ALLOZYME VARIATION AND PHYLOGENETIC RELATIONSHIPS IN TWO-NEEDLE PINES OF THE RUSSIAN FAR EAST

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ABSTRACT

Genetic variation and differentiation in four Scots pine natural populations (*Pinus sylvestris* L.), five funeral pine populations (*P. funebris* Kom.) and two Japanese red pine populations (*P. densiflora* Sieb. et Zucc.) occurring in the Russian Far East were studied on the basis of analysis for 24 allozyme loci. The genetic distances separating *P. sylvestris*, *P. funebris* and *P. densiflora* show close relation *P. funebris* and *P. densiflora* (mean $D_N = 0.033$). Funeral pine had admixtures of allozymes from both *P. sylvestris* and *P. densiflora.* The present results suggest that funeral pine can be regarded as a Japanese red pine variety of hybrid origin. The intrapopulation genetic variation values exhibited by marginal populations of the Far Eastern two-needle pines were lower than those exhibited by the populations from the central segments of the pines' distribution areas. This was supported by the data on the marginal coniferous populations obtained earlier. The level of genetic variation exhibited only by the two northern hybrid populations located in close proximity to the *P. sylvestris'* range appeared to be in general higher than those exhibited by the both parent species. Lower levels of intrapopulation variation in the southern hybrid populations in comparison with those in the northern ones may be explained by gene drift and limited gene flow due to small sizes and scattered distributions of the hybrid populations assayed.

Key words: allozymes, genetic variation, hybridization, *Pinus sylvestris*, *Pinus densiflora.*

INTRODUCTION

Three members of the subgenus *Diploxylon*, Scots pine *Pinus sylvestris* L., Japanese red pine *P. densiflora* Sieb et. Zucc. and funeral pine *P. funebris* Kom., occur in the Russian Far East (VOROB´EV 1968).

Scots pine is one of the most widespread coniferous species and occupies vast areas in Russia. In the Russian Far East it occurs at the limits of its distribution and creates isolated stands, mainly in Amur Territory and partially in Khabarovsk Territory and the Jewish Autonomous Region.

Japanese red pine grows on the Korean peninsula, some Japanese islands (Honshu, Shikoku and Kyushu), in northeast China and in the south of Primorski Territory (Russia). In Russia, *P. densiflora* occurs at the northeastern limit of the continental part of its natural range. It normally creates small stands on the southern exposure of steep slopes, with sufficiently debris-like soil, sometimes directly on the rocks (VOROB`EV 1968).

In 1901 KOMAROV (1949) described *P. funebris* as a novel species found in the southern part of the Ussury River basin and in the northern half of the Korean peninsula. The species differed from both Scots and Japanese red pines. Since this species grew throughout cemeteries in northern Korea, KOMAROV suggested naming it "funeral pine". However, in the book *Flora of the USSR* KOMAROV (1934) speculated that *P. funebris* was close to *P. densiflora* and funeral pine being mentioned as a northern geographic variety of Japanese red pine. Based on the analysis of the formation of generative organs of funeral pine URUSOV (1974) showed that *P. funebris* arose as a result of introgressive hybridization between Scots and Japanese red pines. More recently, based on the allozyme and chloroplast DNA analyses SZMIDT & WANG (1993) inferred that var. *sylvestriformis* (Takenouchi) occurring in China might be regarded as a form of *P. densiflora* arisen from introgressive hybridization between Scots and Japanese red pines. However, in review on vascular plants of the Russian Far East (KOROPACHINSKII 1989) funeral pine did not receive individual mention and NEDOLUZHKO (1995) regarded the funeral pine populations from the vicinity of the Khanka Lake as Scots pine. Hence the question of the taxonomic status of *P. funebris* and its phylogenetic relationships with *P. densiflora* and *P. sylvestris* is still debatable.

The allozyme analysis permits to reveal genetic differences between closely related taxa and to identify hybrids between them (WHEELER & GURIES 1987; YEH

& ARNOTT 1988; WANG *et al.* 1990; KOROL *et al.* 1995; POLITOV *et al.* 1999). However, investigation on genetic differentiation in closely related pines from the former Soviet Union (GONCHARENKO *et al.* 1995) did not resolve the problem of the taxonomic status of funeral pine and its phylogenetic relationships with the other Far Eastern two-needle pines due to the shortage of seed material.

The objectives of the current study were to identify the origin of *P. funebris*, to estimate the degree of similarity among the Far Eastern two-needle pines and to estimate the level of genetic variation in them.

MATERIALS AND METHODS

Seed material was collected from individual trees from four Scots pine natural populations, five funeral pine populations and two Japanese red pine populations. Geographic distributions of the taxa assayed with locations of the populations sampled are shown in Fig. 1 (A, B).

