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Allozyme Variation in Natural Populations of Eurasian Pines

II. Genetic Variation, Diversity, Differentiation, and Gene Flow in Pinus sibirica Du Tour in Some Lowland and Mountain Populations

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Summary

Eight natural populations of *Pinus sibirica* were investigated by starch-gel electrophoresis. A total of 36 alleles were observed at 20 loci. Of the 20 loci, 55% were polymorphic. The mean observed and expected heterozygosity values were 0.173 and 0.176, respectively.

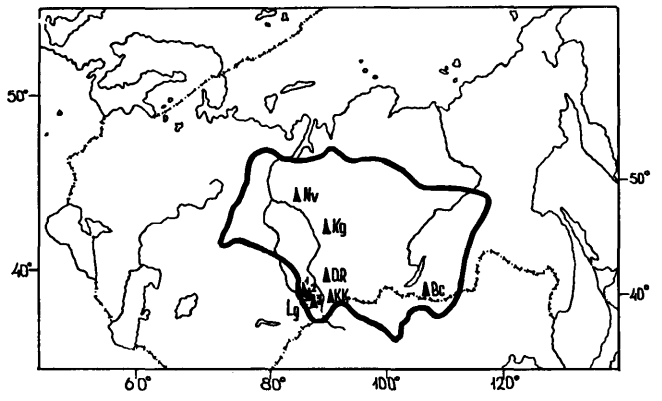

Figure 1. — Natural distribution of Sibirian stone pine (CRITCHFIELD and LITTLE, 1966) with locations of 8 populations samples. Lg1 — Leninogorsk-1, Lg2 — Leninogorsk-2, Lg3 — Leninogorsk-3, KK — Katon-Karagai, AR — Altai Reserve, Kg — Kargasok, Nv — Nizhnevartovsk, Bc — Bichura.

Interpopulation genetic diversity was about 4% of the total genetic diversity and the level of gene flow was 6.2. NEI's genetic distance coefficient ranged from 0.007 to 0.015 among populations and averaged 0.022. The data suggest that populations of *P. sibirica* (at least in the central, south-west, and south-east distribution) exchange genetic material and as a result have similar gene pools.

Key words: Pinus sibirica, isozymes, segregation, population structure, genetic variation, genetic differentiation.

Introduction

Pinus sibirica DU TOUR (Siberian stone pine) is 1 of the 5 species in subsection Cembrae (CRITCHFIELD and LITTLE, 1966). It is an economically important forest species. Almost the entire range of this species is in the territory of the former Soviet Union. *P. sibirica* populations occur both in the mountains and lowland bogs. To date, population studies in *P. sibirica* are based on phenotypic characters (IROSHNIKOV, 1974; KRYLOV et al., 1983) which are apparently controlled by many genes each of which may have different individual effects. These so-called quan-

Table 2. — Segregation of allozyme variants in *P. sibirica.*

Locus	Allele	Ratio	χ^2
Adh	0.85/1.00	140:171	3.09
Aat-2	0.95/1.00	13:22	2.31
Gpi	1.00/1.25	153:167	0.61
Dia-2	0.75/1.00	32:63	10.12*
	1.00/1.10	4:4	0.00
Lap-1	1.00/0	14:3	7.12*
Lap-2	0.70/1.00	74:97	3.09
Mdh-3	1.00/1.05	147:177	2.78
Mdh-4	0.80/1.00	36:41	0.32
	0.80/1.20	37:30	0.73
	1.00/1.20	162:112	9.12*
Fl-Est	1.00/2.00	136:115	1.76
Pgm-1	0.95/1.00	104:127	2.29
Skdh	0.95/1.00	59:60	0.01
	0.95/1.10	3:5	0.50
	1.00/1.10	6:8	0.29
	1.00/0	16:10	1.38

* level of significance < 0.01

Table 1. — Enzymes, their abbreviations (abbr.), Enzyme Comission Numbers (EC), number of loci scored (Scor.), and buffer systems used for electrophoresis.

