, differentiation among regions was not significantly, but higher than differentiation within regions (*Table 5*). To obtain a more sensitive model of population differentiation from different geographic regions it is necessary to use methods of organelle DNA analyses (HIPKINS et al., 1994). In particular RAPDs of mtDNA turn out to be more effective markers in revealing genetic

differences between Douglas-fir races than allozymes (AAGAARD et al., 1995).

As reported by HAMRICK et al. (1992), evolutionary history of the species must have a major influence on genetic diversity. Woody species that originated from a limited number of individuals or have spent a considerable proportion of their evolutionary history as small, isolated populations should have less variation at the species and population level and more variation between populations than species with a history of larger, more continuously distributed populations.

Several Korean pine colonization attempts of the Russian Far East territories in the Holocene period are hypothetised. NEISHTADT's (1957) pollen analysis from Pleistocene peat sediments has shown that *P. koraiensis* grew beyond the present range in the Russian Far East. In the early Holocene period (7700 to 9800 years ago) the limits of distribution had moved north of Hanka lake and in the middle Holocene period (2500 to 7700 years ago) – further north toward the mouth of the Ussury river. The species achived its greatest development in the late Holocene period (0 to 2500 years ago) moving far to the north (NEISHTADT, 1957).

The latest studies of fossil conifer pollen from the lower reaches of the Amur river "clearly give evidence to the presence of Korean pine in the phytocoenoses of the south Soviet Far East during the entire Quaternary. The only change was in the density of the interbreeding populations" (URUSOV, 1995).

The studies of KOROTKII et al. (1997) indicate that 18 000 to 20 000 years ago the vegetation of Sikhote Alin was similar to that of contemporary north-west coast of the Sea of Okhotsk. After the cooling of climate 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 Nima-elen rivers. As can be seen, the range of *P. koraiensis* was much wider than presently (*Figure 2*). Southward decline of Korean pine occured because of the cooler climate periods in the middle and late Holocene, resulting in the expantion of taiga boreal forests with *Picea, Abies,* and *Larix* species.

The mixed birch and larch stands, rare forests combined with tundra phytocenoses and a wide mountain tundra zone were characteristic of the middle Sikhote Alin during the recent period of climate cooling (about 18 000 to 20 000 years ago) as described in the work of GOLUBEVA and KARAULOVA (1983). More than 9800 years ago the Korean pine-broadleaf forests appeared in the low mountain zone of the pre-Boreal Holocene period. The studies of GOLUBEVA and KARAULOVA (1983) and KOROTKII et al. (1997) indicate a recolonization history of *P. koraiensis* as opposed to the colonization hypothesis of NEISHTADT (1957).

We did not find a decline of *P. koraiensis* genetic variation from south to north in Russia as could be expected from the south to north colonization history by NEISHTADT (1957). For example, in the most northern (Galichnoe) population the levels of variation has exceeded some southern populations (*Table 2*), and was comparable with those found in south Korea (KIM et al., 1994).

As the Sikhote Alin and Low Amur mountain range populations have comparable levels of variation to south Korea's, it may serve as confirmation of the hypothesis of longterm existence of Korean pine within these areas (*Figure 2*). The lower values of genetic variation within the Maliy Khingan – Kukan mountain range populations are likely evidence of recent colonization of these territories by *P. koraiensis* and/or of the influence of geographic barriers.

Interestingly the present expansion of the species range (URUSOV, 1995) or a continued expansion of Korean pine (NEISHTADT, 1957) to the north have been suggested by both hypotheses. The discovered lower values of genetic variation in the Maliy Khingan – Kukan mountain range populations do not contradict these assuptions, at least for this part of the *P. koraiensis* range.

Were we to compare the obtained results with the history of the studied species in the recent period of climate cooling, a lack of Sikhote Alin Korean pine refugium would have been corroborated by low F_{ST} (*Table 3*) and D values (*Table 4*).

At the same time in order to reconstruct a more correct model of *P. koraiensis* diversity distribution it is nessesary to research the populations in China and north Korea.