Seed material of Scots pine was collected from 19 trees occurring in the vicinity of the town of Svobodnyi (1) in 1993 and preliminarily analyzed at the Department of Molecular Genetics, Forest Institute of the National Academy of Sciences of Belarus (Gomel). In addition, in 2001 seeds were collected from 23 individuals growing in the vicinity of the village of Chegdomyn (2) and 25 tree individuals occurring on the southern coast of Lake Evoron (3). In 2002 seeds were collected from 24 trees growing in the vicinity of the village of Pashkovo (4).

In 2000 and 2001 cones were collected from 33 funeral pine individuals occurring on the northwestern coast of Lake Khanka in the vicinity of the village of Turii Rog (5), 25 trees growing in the vicinity of the village of Barabash – Levada (6), 27 trees occurring in the vicinity of the village of Nikolaevka (7), 25 trees growing throughout the upper reaches of the Osinovka River (8), and from all of 25 fruit-bearing individuals occurring in the vicinity of the village of Gornotayozhnoye (9).

In 2000 seed material of Japanese red pine was collected from 35 trees in the stands located on the rocks of Telyakovskii Bay on the Gamov Peninsula (11) and from 25 trees growing in the vicinity of the village Petrovka (10).

Individual trees were genotyped using six megagametophytes for every locus. Electrophoresis was carried out in a horizontal chamber according to the methods described by POTENKO & VELIKOV (1998). In the electrophoretic study of the pine populations, we used 24 loci encoding 15 enzymes: aspartate amino-

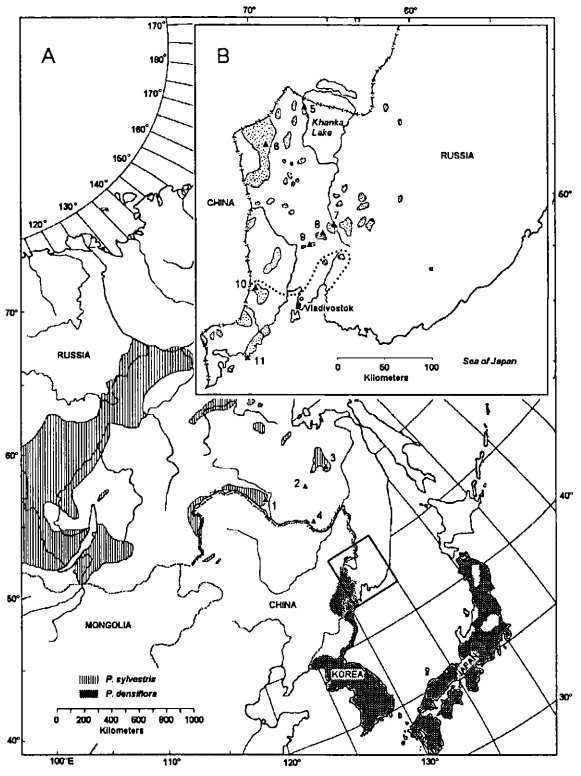

Figure 1. (A) Distributions of *Pinus sylvestris* and *P. densiflora* (modified from CRITCHFIELD & LITTLE (1966)) and locations of the sampled populations of *P. sylvestris*: 1 – Svobodnyi; 2 – Chegdomyn; 3 – Evoron; 4 – Pashkovo. (B) Distributions of *P. funebris* and *P. densiflora* in the Russian Far East. Locations of the sampled populations of *P. funebris*: 5 – Turii Rog; 6 – Barabash-Levada; 7 – Nikolaevka; 8 – Osinovka; 9 – Gornotaezhnoe. Locations of the sampled populations of *P. densiflora*: 10 – Petrovka; 11 – Gamov Peninsula. Dotted line represents the northern limit of the natural range of *P. densiflora* (modified from URUSOV (1995)).

transferase (AAT; E.C. 2.6.1.1), aconitase (ACO; E.C. 4.2.1.3), alcohol dehydrogenase (ADH; E.C. 1.1.1.1), diaphorase (DIA; E.C. 1.8.1.4), fluorescent esterase (FL-EST; E.C. 3.1.1.2), formate dehydrogenase (FDH; E.C. 1.2.1.2), glucose phosphate isomerase (GPI; E.C. 5.3.1.9), glutamate dehydrogenase (GDH; E.C. 1.4.1.2), isocitrate dehydrogenase (IDH; E.C. 1.1.1.42), leucine aminopeptidase (LAP; E.C. 3.4.11.1), malate dehydrogenase (MDH; E.C. 1.1.1.37), phosphoglucomutase (PGM; E.C. 2.7.5.1), 6-phosphogluconate dehydrogenase (6-PGD; E.C. 1.1.1.44), shikimate dehydrogenase (SKDH; E.C. 1.1.1.25) and sorbitol dehydrogenase (SDH; E.C. 1.1.1.14). Recipes for enzyme staining followed standard methods (CHELIAK & PITEL 1984) with insignificant modifications. Within each

locus, the most common allele in Scots pine was designated with the arbitrary value 1.00 symbol. The other alleles at this locus were numbered according to the electrophoretic migration of allozymes relative to the commonest allozyme. The inheritance pattern of enzyme electrophoretic variants of pines was determined earlier (GONCHARENKO *et al.* 1994).