Enzyme	Abbr.	EC no.	Scor. loci	Buffer
Alcohol dehydrogenase	ADH	1.1.1.1	1	A
Aspartate aminotransferase	AAT	2.6.1.1	3	A
Diaphorase	DIA	1.6.4.3	3	B
Fluorescent esterase	FL-EST	3.1.1.2	1	B
Glutamate dehydrogenase	GDH	1.4.1.2	1	A
Isocitrate dehydrogenase	IDH	1.1.1.42	1	B
Leucine aminopeptidase	LAP	3.4.11.1	2	A
Malate dehydrogenase	MDH	1.1.1.37	4	B
Phosphoglucomutase	PGM	2.7.5.1	2	A
Phosphoglucose isomerase	GPI	5.3.1.9	1	B
Shikimate dehydrogenase	SKDH	1.1.1.25	1	B

titative characters are also often strongly influenced by environmental variation. *P. sibirica*, like other species of Asian pines, is almost unstudied with respect to the widely used and exact methods of isozyme analysis.

In a previous study, we performed a genetic analysis of natural populations of *P. pumila* using a large set of allozyme loci (Goncharenko et al., 1993). In the present study, we used 20 allozyme loci to analyze genetic variation, differentiation, and gene flow among natural lowland and mountain populations of *P. sibirica*.

Materials and Methods

Materials

This study was based on seeds collected in 1983 to 1990 from 103 individual trees in two lowland populations of *P. sibirica* from the Tyumen Region (the Nizhnevartovsk forest division) and the Tomsk Region (the Kargasok forest division), and 6 mountain populations from the Rudny Altai (the Leninogorsk forest division), the South Altai (the Katon-Karagai forest division), the Central Altai (the Altai reserve), and Zabaikalye (the Bichura forest division). Locations of the populations sampled are shown in *figure 1*.

Isozyme analysis

Individual trees were genotyped using 8 to 20 megagametophytes plus some embryos for every locus. The megagametophytes and embryos were sampled randomly from a set of not less than 50 seeds extracted from 2 to 30 cones from each of the 103 trees. One of the Mdh loci was

Table 3. — Allele frequencies for 11 polymorphic loci in 8 populations of P. *sibirica*.

Locus[a]	Allele	Populations[b]							
		Lg1	Lg2	Lg3	KK	Kg	AR	Bc	Nv
		n[c]=11	n=16	n=10	n=9	n=11	n=23	n=11	n=12
Aat-2	0.95	0.045	0.000	0.000	0.056	0.000	0.000	0.000	0.000
	1.00	0.955	1.000	1.000	0.944	1.000	1.000	1.000	1.000
	H_o[d]	0.091	0.000	0.000	0.111	0.000	0.000	0.000	0.000
Adh	0.85	0.364	0.344	0.200	0.111	0.273	0.261	0.227	0.350
	1.00	0.636	0.656	0.800	0.889	0.727	0.739	0.773	0.650
	H_o	0.818	0.313	0.200	0.000	0.364	0.188	0.455	0.500
Gpi	1.00	0.545	0.625	0.650	0.833	0.864	0.727	0.955	0.708
	1.25	0.455	0.375	0.350	0.167	0.136	0.273	0.045	0.292
	H_o	0.727	0.375	0.700	0.333	0.091	0.545	0.091	0.417
Dia-2	0.75	0.091	0.156	0.100	0.111	0.136	0.000	0.000	0.000
	1.00	0.909	0.844	0.900	0.889	0.818	1.000	1.000	1.000
	1.10	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000
	H_o	0.182	0.313	0.200	0.222	0.364	0.000	0.000	0.000
Lap-1	0	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000
	1.00	1.000	1.000	1.000	1.000	0.955	0.957	1.000	1.000
	1.10	0.000	0.000	0.000	0.000	0.000	0.043	0.000	0.000
	H_o	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.000
Lap-2	0.70	0.045	0.188	0.100	0.278	0.045	0.174	0.227	0.000
	1.00	0.955	0.812	0.900	0.722	0.955	0.826	0.773	1.000
	H_o	0.091	0.438	0.200	0.333	0.091	0.348	0.273	0.000
Skdh	0	0.000	0.100	0.083	0.000	0.045	0.045	0.000	0.000
	0.95	0.333	0.500	0.417	0.667	0.227	0.114	0.318	0.125
	1.00	0.500	0.350	0.500	0.333	0.636	0.795	0.182	0.875
	1.10	0.167	0.050	0.000	0.000	0.091	0.045	0.500	0.000
	H_o	0.833	0.400	0.500	0.667	0.636	0.409	0.545	0.250