Acknowledgements

Literature

AAGAARD, J. E., VOLLMER, S. S., SORENSEN, F. C. and STRAUSS, S. H.: Mitochondrial DNA products among RAPD profiles are frequent and strongly differentiated between races of Douglas-fir. Molecular Ecology **4**: 441–447 (1995). — ADAMS, W. T. and JOLY, R. J.: Genetics of allozyme variants in loblolly pine. Heredity **71**: 33–40 (1980). — CHELIAK, W. M. and PITEL, J. A.: Techniques for starch gel electrophoresis of enzymes from forest tree species. Petawawa National For. Institute, Can. For. Serv., Information Rep. PI-X-42 (1984). — GOLUBEVA, L. V. and KARAULOVA, L. P.: Vegetation and climatostratigraphy of pleistocene and holocene of the USSR Far East South. Nauka, Moskow (1983). — GONCHARENKO, G. G., PADUTOV, V. E. and POTENKO, V. V.: Guide to conifer species research by isozyme methods. Byelorussian For. Res. Inst., Gomel (1989). — GONCHARENKO, G. G. and POTENKO, V. V.: Genetic diversity and differentiation in populations of Norway spruce *Picea abies* (L.) KARST. from Ukraine, Byelorussia, and Latvia. Proc. Acad. Sci. USSR **314**: 492–496 (1990). — GURIES, R. P. and LEDIG, F. T.: Inheritance of some polymorphic isoenzymes in Pitch pine (*Pinus rigida* MILL.). Heredity **40**: 27–32 (1978). — HAMRICK, J. L., GODT, M. J. W. and SHERMAN-BROYLES, S. L.: Factors influencing levels of genetic diversity in woody plant species. New Forests **6**: 95–124 (1992). — HIPKINS, V. D., KRUTOVSKII, K. V. and STRAUSS, S. H.: Organelle genomes in conifers: structure, evolution, and diversity. Forest Genetics **1**: 179–189 (1994). — KIM, Z.-S., LEE, S.-W., LIM, J.-H., HWANG, J.-W. and KWON, K.-W.: Genetic diversity and structure of natural populations of *Pinus koraiensis* (SIEB. et ZUCC.) in Korea. Forest Genetics **1**: 41–49 (1994). — KOROTKII, A. M., GREBENNIKOVA, T. A., PUSHKAR, V. S., RAZJIGAEVA, N. G., VOLKOV, V. G., GANZEY, L. A., MOKHOVA, L. M., BAZAROVA, V. B. and MAKAROVA, T. R.: Climatic changes in the Russian Far East during late Pleistocene-Holocene. Vestnik FEBRAS **3**: 121–143 (1997). — KRUTOVSKII, K. V., POLITOV, D. V. and ALTUKHOV, YU. P.: Genetic variability in Siberian cedar pine *Pinus sibirica* DU TOUR. I. Inheritance of isoenzyme systems. Soviet Genetics **23**: 2216–2228 (1987). — KRUTOVSKII, K. V., POLITOV, D. V. and ALTUKHOV, YU. P.: Genetic differentiation between Eurasian cedar pines for isozyme loci. Soviet Genetics **26**: 694–707 (1990). — MILLAR, C. I. and LIBBY, W. J.: Strategies for conserving clinal, ecotypic, and disjunct population diversity in widespread species. Genetics and conservation of rare plants. Ed. by FALK, D. A. and HOLSINGER, K. E.. Oxford Univ. Press. 149–170 (1991). — NEI, M.: F-statistics and analysis of gene diversity in subdivided populations. Ann. Hum. Genet. **41**: 225–233 (1977). — NEI, M.: Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**: 583–590 (1978). — NEISHTADT, M. I.: History of forests and paleogeography of the USSR in Holocene. Acad. Sci. USSR, Moskow (1957). — POLITOV, D. V., KRUTOVSKII, K. V. and ALTUKHOV, YU. P.: Characterization of gene pools of cembrae pines on the set of isozyme loci. Soviet Genetics **28**: 93–114 (1992). — SCHMIDT, W. C.: Distribution of stone pines. Proceedings of the International Workshop on Subalpine Stone Pines and Their Environment, the Status of Our Knowledge, 5 to 11 Sept. 1992, St. Moritz, Switzerland. USDA For. Serv. Gen. Tech. Rep. INT-GTR-309: 1, 6 (1994). — SWOFFORD, D. L. and SELANDER, R. B.: BIOSYS-1: A computer program for the analysis of allelic variation in population genetics and biochemical systematics. Release 1.7. Illinois Natural History Survey, IL (1989). — URUSOV, V. M.: Geographical distribution and variability of coniferous of the Russian Far East. Dalnauka, Vladivostok (1995). — USENKO, N. V.: Trees, shrubs and lianas of the Far East. Far East For. Res. Inst., Khabarovsk (1969).

Juvenile-Mature Correlations and Selection Effects on Clone Level After Stratified Family and Individual Selection of *Picea abies* (L.) KARST. Seedlings

By B. KARLSSON[1], K. LUNDKVIST[2] and G. ERIKSSON[2]

1. Abstract

A set of 130 clones of *Picea abies* planted in three field sites has been studied after 8 years. The ortets were representatively selected in 5 full-sib families. Estimates of ortet (nursery) – ramet (field) correlation were significant but low, r = 0.23. Correlation estimates vary dramatically between families. Different degrees of adaptation on family level are discussed as one possible explanation.

Clone x field site interaction components were significant and twice the size of the clone component for height increment, which is larger than found in other studies.

The gain in height increment in the field after selection for the 20% best ortets in the nursery was only 5.6%, which is only 15% of the gain achieved after field tests. That is considered too low to justify nursery selection. The selection response showed a large variation between full-sib families.

The conclusion of the study is that nursery selection of young ortets is too unprecise. Field testing on clonal level is required to obtain a sufficient genetic gain.

[1] The Forestry Research Institute of Sweden (SkogForsk), Ekebo, S-268 90 Svalöv, Sweden

[2] Department of Forest Genetics, Swedish University of Agricultural Sciences, Box 7027, S-750 07 Uppsala, Sweden