Allozyme Variation in Natural Populations of Eurasian Pines

III. Population Structure, Diversity, Differentiation and Gene Flow in Central and Isolated Populations of Pinus sylvestris L. in Eastern Europe and Siberia

By G. G. Goncharenko, A. E. Silin and V. E. Padutov

Department of Molecular Genetics, Institute of Forestry of the Academy of Sciences of Belarus, 71 Proletarskaya Str., 246654, Gomel, Belarus, Russia

(Received 29th November 1993)

Summary

Eighteen natural populations of *Pinus sylvestris* L. were investigated by starch-gel electrophoresis. A total of 88 alleles were observed at 21 loci. More than 90% of the loci in *P. sylvestris* were polymorphic and the mean observed and expected heterozygosity values were 0.282 and 0.286, respectively. Interpopulation genetic diversity was about 3 % of the total genetic diversity and the level of gene flow was 8.68. Nei's genetic distance coefficient ranged from 0.005 to 0.046 among populations and averaged 0.017. From the dendrogram it can be seen that clustering did not reflect geographical proximity. The data also show that *P. cretacea*, which occurs on ancient carbonate rock outcrops, can be regarded simply as a race of *P. sylvestris*. On the whole, our results demonstrate that sufficient genetic variation exists in *P. sylvestris* to explain its great ecological plasticity and evolutionary potential. Estimated parameters of gene diversity, genetic distance and gene flow show that the *P. sylvestris* populations we studied share a common gene pool.

Key words: *Pinus sylvestris*, isozymes, inheritance, segregation, population structure, gene diversity, gene flow, genetic differentiation.

FDC: 165.3; 165.5; 174.7 *Pinus sylvestris*; (4); (571).

Introduction

Scotch pine (*Pinus sylvestris* L.) is one of the most widespread of the Eurasian coniferous species (Bobrov, 1978). *P. sylvestris* is distributed from the Atlantic to the Pacific Ocean (Pravdin, 1964; Critchfield and Little, 1966). Within its distribution, Scotch pine can grow in absolutely different ecological conditions, including extreme ones such as the bounds of the tundra, bogs, steppes, and mountains. Such a wide range of ecological conditions could favour the formation of variety of ecotypes. Within *P. sylvestris* a few geographical races with numerous types have been described (Komarov, 1934; Sukhachyov, 1938; Kapper, 1954; Pravdin, 1964; Bobrov, 1978; Kozubov and Muratova, 1986).

Till recently, researchers analyzed only phenotypical traits of trees in *P. sylvestris* populations. However, these traits are influenced by the environment and by multiple genes.

In recent years, it has become evident that use of isozyme electrophoresis may be valuable in the study of genetic structure of coniferous species. By the end of the 80s, in different countries of the world gene pools of more than 40 coniferous species were analyzed by means of isozyme electrophoresis (Hamrick et al., 1981; Ledig, 1986; Goncharenko et al., 1989). However, population and genetic study of *P. sylvestris* by isozyme electrophoresis was fragmentary because researchers used either few loci or few populations (Krzakowa et al., 1977; Gullberg et al., 1982 and 1985; Krzakowa, 1982; Szmidt, 1984; Mejnartowicz and Bergmann, 1985; Müller-Starck and Gregorius, 1986; Dukharev et al., 1987; Padutov et al., 1989; Starova et al., 1990; Filppula et al.,1992). Only scotch populations of *P.* sylvestris were studied more intensively; 16 allozyme loci were used to assay genetic structure, including gene diversity and genetic differentiation (Kinloch et al., 1986).

The purpose of our study was to investigate genetic structure, levels of variation, and differentiation in 18 central and isolated populations of *P. sylvestris* in eastern Europe and western Siberia.