Locus	Allele	Populations							
		Lg1	Lg2	Lg3	KK	Kg	AR	Bc	Nv
Mdh-3	1.00	0.455	0.656	0.550	0.625	0.864	0.841	0.636	0.833
	1.05	0.545	0.344	0.450	0.375	0.136	0.159	0.364	0.167
	H_o	0.909	0.688	0.500	0.500	0.273	0.227	0.727	0.333
Mdh-4	0.80	0.091	0.063	0.150	0.000	0.045	0.091	0.091	0.150
	1.00	0.682	0.656	0.450	0.500	0.909	0.727	0.409	0.550
	1.20	0.227	0.281	0.400	0.500	0.045	0.182	0.500	0.300
	H_o	0.636	0.563	0.700	0.778	0.182	0.455	0.727	0.500
Fl-Est	1.00	0.500	0.611	0.375	0.667	0.636	0.565	0.636	0.273
	2.00	0.500	0.389	0.625	0.333	0.364	0.435	0.364	0.727
	H_o	0.333	0.111	0.250	0.333	0.364	0.609	0.727	0.545
Pgm-1	0.95	0.273	0.250	0.100	0.111	0.091	0.159	0.000	0.375
	1.00	0.727	0.750	0.900	0.889	0.909	0.841	1.000	0.625
	H_o	0.182	0.500	0.200	0.222	0.182	0.227	0.000	0.583

[a]) Locus of Aat-1, Aat-3, Gdh, Dia-1, Dia-3, Idh, Mdh-1, Mdh-2, Pgm-2 were monomorphic
[b]) Lg1 — Leninogorsk-1, Lg2 — Leninogorsk-2, Lg3 — Leninogorsk-3, KK-Katon-Karagai, Kg — Kargasok, AR — Altai reserve. Bc — Bichura, Nv — Nizhnevartovsk
[c]) number of trees analysed for a locus; in populations of Leninogorsk-1 and Katon-Karagai from 6 to 9 trees were analysed for the Skdh and Fl-Est loci
[d]) observed heterozygosity

expressed in the embryo tissues, and in this case, no less than 8 embryos from each tree were assayed.

Methods of enzyme extraction and electrophoresis followed Conkle et al. (1982), Cheliak and Pitel (1984), and Goncharenko et al. (1989). The enzymes were electrophoresed in a vertical chamber on 13% to 14% starch gel. For electrophoresis, two buffer systems were used: A) tris-EDTA-borate, pH 8.6, B) tris-citrate, pH 6.2 (electrode buffer)/tris-HCl, pH 8.0 (gel buffer) (Goncharenko et al., 1992).

Table 4. — Genetic variation in natural population of *P. sibirica*.

Populations	Percentage of loci polymorhic		Average number of alleles per locus	Percentage of heterozygous loci per individual	
	P_{95}	P_{99}	A	Expected (H_e)	Observed (H_o)
Leninogorsk-1	0.400	0.500	1.600	0.189	0.240
Leninogorsk-2	0.450	0.450	1.600	0.195	0.185
Leninogorsk-3	0.450	0.450	1.550	0.173	0.172
Katon-Karagai	0.500	0.500	1.500	0.162	0.175
Kargasok	0.400	0.500	1.700	0.134	0.132
Altai reserve	0.400	0.450	1.600	0.148	0.154
Bichura	0.300	0.350	1.450	0.145	0.177
Nizhnevartovsk	0.350	0.350	1.400	0.141	0.156
In total in the species	0.450	0.550	1.800	0.176	0.173

Table 5. — Genetic variation in pines.