Allele frequencies were analyzed using the BIOSYS-1 computer program (SWOFFORD & SELANDER 1989). We estimated genetic variation in the populations studied by the use of the following parameters: mean number of alleles per locus (A), percent of polymorphic loci (P), observed heterozygosity (H_o), expected heterozygosity (H_e), and Wright's fixation index (F). Values of F for each polymorphic locus in each population were tested for significance with chi-square analysis, $\chi^2 = NF^2(a-1)$, with df = $a(a-1)/2$, where N is the total sample size, and a is the number of alleles at a locus (LI & HORVITZ 1953). The analysis of gene diversity followed NEI (1973). Data on the total genetic diversity (H_T), genetic diversity within populations (H_S), and proportion of genetic variation found among populations (G_{ST}) were calculated on the basis of analysis for 13 loci that appeared to be polymorphic in all the taxa assayed. A cluster analysis using UPGMA and NEI's unbiased genetic distances (NEI 1978) was performed.

RESULTS

The electrophoretic analysis of the Scots, Japanese red and funeral pine populations revealed 85 allelic variants at 24 loci. Allelic frequencies were determined for each locus in each population (Table 1). The most pronounced differences were revealed between Scots and Japanese red pines at 17 loci: *Aat*-2, *Aat*-3, *Adh*-1, *Adh*-2, *Dia*-1, *Pgm*-1, *Pgm*-2, *Lap*-1, *Mdh*-2, *Mdh*-3, *Mdh*-4, *Gdh*, *Idh*, *Aco*, *Fl-Est*, *Skdh*-1 and 6-*Pgd*-1. Allele frequencies for these loci were inter-mediate in most of the funeral pine populations studied. The *Mdh*-$1^{0.85}$ allele was present in most of the *P. funebris* populations and had a sufficient frequency. However, this allele was not revealed in the populations of Scots and Japanese red pines.

On the basis of allele frequencies for 24 loci, we computed parameters of intrapopulation genetic variation in Scots, funeral and Japanese red pines. Most of them were the highest in two funeral pine populations from the northwestern coast of Lake Khanka in the vicinity of the village of Turii Rog (5) and in the vicinity of the village of Barabash-Levada (6) (Table 2). The mean number of alleles per locus in the *P. funebris* populations averaged 2.18, percent of polymorphic loci (P_{99}) averaged 73.3%, and expected and observed heterozygosities averaged 0.239 and 0.234, respectively. The genetic variation parameter values obtained for *P. funebris* were similar to those exhibited by the Scots pine populations assayed. In the latter ones the mean number of alleles per locus averaged 2.11, percent of polymorphic loci (P_{99}) averaged 71.9 %, and expected and observed heterozy-gosities averaged 0.249 and 0.223, respectively. The genetic variation values were the lowest in the Japanese red pine populations; the mean number of alleles per locus averaged 1.98, percent of polymorphic loci (P_{99}) averaged 62.5 %, and expected and observed heterozygosities averaged 0.201 and 0.196, respectively.

Observed genotype frequencies confirmed to Hardy-Weinberg expectations for the most loci in most populations. Eleven of 184 χ^2-tests (6 %) for polymorphic loci in individual pine populations indicated a significant deviation from Hardy-Weinberg expectations of genotype frequencies. For *Aat*-3, *Adh*-1, *Pgm*-2, *Lap*-1, *Mdh*-1, *Mdh*-3, *Fdh*, *Skdh*-1 and 6-*Pgd*-1 loci where genotype frequencies departed from Hardy-Weinberg a deficiency of heterozygotes showed while for *Idh* locus an excess of heterozygotes showed (Table 1). For individual populations, the mean F values were from -0.048 to 0.070 (Table 2), indicating little deviation from Hardy-Weinberg expectations. In nine of the eleven populations investigated the average proportion of observed heterozygotes per population was lower than expected under the Hardy-Weinberg equilibrium. Only in the funeral pine populations from the vicinity of the villages of Nikolaevka (7) (F = -0.007) and Gornotayezhnoe (9) (F = -0.048) an excess of heterozygotes was revealed.

The taxa assayed demonstrated distinct differences in interpopulation diversity (Table 3). Funeral pine exhibited higher differentiation values (G_{ST} = 0.098) than Scots pine (0.064) and Japanese red pine (0.030). To estimate the level of genetic differentiation between the populations and taxa studied, the genetic distances were calculated according to NEI (1978) (Table 4). The least distance values were between the Japanese red pine populations (D_N = 0.011) and between the Scots pine populations (mean D_N = 0.026). The D_N value between the funeral and Japanese red pine populations averaged 0.033, whereas that between funeral and Scots pines averaged 0.160, i.e. genetic differences between funeral and Scots pines were almost five times as high as those between funeral and Japanese red pines. The mean D_N value of 0.201 between Scots and Japanese red pines was the highest.