Materials and Methods

Materials

This study was based on seeds collected in 1986 to 1992 from 586 individual trees in seven *P. sylvestris* natural populations from the continuous part of its distribution (i. e., Tukumus in Latvia; Albertin, Podsvilye, and Markovichi in Belarus; Polessky reserve in Ukraine; Karaulnoye and Uyarskoye in Russia) and in 11 Scotch pine populations isolated from the main distribution (i.e., 4 populations from Vygodskaya, Mizunskaya, Zelenskaya, and Gorganskaya in the Ukrainian Carpathians; 1 population from the Novomoskovsk pine forest in the Ukrainian forest-steppe zone; 2 populations from the Kursk pine forest and Usman pine forest in the Russian forest-steppe zone; and 4 populations grown on cretaceous soils from Slavyansk in Ukraine and from Shebekino, Khvalynsk, and Zhygulyovsk in Russia). Some scientists (Lypa, 1955; Mashkin, 1971; Artamonov, 1989) consider that trees of the latter 4 populations belong to another species, *Pinus cretacea* Kalen. Locations of the populations sampled are shown in *figure 1.*

Isozyme analysis

Individual trees were genotyped using 8 to 20 megagametophytes for every locus. The megagametophytes were sampled randomly from a set of not less than 40 seeds extracted from 3 to 20 cones from each of the 586 trees.

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 vertical and horizontal chambers on 13 % to 14 % starch gel. For electrophoresis, 3 buffer systems were used: A) tris-EDTA-borate, pH 8.6, B) tris-citrate, pH 6.2, C) tris-citrate, pH 6.2 (electrode buffer)/tris-HCl, pH 8.0 (gel buffer) (Goncharenko et al., 1992).

Recipes for histochemical enzyme staining followed the standard methods (Conkle et al., 1982; Cheliak and Pitel, 1984) with insignificant modifications. The enzymes as-

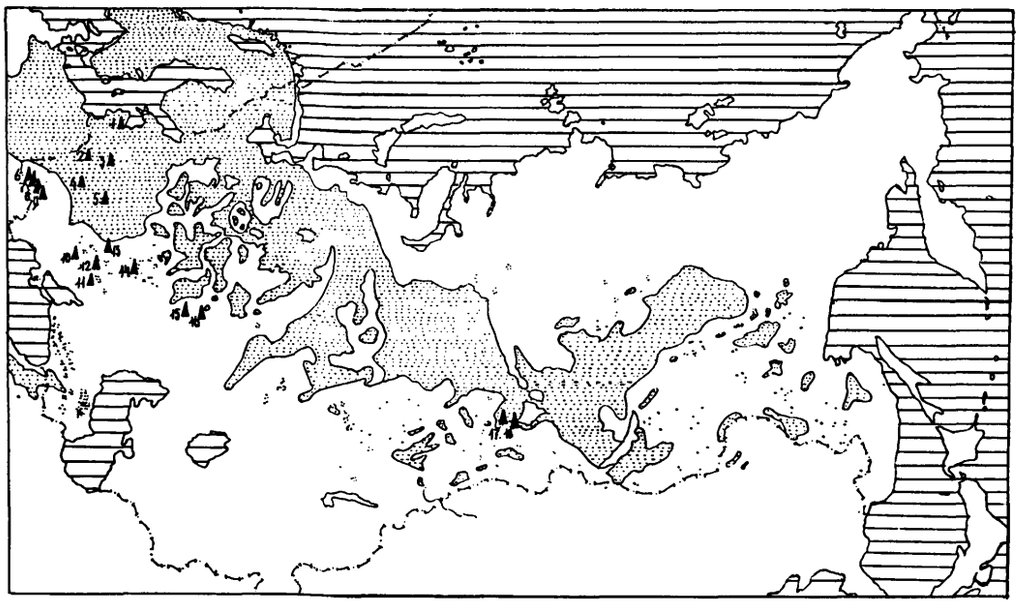

Figure 1. — Natural distribution of *Pinus sylvestris* — stippled area — in eastern Europe and Asia (PRAVDIN, 1964; CRITCHFIELD and LITTLE, 1966) with locations of 18 sampled populations: 1 — Tukumus, 2 — Albertin, 3 — Podsvilye, 4 — Polesye, 5 — Markovichi, 6 — Vygodskaya, 7 — Mizunskaya, 8 — Zelenskaya, 9 — Gorganskaya, 10 — Novomoskovsk, 11 — Slavyansk, 12 — Shebekino, 13 — Kursk, 14 — Usmansk, 15 — Khvalynsk, 16 — Zhygulyovsk, 17 — Karaulnoye, 18 — Uyarskoye.