Species	Percentage of loci polymorhic	Percentage of heterozygous loci per individual		References[a]
		Observed	Expected	
P.aristata	0.34	–	0.139	9
P.attenuata	0.55–0.73	–	0.087–0.131 (0.090)[b]	2,13,20
P.balfouriana	0.46	–	0.208	9
P.banksiana	0.58–0.70	0.115–0.207	0.104–0.192 (0.118)	4,18,22
P.brutia	0.43	–	0.118	3
P.contorta	0.59–0.90	0.161–0.184	0.120–0.185 (0.121)	2,4,9,21, 22,24
P.coulteri	0.36	–	0.148	10
P.eldarica	0.37	–	0.075	3
P.halepensis	0.15–0.23	–	0.040–0.042 (0.041)	3,19
P.jeffrey	0.68–0.90	–	0.203–0.261 (0.218)	2,6
P.muricata	0.31–0.47	–	0.085–0.141 (0.130)	12,13
P.oocarpa	0.65	–	0.270	13
P.pithyusa	0.30	–	0.097	3
P.ponderosa	0.54–0.71	0.138	0.124–0.226 (0.173)	1,9,15,16, 23
P.pumila	0.77	0.288	0.255	7
P.radiata	0.46–0.58	0.085–0.115	0.098–0.141 (0.122)	13,14,17
P.resinosa	0.11	–	0.007	1
P.rigida	0.73	0.138	0.146	8
P.sabiniana	0.73	–	0.128	10
P.sibirica	0.55	0.176	0.176	
P.stankewiczii	0.40	–	0.118	3
P.sylvestris	–	0.167	–	5
P.torreyana	0	0	0	11
P.washoensis	0.54	–	0.147	15
Mean			0.132	

[a]) 1. Allendorf et al., 1982. 2. Conkle, 1981. 3. Conkle et al., 1988. 4. Dancik and Yeh, 1983. 5. Dukharev et al., 1987. 6. Furnier and Adams, 1986. 7. Goncharenko et al., 1993. 8. Guries and Ledig, 1982. 9. Hamrick et al., 1981. 10. Ledig, 1986. 11. Ledig and Conkle, 1983. 12. Millar, 1983. 13. Millar et al., 1988. 14. Moran et al., 1988. 15. Niebling and Conkle, 1990. 16. O'Malley et al., 1979. 17. Plessas and Strauss, 1936. 18. Ross and Hawkins, 1986. 19. Schiller et al., 1986. 20. Strauss and Conkle, 1986. 21. Wheeler and Guries, 1982. 22. Wheeler and Guries, 1987. 23. Woods et al., 1983. 24. Yeh and Layton, 1979.

[b]) Average expected heterozygosity value in the species.

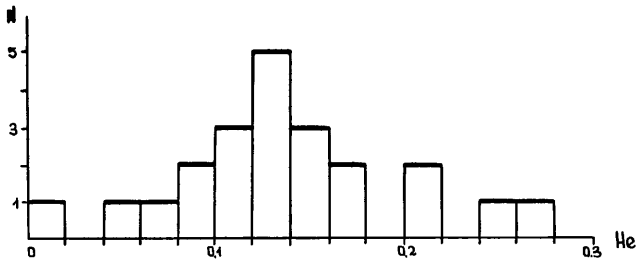

Figure 2. — Distribution of expected heterozygosity values for pines. N — number of species, H_e — expected heterozygosity.