The UPGMA cluster analysis (Fig. 2) split the populations investigated into two major clusters. One of them is comprised of all the *P. funebris* and *P. densiflo-*

Table 1. Allele frequencies in the natural populations of *P. sylvestris, P. funebris,* and *P. densiflora*.

Locus, allele	Species / Population										
	P. sylvestris				*P. funebris*					*P. densiflora*	
	1	2	3	4	5	6	7	8	9	10	11
Aat–1											
1.00	1.000	.978	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
1.10	.0	.022	.0	.0	.0	.0	.0	.0	.0	.0	.0
Aat–2											
0	.026	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
1.00	.789	.652	.440	.688	.273	.320	.037	.0	.560	.040	.014
1.10	.184	.348	.200	.250	.470	.400	.537	.800	.220	.640	.900
1.25	.0	.0	.340	.063	.182	.280	.296	.140	.0	.280	.043
1.40	.0	.0	.020	.0	.076	.0	.130	.060	.220	.040	.043
Aat–3											
1.00	.579	.609	.240	.563	.924	.960	.852	.980	1.000	.980	.957
1.20	.026	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
1.40	.395	.391	.760	.438	.076	.040	.148	.020	.0	.020	.043
Adh–1											
1.00	.737	.500	.820	.479	.167	.100	.093	.540	.0	.0	.0
1.10	.263	.500	.180	.521	.833	.900	.870	.460	1.000	1.000	1.000
1.25	.0	.0	.0	.0	.0	.0	.037	.0	.0	.0	.0
Adh–2											
0.30	.447	.478	.160	.313	.136	.0	.0	.400	.0	.0	.0
0.60	.0	.0	.0	.0	.045	.0	.0	.0	.0	.0	.0
1.00	.368	.478	.660	.688	.333	.460	.481	.260	.760	.500	.329
1.30	.0	.0	.0	.0	.015	.060	.037	.100	.0	.080	.129
1.40	.184	.022	.180	.0	.470	.480	.481	.240	.240	.420	.543
1.60	.0	.022	.0	.0	.0	.0	.0	.0	.0	.0	.0
Dia–1											
0	.0	.087	.0	.042	.015	.0	.0	.0	.0	.0	.0
0.85	.316	.022	.100	.167	.015	.0	.0	.080	.0	.0	.0
0.90	.053	.0	.100	.021	.0	.0	.0	.0	.0	.0	.0
1.00	.632	.891	.800	.750	.970	.980	1.000	.720	1.000	1.000	1.000
1.10	.0	.0	.0	.021	.0	.020	.0	.200	.0	.0	.0
Pgm–1											
0.95	.0	.0	.0	.0	.015	.040	.167	.040	.0	.240	.014
1.00	.947	1.000	1.000	1.000	.955	.960	.815	.940	.960	.760	.957
1.05	.053	.0	.0	.0	.030	.0	.019	.020	.040	.0	.029
Pgm–2											
0.90	.0	.0	.0	.0	.0	.020	.0	.0	.0	.0	.0
1.00	1.000	1.000	1.000	1.000	.712	.600	.889	.920	.600	.780	.743
1.10	.0	.0	.0	.0	.273	.360	.111	.080	.400	.220	.257
1.15	.0	.0	.0	.0	.015	.020	.0	.0	.0	.0	.0
Lap–1											
0	.026	.022	.0	.0	.030	.080	.0	.0	.0	.0	.014
0.90	.0	.0	.0	.0	.152	.080	.167	.140	.280	.200	.157
0.95	.0	.0	.0	.0	.0	.020	.0	.0	.0	.0	.0
1.00	.974	.974	1.000	1.000	.818	.820	.833	.860	.720	.800	.829

Table 1. (continued).