Table 1. — Enzymes, their Abbreviations (Abbr.), Enzyme Commission 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	2	A
Aspartate aminotransferase	AAT	2.6.1.1	3	A
Diaphorase	DIA	1.6.4.3	2	C
Fluorescent esterase	FL-EST	3.1.1.2	1	B
Glucose phosphate isomerase	GPI	5.3.1.9	1	B,C
Glutamate dehydrogenase	GDH	1.4.1.2	1	A
Isocitrate dehydrogenase	IDH	1.1.1.42	1	C
Leucine aminopeptidase	LAP	3.4.11.1	2	A,C
Malate dehydrogenase	MDH	1.1.1.37	4	C
Phosphoglucomutase	PGM	2.7.5.1	2	A
6-Phosphogluconate dehydrogenase	6-PGD	1.1.1.44	2	B,C

sayed, 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. sylvestris* 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, diversity, differentiation, and gene flow in the populations studied we used all the parameters earlier applied to *P. pumila* (GONCHARENKO et al., 1993a): expected heterozygosity (H_e), observed heterozygosity (H_o), percent polymorphic loci (P_{99}

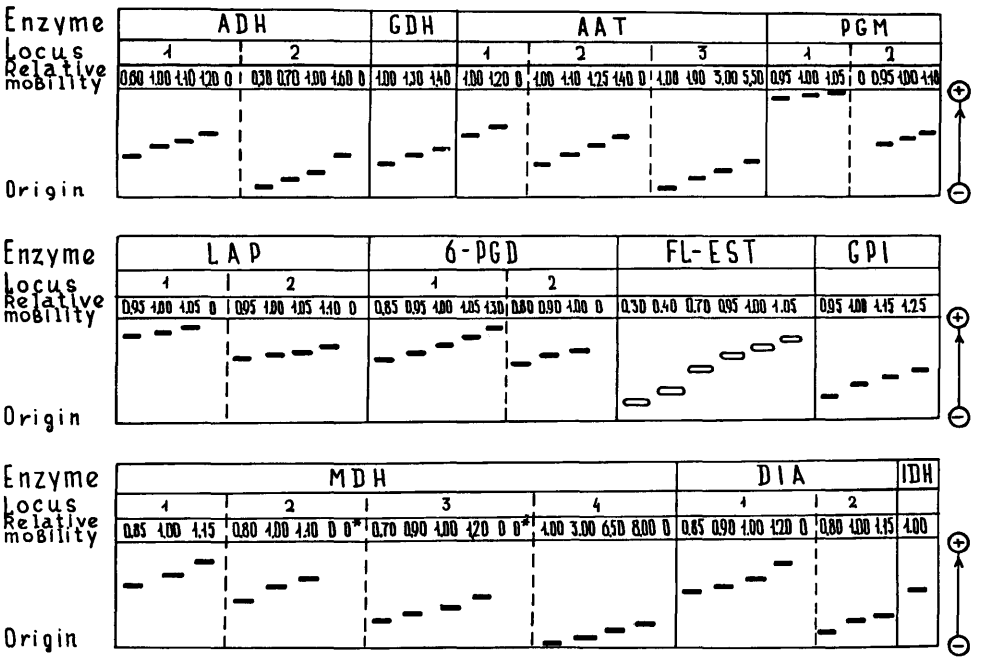

Figure 2. — Relative mobility and designation of the electrophoretic variants at 21 loci found in *P. sylvestris*.

— 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

Enzyme phenotypes

All the electrophoretic allelic variants revealed in our study of *P. sylvestris* are shown schematically in *figure 2.*

Alcohol dehydrogenase (ADH)

Two zones of activity were observed on gels stained for ADH. In both zones (ADH-1 and ADH-2) 4 electrophoretic variants were observed. In addition both zones had null variants.

Aspartate aminotransferase (AAT)

Gels stained for AAT had 3 zones of activity, the lower zone being double-banded. These 3 zones, AAT-1, AAT-2, AAT-3, were all variable in *P. sylvestris*. The fastest migrating zones, AAT-1 and AAT-2, had null variants.

Diaphorase (DIA)

There were 4 zones of activity on gels stained for DIA but only 2 zones, the fastest zone (DIA-1) and the slowest zone (DIA-2), produced clear bands. These zones were polymorphic. The middle zones were not scored. The faster migrating zone (DIA-1) had null variants.

Fluorescent esterase (FL-EST)

There was 1 variable zone of activity on gels stained for FL-EST. Other zones of fluorescent activity were diffused and inconsistently resolved.

Glucose phosphate isomerase (GPI)

Gels stained for GPI had 2 zones of activity. Bands of the faster migrating zone sometimes blurred and stained faintly, therefore, this zone was difficult to interpret and not scorable in our study. The slower zone had 4 different variants.