Recipes for histochemical enzyme staining followed the standard methods (Conkle et al., 1982; Cheliak and Pitel, 1984) with insignificant modifications. The enzymes assayed, their abbreviations, the buffer systems used, and the number of loci consistently scorable are given in *table 1.*

Alleles were designated as described by Prakash et al. (1969). Within each locus, the most common allele and the corresponding allozyme in *P. sibirica* were designated with the arbitrary value 1.00. The other alleles were numbered according to the electrophoretic migration of allozymes relative to the commonest allozyme. Null alleles were designated by the symbol 0.

Statistical analysis

To estimate levels of genetic variation, differentiation, and gene flow in the populations studied, we used all the statistics earlier applied to *P. pumila* (Goncharenko et al., 1993): expected heterozygosity (H_e), observed heterozygosity (H_o), percent polymorphic loci (P_{99} — the frequency of the most common allele was not greater than 99% — and P_{95}), the mean number of alleles per locus (A), Nei's genetic distance coefficient (D_N), parameters of Wright's F-statistics and Nei's G-statistics (F_{IS}, F_{IT}, F_{ST}, and G_{ST}), and the gene flow parameter (N_em).

Results and Discussion

Segregation

All enzyme systems of *P. sibirica* studied were similar to those of *P. pumila* described earlier (Goncharenko et al., 1993), with the exception of ADH and SKDH. With regard to *P. sibirica*, we took into account only 1 locus for each of the above 2 enzymes.

Electrophoresis of 11 enzymes in the 8 populations of *P. sibirica* revealed 36 different electrophoretic variants. Analysis for segregation of haploid megagametophytes in heterozygous trees enabled us to establish that these variants were under gene control. A parent tree heterozygous for any locus produces haploid megagametophytes in a 1:1 segregation of allelic variants. The data on segregation of variants are presented in *table 2.* As a whole, the data support genetic control of the electrophoretic variants revealed in *P. sibirica.* Cases of distortion of the expected 1:1 segregation occurred for some allelic combinations at Dia-2, Lap-1, and Mdh-4.

Genetic variation

Allelic frequencies at 11 polymorphic loci of *P. sibirica* are listed in *table 3.* From this table it is obvious that in practically all the populations surveyed, 6 loci (Adh, Gpi, Mdh-3, Mdh-4, Skdh, Fl-Est) appeared to be highly polymorphic. Five more loci (Pgm-1, Dia-2, Lap-2, Aat-2 and Lap-1) were weakly polymorphic, and in some populations no variation was observed at these loci. In the case of Aat-2 and Lap-1, even in variable populations the frequency of the most common allele approximated to 95% (*Table 3*). Nine loci (Aat-1, Aat-3, Gdh, Dia-1, Dia-3, Idh, Mdh-1, Mdh-2 and Pgm-2) appeared to be entirely monomorphic.

On the basis of allelic frequencies for 20 loci, we computed parameters of genetic variation in the 8 populations of *P. sibirica* (*Table 4*). From the table it is seen that all parameters vary among the populations. The greatest differences were revealed for observed heterozygosity. In the Kargasok population, for example, observed heterozygosity was 0.132, while in one of the populations from the Leninogorsk forestry division, observed heterozygosity was 0.240. Such great differences in heterozygosity are apparently due to the restricted sample size analyzed in each population. The marginal populations of *P. sibirica* surveyed (Leninogorsk, Katon-Karagai, and Bichura forest divisions) appeared in total, more heterozygous, especially in observed heterozygosity, than those located in the main part of the species' distribution (Altai reserve, Kargasok and Nizhnevartovsk forest divisions). The small population sample sizes do not allow us to draw definitive conclusions from this interesting fact.