Locus, allele	Species / Population										
	P. sylvestris					*P. funebris*				*P. densiflora*	
	1	2	3	4	5	6	7	8	9	10	11
Lap–2											
0.95	.026	.022	.0	.042	.0	.0	.185	.0	.0	.020	.100
1.00	.974	.978	1.000	.958	1.000	1.000	.815	1.000	1.000	.980	.900
Mdh–1											
0.85	.0	.0	.0	.0	.197	.240	.148	.140	.0	.0	.0
1.00	1.000	.957	.980	.896	.803	.720	.852	.860	1.000	1.000	1.000
1.11	.0	.043	.020	.104	.0	.040	.0	.0	.0	.0	.0
Mdh–2											
0	.079	.043	.260	.0	.106	.020	.0	.0	.0	.0	.0
0.65	.0	.0	.040	.0	.045	.040	.0	.0	.0	.0	.0
1.00	.921	.957	.700	1.000	.848	.940	1.000	1.000	1.000	1.000	1.000
Mdh–3											
0.70	.0	.174	.080	.021	.667	.860	.889	.920	1.000	1.000	1.000
1.00	1.000	.804	.920	.938	.333	.140	.111	.0	.0	.0	.0
1.05	.0	.022	.0	.042	.0	.0	.0	.080	.0	.0	.0
Mdh–4											
1.00	.579	.500	.640	.604	.333	.140	.056	.020	.0	.0	.0
6.50	.342	.457	.260	.396	.667	.860	.944	.960	.960	.980	.986
7.00	.0	.0	.0	.0	.0	.0	.0	.0	.0	.020	.0
8.00	.079	.043	.100	.0	.0	.0	.0	.020	.040	.0	.014
Gdh											
0.40	.0	.0	.0	.0	.0	.0	.0	.020	.0	.0	.0
1.00	.842	.783	.860	.854	.818	.600	.981	.980	1.000	1.000	1.000
1.20	.158	.217	.140	.146	.182	.400	.019	.0	.0	.0	.0
Fdh											
0.50	.0	.065	.0	.104	.015	.020	.0	.0	.0	.0	.0
1.00	.974	.848	.920	.854	.985	.980	1.000	1.000	.920	1.000	1.000
1.60	.026	.087	.080	.042	.0	.0	.0	.0	.080	.0	.0
Sdh											
0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.100	.0
1.00	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.900	1.000
Gpi											
0.50	.0	.0	.0	.0	.0	.0	.019	.0	.120	.020	.0
0.70	.0	.130	.0	.0	.0	.0	.0	.0	.0	.0	.029
1.00	1.000	.848	1.000	1.000	1.000	1.000	.981	1.000	.880	.820	.971
1.30	.0	.022	.0	.0	.0	.0	.0	.0	.0	.160	.0
Idh											
0.80	.0	.0	.0	.0	.182	.100	.222	.020	.220	.300	.100
1.00	1.000	1.000	1.000	1.000	.818	.900	.778	.980	.780	.700	.900
Aco											
0.80	.0	.0	.0	.0	.0	.0	.0	.120	.0	.020	.029
0.90	.139	.174	.140	.188	.500	.604	.241	.240	.120	.380	.324
1.00	.861	.826	.860	.813	.439	.313	.296	.640	.700	.560	.397
1.10	.0	.0	.0	.0	.061	.083	.463	.0	.180	.040	.250

Table 1. (continued).

Locus, allele	Species / Population										
	P. sylvestris					*P. funebris*				*P. densiflora*	
	1	2	3	4	5	6	7	8	9	10	11
Fl–Est											
0.70	.105	.435	.160	.208	.091	.021	.019	.0	.0	.0	.0
0.85	.079	.043	.060	.0	.136	.167	.278	.680	.160	.240	.329
0.95	.184	.174	.280	.438	.242	.167	.056	.0	.0	.0	.0
1.00	.632	.348	.500	.354	.485	.646	.389	.220	.840	.680	.671
1.10	.0	.0	.0	.0	.045	.0	.259	.100	.0	.080	.0
Skdh–1											
0	.0	.0	.0	.0	.045	.0	.0	.0	.0	.0	.0
0.90	.0	.0	.0	.167	.030	.0	.0	.0	.0	.0	.0
0.95	.053	.043	.080	.167	.273	.080	.241	.060	.300	.280	.286
1.00	.895	.913	.840	.646	.561	.840	.333	.340	.440	.360	.471
1.05	.053	.0	.060	.021	.091	.060	.407	.600	.260	.360	.229
1.15	.0	.0	.0	.0	.0	.020	.019	.0	.0	.0	.014
1.15/1.05	.0	.043	.020	.0	.0	.0	.0	.0	.0	.0	.0
6–Pgd–1											
0.95	.395	.457	.800	.146	.864	.780	1.000	1.000	1.000	1.000	.957
1.00	.605	.543	.200	.854	.136	.220	.0	.0	.0	.0	.043
6–Pgd–2											
0.70	.0	.0	.0	.042	.0	.0	.0	.0	.0	.0	.0
0.85	.447	.478	.300	.500	.136	.140	.167	.100	.200	.220	.343
1.00	.553	.522	.700	.458	.864	.860	.833	.900	.800	.780	.657

Table 2. Genetic variation in populations of *P. sylvestris*, *P. funebris* and *P. densiflora*.