Glutamate dehydrogenase (GDH)

One zone of activity was observed on gels stained for GDH with 3 variants. Two additional zones of activity, ADH-1 and ADH-2, were often observed.

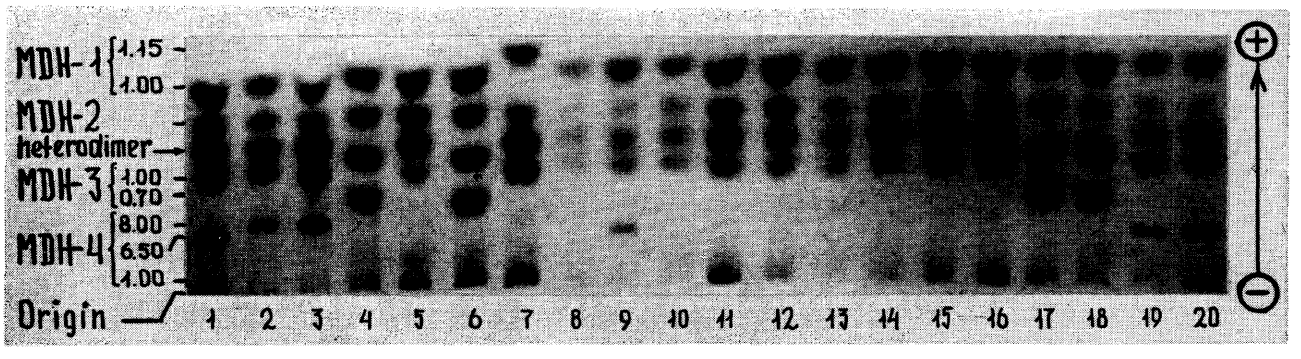

Figure 3. — Zymogram of MDH allozymes of megagametophytes *P. sylvestris*: slots 1,9,19,20 — Mdh—$1^{1.00}$Mdh-$2^{1.00}$Mdh-$3^{1.00}$Mdh-$4^{8.50}$; slots 2,3 — Mdh-$1^{1.00}$Mdh-$2^{1.00}$Mdh-$3^{1.00}$ Mdh-$4^{8.00}$; slots 4,6,17,18 —Mdh-$1^{1.00}$ Mdh-$2^{1.00}$ Mdh-$3^{0.70}$ Mdh-$4^{1.00}$; slots 5, 8, 10 to 16 — Mdh—$1^{1.00}$ Mdh-$2^{1.00}$ Mdh-$3^{1.00}$ Mdh-$4^{1.00}$; slot 7 — Mdh-$1^{1.15}$ Mdh-$2^{1.00}$Mdh-$3^{1.00}$Mdh-$4^{1.00}$.

Isocitrate dehydrogenase (IDH)

Gels stained for IDH had 2 zones of activity. The slower zone was weak and inconsistently resolved. The faster zone demonstrated clear bands but showed no variability.

Leucine aminopeptidase (LAP)

Two zones of activity occurred on gels stained for LAP. Three electrophoretic variants for LAP-1 and four variants for LAP-2 were observed. In addition, both zones had null variants.

Malate dehydrogenase (MDH)

Gels stained for MDH had 4 zones of activity. All zones were polymorphic. There was an interlocus heterodimer formed as the result of interaction of MDH-2 and MDH-3. The heterodimeric band was always midway between MDH-2 and MDH-3. The heterodimeric bands and some electrophoretic variants of MDH-1, MDH-3, and MDH-4 are shown in *figure 3*. MDH-2 zone had 2 different null variants. When in the presence of the 1st null variant (designated by symbol 0),both MDH-2 and the hetero-