Despite differences among populations, the data allows us to objectively estimate the level of variation that is characteristic of *P. sibirica.* It appears that in *P. sibirica*

Table 6. — Estimates of F_{IS}, F_{IT}, F_{ST} and G_{ST} for 20 loci in *P. sibirica.*

Locus	F_{IS}	F_{IT}	F_{ST}	G_{ST}
Aat-1	0.000	0.000	0.000	0.000
Aat-2	-0.011	0.030	0.041	0.041
Aat-3	0.000	0.000	0.000	0.000
Adh	0.132	0.156	0.027	0.027
Gdh	0.000	0.000	0.000	0.000
Gpi	-0.111	-0.029	0.073	0.073
Dia-1	0.000	0.000	0.000	0.000
Dia-2	-0.084	-0.022	0.057	0.064
Dia-3	0.000	0.000	0.000	0.000
Idh	0.000	0.000	0.000	0.000
Lap-1	0.217	0.244	0.035	0.033
Lap-2	-0.099	-0.029	0.063	0.063
Mdh-1	0.000	0.000	0.000	0.000
Mdh-2	0.000	0.000	0.000	0.000
Mdh-3	-0.252	-0.137	0.092	0.092
Mdh-4	-0.159	-0.075	0.073	0.087
Fl-Est	0.034	0.096	0.065	0.065
Pgm-1	-0.023	0.059	0.080	0.080
Pgm-2	0.000	0.000	0.000	0.000
Skdh	-0.085	0.099	0.170	0.196
Mean	-0.022	0.020	0.039	0.041

Table 7. — Estimates of Nei's genetic distance coefficient, D_N, based upon data from 20 loci.

Populations	Lg1	Lg2	Lg3	KK	Kg	AR	Bc	Nv
Leninogorsk-1	–	0.008	0.010	0.026	0.024	0.019	0.032	0.022
Leninogorsk-2		–	0.010	0.012	0.017	0.016	0.024	0.027
Leninogorsk-3			–	0.014	0.026	0.018	0.024	0.020
Katon-Karagai				–	0.029	0.028	0.016	0.046
Kargasok					–	0.007	0.033	0.024
Altai reserve						–	0.032	0.014
Bichura							–	0.051
Nizhnevartovsk								–

55% of the loci are polymorphic and the mean number of alleles per locus is 1.8. The mean observed and expected heterozygosity values for 20 loci are 0.173 and 0.176, respectively. Among the pine species analyzed for 20 or more loci (*Table 5*), heterozygosity averages 0.132. The histogram (*Fig. 2*) constructed on the basis of the data from *table 5* shows that pines have a very wide spectrum of heterozygosity values that range from 0 (*P. torreyana*) to 0.27 (*P. oocarpa*). Thus, according to our data, *P. sibirica* is one of the most polymorphic species in the *Pinus* genus.

Analysis of genetic structure

On the basis of all 20 loci, the genetic structure of *P. sibirica* can be quantified by describing the correlation between uniting gametes within populations (F_{IS}), between populations (F_{ST}), for the species as a whole (F_{IT}), and by the ratio of diversity among populations to the total diversity (G_{ST}). The values of the parameters are presented in *table 6*.

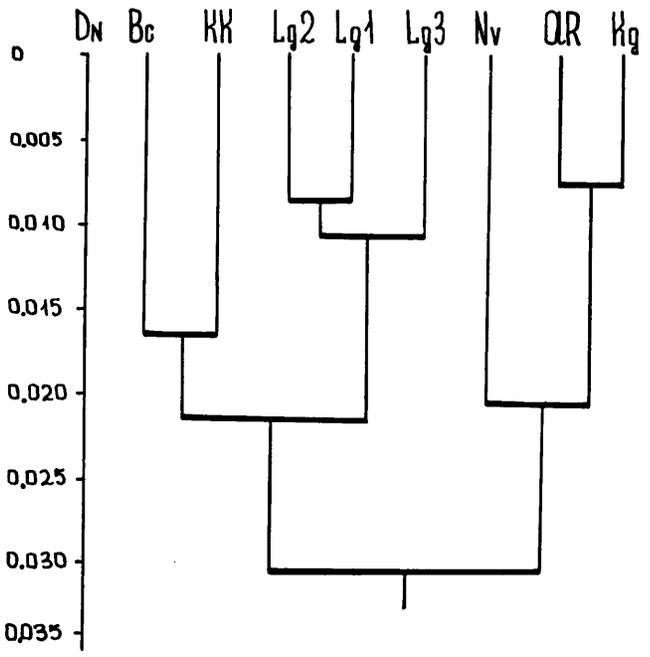

Figure 3. — Dendrogram showing the clustering of the 8 natural populations of *P. sibirica* based on Nei's genetic distance coefficient.