Population	A	P_{95}	P_{99}	H_e[a]	H_o	F
1 Svobodnyi	2.04	58.3	70.8	0.237	0.196	0.055
2 Chegdomyn	2.25	62.5	83.3	0.268	0.226	0.070
3 Evoron	2.08	62.5	66.7	0.238	0.225	0.041
4 Pashkovo	2.08	62.5	66.7	0.252	0.243	0.011
Mean for *P. sylvestris*	**2.11**	**61.5**	**71.9**	**0.249**	**0.223**	**0.044**
5 Turii Rog	2.54	70.8	83.3	0.296	0.280	0.033
6 Barabash-Levada	2.38	66.7	83.3	0.257	0.245	0.023
7 Nikolaevka	2.21	66.7	75.0	0.253	0.255	-0.007
8 Osinovka	2.08	54.2	70.8	0.202	0.187	0.029
9 Gornotaezhnoe	1.67	45.8	54.2	0.187	0.202	-0.048
Mean for *P. funebris*	**2.18**	**60.8**	**73.3**	**0.239**	**0.234**	**0.006**
10 Petrovka	1.96	50.0	62.5	0.219	0.212	0.013
11 Gamov Peninsula	2.00	41.7	62.5	0.183	0.179	0.026
Mean for *P. densiflora*	**1.98**	**45.9**	**62.5**	**0.201**	**0.196**	**0.020**

[a] Unbiased estimate (see NEI, 1978)

ra populations sampled, while the other is comprised of the *P. sylvestris* populations. The former cluster in its turn is represented by two clusters. The northern funeral pine populations formed one of them and the Japanese red pine populations and the funeral pine southern populations formed the other. The Osinovka

Table 3. Mean values of Nei's (1973) genetic diversity statistics for populations of *P. sylvestris*, *P. funebris*, and *P. densiflora*.

Species	H_T	H_S	G_{ST}
P. sylvestris	0.349	0.322	0.064
P. funebris	0.347	0.308	0.098
P. densiflora	0.313	0.304	0.030

(8) population appeared to be rather divergent from the above two clusters.

DISCUSSION

Despite the fact that Komarov described funeral pine in 1901, the Russian scientists continued making attempts to revise its taxonomic ranking. Using a diversity of morphological traits it was described as either a distinct species *P. funebris* (KOMAROV 1949; VOROB'EV 1968), Japanese red pine (KHARKEVICH & KACHURA 1981), or Scots pine (NEDOLUZHKO 1995). Based on an examination of generative organs of funeral pine, URUSOV (1974) recognized it as a distinct species of hybrid origin.

Our data show that funeral pine can be regarded as a Japanese red pine variety of hybrid origin due to past hybridization between *P. densiflora* and *P. sylvestris*. This suggestion has the genetic evidence. Firstly, allele frequencies for 17 loci in the funeral pine populations were intermediate in comparison with those in the Japanese red and Scots pine ones. Secondly, in the funeral and Scots pine populations we found similar alleles that was not revealed in the Japanese red pine, *Adh*-$1^{1.00}$, *Adh*-$2^{0.30}$, *Dia*-$1^{0.85}$, *Mdh*-2^{0}, *Mdh*-$3^{1.00}$, *Mdh*-$4^{1.00}$, *Gdh*$^{1.20}$, *Fl-Est*$^{0.70}$, and *Fl-Est*$^{0.95}$, while in the *P. funebris* and *P. densiflora* populations the similar alleles revealed were *Adh*-$2^{1.30}$, *Pgm*-$1^{0.95}$, *Pgm*-$2^{1.10}$, *Lap*-$1^{0.90}$, *Idh*$^{0.80}$, and *Aco*$^{1.10}$. Hence funeral pine possesses a mixture of genes of Scots and Japanese red pines. Thirdly, two of the five funeral pine populations had higher levels of genetic variation than the populations of the parent species, which may represent an additive effect of gene introgression from the both parent species. Fourthly, allele frequencies for funeral pine are much more similar with those for Japanese red pine than for Scots pine, as shown in the dendrogram (Fig. 2). The mean Nei's genetic distance value of 0.033 between funeral and Japanese red pines is considerably lower than those between *P. sylvestris* and *P. densiflora* and between other closely related coniferous species with partial reproduction isolation (DANCIK & YEH 1983; WHEELER & GURIES 1987; MILLAR *et al.* 1988; YEH & ARNOTT 1988; WANG *et al.* 1990; KOROL *et al.* 1995; POLITOV *et al.* 1999).

Our data and conclusions are very close to those obtained and drawn by SZMIDT & WANG (1993) who demonstrated that in China var. *sylvestriformis* (Takenouchi) was of hybrid origin and had arisen from introgressive hybridization between Scots and Japanese red pines. Thus we may suppose that var. *sylvestriformis* and *P. funebris* are the same taxon distributed in China, northern Korea and Russia.