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

Locus	Allele	Ratio	χ^2	Locus	Allele	Ratio	χ^2
Adh-1	0.80/1.00	7:6	0.08	Gdh	1.00/1.30	837:856	0.21
	1.00/1.10	951:880	2.75		1.00/1.40	3:5	0.50
	1.00/1.20	2:6	2.00	Lap-1	0.95/1.00	160:190	2.57
	1.00/0	6:2	2.00		0.95/0	3:5	0.50
Adh-2	0.30/0.80	9:8	0.06		1.00/0	89:83	0.21
	0.30/0	14:21	1.40		1.00/1.05	27:31	0.28
	0.30/1.00	429:502	5.72*	Lap-2	0.95/1.00	143:166	1.71
	0.30/1.60	12:23	3.46		1.00/0	5:9	1.14
	0.80/1.00	13:11	0.17		1.00/1.05	156:127	2.97
	1.00/0	39:45	0.43	Mdh-1	0.85/1.00	4:4	0.00
	1.00/1.60	134:134	0.00		1.00/1.15	166:155	0.38
	1.60/0	5:9	1.14	Mdh-2	0.80/1.00	86:81	0.15
Aat-1	1.00/1.20	46:40	0.42		1.00/0	7:5	0.33
	1.00/0	9:3	3.00		1.00/0	58:36	5.15*
Aat-2	1.00/0	71:63	0.48		1.00/1.10	4:8	1.33
	1.00/1.10	643:627	0.20	Mdh-3	0.70/0	3:5	0.50
	1.10/0	30:20	2.00		0.70/1.00	628:679	1.99
	1.00/1.25	64:66	0.03		0.90/0	12:8	0.80
	1.10/1.25	34:24	1.72		1.00/0	12:12	0.00
	1.25/1.40	5:3	0.50		1.00/0	11:9	0.20
Aat-3	1.00/1.90	41:41	0.00		1.00/1.20	19:21	0.10
	1.90/3.00	25:13	3.79	Mdh-4	1.00/3.00	30:33	0.14
	1.00/3.00	641:613	0.63		1.00/6.50	655:676	0.33
	3.00/5.50	2:10	5.33*		1.00/8.00	175:144	3.01
Gpi	0.95/1.00	131:144	0.61		1.00/0	5:3	0.50
	1.00/1.15	24:16	1.60		3.00/6.50	5:3	0.50
	1.00/1.25	123:116	0.21		6.50/8.00	110:118	0.28

Locus	Allele	Ratio	χ^2	Locus	Allele	Ratio	χ^2
Dia-1	0.85/0.90	27:21	0.75	Pgm-1	0.95/1.00	102:164	14.45**
	0.85/1.00	511:568	3.01		1.00/1.05	139:135	0.06
	0.85/1.20	9:9	0.00	Pgm-2	0.95/1.00	12:18	1.20
	0.90/1.00	135:160	2.12		1.00/1.10	57:49	0.60
	0.90/1.20	7:1	4.50*		1.00/0	9:2	4.45*
	1.00/0	40:34	0.49	6-Pgd-1	0.85/0.95	14:22	1.78
	1.00/1.20	11:14	0.36		0.85/1.00	20:34	3.63
Dia-2	0.80/1.00	22:13	2.31		0.85/1.30	4:4	0.00
	1.00/1.15	4:4	0.00		0.95/1.00	802:805	0.01
Fl-Est	0.30/0.70	82:77	0.16		0.95/1.30	3:5	0.50
	0.30/1.00	357:338	0.52		1.00/1.05	8:6	0.29
	0.30/1.20	4:4	0.00		1.00/1.30	7:11	0.89
	0.70/1.00	288:284	0.03	6-Pgd-2	0.80/0.90	20:24	0.36
	1.00/1.20	8:10	0.22		0.80/1.00	14:16	0.13
					0.90/1.00	642:700	2.51

*) level of significance < 0.05
**) level of significance < 0.001

dimeric bands showed no activity. In the presences of the 2nd null variant (designated by symbol 0') only the MDH-2 band disappeared, whereas the heterodimeric band was conserved. An analogous situation was found for the MDH-3 zone.

Phosphoglucomutase (PGM)

There were 2 zones of activity on gels stained for PGM, with the more anodal zone (PGM-1) staining most intensely. Both zones, PGM-1 and PGM-2, were variable with 3 and 4 variants, respectively. A null variant was observed only in the PGM-2 zone.

6-Phosphogluconate dehydrogenase (6-PGD)

Two zones of activity were evident on gels stained for 6-PGD. Both zones were polymorphic. The faster zone, 6-PGD-1, consisted of 5 isozyme variants. The slower zone, 6-PGD-2, consisted of 4 variants. 6-PGD zones may overlap each other, depending upon their variants (*Fig. 2*).