In *P. sibirica*, F_{IS} ranged from —0.252 at Mdh-3 to 0.217 at Lap-1 and averaged —0.022. F_{IT} averaged 0.020; i. e. there was a 2% deficiency of heterozygotes in *P. sibirica* as a whole.

For polymorphic loci, F_{ST} ranged from 0.027 (Adh) to 0.170 (Skdh). Mean F_{ST} for all the 20 loci assayed was equal to 0.039 (3.9%). G_{ST} was 0.041. This means that about 96% of the total genetic diversity is contained within populations of *P. sibirica* and only about 4% is due to interpopulation diversity.

F_{ST} and G_{ST} values listed in *table 6* show that there is no meaningful differentiation among natural populations of *P. sibirica*. To date, significant genetic diversity was revealed in a few pine species such as *P. attenuata, P. jeffreyi, P. monticola, P. muricata,* and *P. radiata* (Brown and Moran, 1981; Steinhoff et al., 1983; Furnier and Adams, 1986; Millar et al., 1988; Moran et al., 1988) in which from 12% to 22% is due to interpopulation variation. The highest value of diversity calculated using polymorphic loci, 100%, occurred in *P. torreyana* (Ledig and Conkle, 1983). In this species, among the 59 loci analyzed only 2 were polymorphic and alternative alleles were fixed in the two *P. torreyana* populations.

F_{ST} enables us to estimate the level of gene flow (N_em). Wright (1951) showed that F_{ST} and N_em are mathematically related as follows:

$$F_{ST} = 1/(1 + 4N_em)$$

(Slatkin, 1985). According to our data, N_em is 6.2. This means that the number of migrants exchanged between *P. sibirica* populations is over 6 per generation. Gene flow can be mediated both by seeds and pollen migration.

In any case, from our data it is evident that populations of P. sibirica, at least in the center, south-west and south-east of its distribution (*Fig. 1*), exchange genetic material and as a result maintain a cohesive gene pool.

Genetic distance

Using Nei's genetic distance coefficient (Nei, 1972), we estimated the level of genetic differentiation (D_N) among the populations of *P. sibirica*. Values of D_N based on the 20 loci are listed in *table 7*. Distance values among the populations ranged from 0.007 (Kargasok and Altai reserve) to 0.051 (Bichura and Nizhnevartovsk), averaging 0.022.

To better visualize the results, a dendrogram was constructed (*Fig. 3*). The D_N values were clustered using the unweighted pair group method (UPGMA). Contrary to

our expectations, the mountain and lowland populations did not form independent groups because the latter (Kargasok and Nizhnevartovsk) formed one cluster with the mountain population from the Altai reserve (*Fig. 3*). Moreover, in this cluster, populations from the Altai reserve and the Kargasok forest division are most similar (0.007). Clustering demonstrated that genetic distance among all the 8 populations does not, on average, exceed 0.030, which is a characteristic of geographically related pine populations within one species (GURIES and LEDIG, 1982; WHEELER and GURIES, 1982; DANCIK and YEH, 1983; LOUKAS et al., 1983; WOODS et al., 1983; Ross and HAWKINS, 1986; GONCHARENKO et al., 1989). Such an insignificant genetic distance value indicates that these populations of *P. sibirica* are genetically similar and supports our earlier suggestion that they share a common gene pool within the part of the range that we investigated.

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