In spite of the detailed description of the history of *P. densiflora* and *P. sylvestris* in the Far East and genetic analysis of the Japanese red pine and Scots pine varieties made by SZMIDT & WANG (1993), the question of whether the hybrid is returning to the sympatric parent species *P. densiflora* or it is a stable introgressant such as *P. densata* (WANG *et al.* 1990) still remains debatable. In the event that the hybrid is returning to the parent species, differentiation between the hybrid and the *P. densiflora* typical populations is supposed to become less pronounced with decreasing geographical distance between them. Differentiation between the hybrid and *P. sylvestris* populations

Table 4. Estimates of Nei's (1978) genetic distances.

Population	1	2	3	4	5	6	7	8	9	10	11
1 Svobodnyi	***	.013	.028	.015	.118	.157	.204	.182	.190	.220	.210
2 Chegdomyn		***	.036	.012	.087	.118	.158	.149	.150	.170	.161
3 Evoron			***	.049	.114	.160	.180	.175	.191	.207	.209
4 Pashkovo				***	.120	.158	.199	.202	.190	.218	.210
5 Turii Rog					***	.010	.029	.057	.041	.031	.031
6 Barabash-Levada						***	.042	.078	.044	.039	.037
7 Nikolaevka							***	.042	.039	.014	.017
8 Osinovka								***	.080	.048	.047
9 Gornotaezhnoe									***	.024	.035
10 Petrovka										***	.011
11 Gamov Peninsula											***

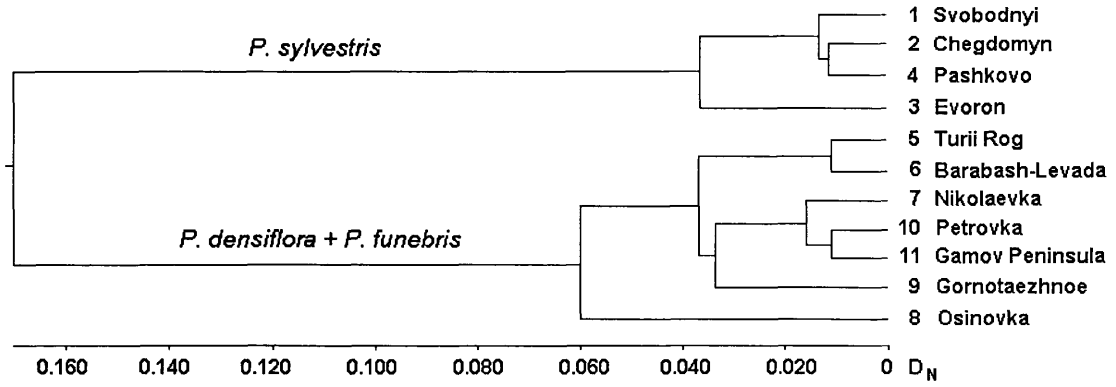

Figure 2. UPGMA dendrogram based on NEI's (1978) genetic distances between the eleven populations of the Far Eastern two-needle pines.

Table 5. Averaged estimates of NEI's (1978) genetic distances between *P. sylvestris*, *P. densiflora* and populations of their hybrid. Hybrid populations put in an order to decrease of geographic distances to sampled populations of *P. densiflora*.

Species, population	*P. sylvestris*	*P. densiflora*
P. sylvestris	0.026	0.201
5 Turii Rog	0.110	0.031
6 Barabash-Levada	0.148	0.038
7 Nikolaevka	0.185	0.016
8 Osinovka	0.177	0.048
9 Gornotaezhnoe	0.180	0.030
P. densiflora	0.201	0.011

therewith should increase. Table 5 shows that such peculiarity is not common to the five hybrid populations, which may be an indicative of the stability of the hybrid, at least in certain of the Russian populations. Also, the presence of the *Mdh*-$1^{0.85}$ allele with high frequency in the hybrid populations alone confirms the stability of the hybrid. If the hybrid is returning to the parent species, *Mdh*-$1^{0.85}$ allele should be presented in adjacent populations of the parent species at least in minor frequencies and/or the allele frequency in hybrid populations should decrease in the direction to the parent species populations due to gene flow.

To estimate the relationships among the pines assayed more completely, it is interesting to compare genetic variation in the populations (Table 2). Genetic variation is normally higher in the hybrid natural and artificial pine and spruce populations than in the parent species (WHEELER & GURIES 1987; YEH & ARNOTT 1988; WANG *et al.* 1990; KOROL *et al.* 1995; EDWARDS-BURKE *et al.* 1997; YU *et al.* 2000). The level of genetic variation exhibited only by the two northern hybrid populations located in close proximity to the *P. sylvestris*' range appeared to be in general higher than those exhibited by the both parent species. The lower level of intrapopulation genetic variation demonstrated by the southern hybrid populations Osinovka (8) and Gornotaezhnoe (9) as compared to that exhibited by the northern ones can be explained by gene drift and limited gene flow due to small sizes and scattered distributions of the hybrid populations assayed. The hybrid's range consists of small (up to 25 fruit-bearing trees in Gornotayezhnoe population (9)) geographically isolated populations (Fig. 1B). Frequencies of such alleles as *Pgm*-$2^{1.10}$, *Fl-Est*$^{1.10}$ and *Aco*$^{1.10}$, which may not be ascribed to hybridization alone, may be proof of gene drift in the hybrid populations. It should be noted that hybrid pine in China exhibited a rather low interpopulation variation level (G_{ST} = 0.026) and high intrapopulation variability (SZMIDT & WANG 1993). Unfortunately, the authors gave a superficial description of the pine's range and sampling areas, and thus it is impossible to reliably explain the differences in variation in the hybrid growing in various habitats.

The genetic variation values in the Far Eastern *P. sylvestris* populations were somewhat lower than those in the East European and Siberian populations (GONCHARENKO *et al.* 1994) obtained for the same number of sampled trees analyzed for almost identical set of loci. However, a higher interpopulation variation level is typical for *P. sylvestris* from the Russian Far East. The data obtained may be explained by the recent colonization of the Far East with Scots pine (BOBROV 1978) and the limited gene flow between geographically isolated populations as compared to that between populations from the continuous range in Eastern

Europe and Siberia. Similar differences in intra- and interpopulation variation were revealed between Scots pine occurring at the western limit of its distribution in Spain where it typically grows in isolated populations and Scots pine occurring in northern and eastern Europe (PRUS-GŁOWACKI & STEPHAN 1994). At the same time, allele diversity in the populations from the eastern limit of the pine's distribution was lower than in the populations from western limit (Spain) where Scots pine is a Tertiary relict and represents "old" gene pool, as indicated by PRUS-GŁOWACKI & STEPHAN (1994).

The level of genetic variation in the Japanese red pine populations from Russia was lower than that in the populations from south Korea, Japan and China. In the south Korean populations the mean number of alleles per locus averaged 2.4, percent of polymorphic loci averaged 80.2 % and expected and observed heterozygosities averaged 0.262 and 0.258, respectively (KIM & LEE 1995). In the Japanese and Chinese populations the values of these parameters averaged 3.5, 64.3 %, 0.275 and 0.255, respectively (SZMIDT & WANG 1993). The recent colonization of the Russian Far East (GOLUBEVA & KARAULOVA 1983) may be responsible for the lower intrapopulation variation values exhibited by the Japanese red pine populations. In the Russian populations, the level of interpopulation genetic variation was lower than that in the Japanese ones (G_{ST} = 0.058). In SZMIDT & WANG's (1993) opinion, more sufficient isolation of the *P. densiflora* island populations compared to the continental ones can decrease the gene flow, and thus to increase the level of differentiation among the populations from the Japanese isles.

The positive F values exhibited by nine of the eleven populations assayed indicate a slight deficiency of heterozygotes relative to the Hardy-Weinberg equilibrium for the most populations investigated and for pine taxa in total. 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 & LEDIG 1982; DANCIK & YEH 1983; KIM *et al.* 1994; POLITOV & KRUTOVSKII 1994; CHANGTRAGOON & FINKELDEY 1995; LEE *et al.* 1998). Unfortunately, without a study of the mating system of investigated taxa it is now difficult to answer the question as to the reasons for the deficiency of heterozygotes in populations. It should be noted that deficiency of heterozygotes was observed in Spanish, eastern and northern European populations of *P. sylvestris* (MEJNARTOWICZ & PALOWSKI 1989; PRUS-GŁOWACKI & STEPHAN 1994), while slight excess of heterozygotes was observed in other Russian and northern Sweden populations of *P. sylvestris* (GONCHARENKO *et al.* 1994; SZMIDT *et al.* 1996) and the Korean populations of *P. densiflora* (KIM & LEE 1995). Interestingly, the negative F value and a high proportion of inbred progeny (s = 26.4 %; POTENKO unpublished) were found in the Gornotayezhnoe population (9). This suggests that selection against inbred progeny caused by inbreeding depression occurs in the population. The excess of heterozygotes and low proportion of outcrossed progeny in conifers were also found in one of four populations of *Abies balsamea* (NEALE & ADAMS 1985), one population of *Pinus cembra* (POLITOV & KRUTOVSKII 1994) and two of five populations of *P. koraiensis* (POTENKO & VELIKOV 2001).

In general, the intrapopulation genetic variation values exhibited by the marginal Far Eastern two-needle pine populations were lower than those in the central populations. This is consistent with the data on the marginal coniferous populations obtained earlier (BERGMANN & GREGORIUS 1979; YEH & LAYTON 1979; GURIES & LEDIG 1982; HAWLES & DEHAYES 1994; POTENKO & VELIKOV 2001). The variety of hybrid origin (= *P. sylvestris* × *P. densiflora*) was found in the northern segment of the *P. densiflora*'s distribution.

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