Segregation

Electrophoresis of 11 enzyme systems revealed 88 different electrophoretic variants in the 18 *P. sylvestris* populations investigated. Analysis for segregation of haploid megagametophytes in heterozygous trees enabled us to establish that these variants were under gene control. Data on segregation of electrophoretic variants in *P. sylvestris* heterozygous trees are presented in *table 2*. As seen in the table, cases of distortion of the expected 1:1 segregation occurred for some allelic combinations at Adh-2, Aat-3, Dia-1, Mdh-2, Pgm-1, and Pgm-2. If the same alleles formed other allelic combinations, the 1:1 segregation ratio was observed (*Table 2*). Other researchers who studied *P. sylvestris* also detected deviation from a 1:1 segregation ratio. For example, Rudin and Ekberg (1978) found deviations from the expected 1:1 segregation ratio at GOT-B, GOT-MC, LAP-A, LAP-B, MDH-MB, and PHOS-ME. Furthermore, Szmidt and Muona (1989) observed similar deviations at ADH-1, ADH-2, GOT-2, Dia-1, Dia-2, Dia-3, Lap-2, Pgm-1, Fl-Est, Shd-1, and Shd-2 in certain individuals. On the whole, the data presented in *table 2* are in good agreement with the supposition that the electrophoretic variants revealed in *P. sylvestris* are under gene control.

All the allelic variants at the 21 structural loci assayed in the current study are shown in *figure 2*. At most of the genes analysed in *P. sylvestris*, 3 and more alleles were present (*Fig. 2*). At 11 loci, null alleles were found.

Genetic variation

Allelic frequencies for all the 88 alleles at 21 loci and observed heterozygosity values are listed in *table 3*. From the table it can be seen that the 1.00 allele is the most frequent in each locus in all the populations studied except for Aat-3, Adh-1, Gdh, Mdh-4, and 6-Pgd-1 at which other alleles were predominant in some populations. It should be noted that of 88 alleles 31 appeared to be rare, i.e. in *P. sylvestris* their frequencies are lesser than 1 %. Aat-2, Aat-3, Adh-1, Adh-2, Gdh, Dia-1, Mdh-3, Mdh-4, Fl-Est,

Table 3. — Allele frequencies for 21 loci in 18 populations of *P. sylvestris*.

Locus / Allele	Populations: 1 n=45	2 n=61	3 n=57	4 n=30	5 n=44	6 n=30	7 n=30	8 n=31	9 n=34	10 n=30	11 n=32	12 n=30	13 n=12	14 n=25	15 n=30	16 n=30	17 n=22	18 n=13
Adh-1																		
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000
0.80	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.00	0.589	0.516	0.605	0.700	0.568	0.683	0.750	0.774	0.529	0.667	0.672	0.700	0.625	0.680	0.533	0.783	0.455	0.423
1.10	0.400	0.484	0.395	0.300	0.432	0.300	0.250	0.226	0.471	0.333	0.328	0.300	0.375	0.320	0.467	0.200	0.545	0.577
1.20	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ho***	0.733	0.607	0.579	0.333	0.591	0.533	0.433	0.258	0.559	0.400	0.406	0.467	0.250	0.560	0.400	0.367	0.545	0.538
Adh-2																		
0	0.011	0.000	0.009	0.050	0.024	0.000	0.050	0.000	0.000	0.083	0.000	0.000	0.083	0.000	0.033	0.000	0.000	0.000
0.30	0.200	0.176	0.255	0.283	0.256	0.333	0.083	0.161	0.265	0.167	0.203	0.000	0.208	0.240	0.250	0.133	0.114	0.308
0.70	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.016	0.017	0.000	0.000	0.050	0.017	0.023	0.000
1.00	0.689	0.676	0.675	0.617	0.659	0.550	0.800	0.790	0.706	0.667	0.719	0.967	0.625	0.740	0.650	0.800	0.795	0.692
1.60	0.100	0.148	0.061	0.050	0.061	0.117	0.067	0.048	0.029	0.067	0.063	0.017	0.083	0.020	0.017	0.050	0.068	0.000
Ho	0.533	0.556	0.456	0.567	0.488	0.600	0.400	0.290	0.529	0.433	0.406	0.067	0.667	0.440	0.400	0.367	0.227	0.462
Aat-1																		
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.000
1.00	0.989	0.977	0.982	0.983	0.986	0.983	0.917	1.000	1.000	0.983	1.000	0.950	1.000	1.000	1.000	0.983	0.955	1.000
1.20	0.011	0.023	0.018	0.017	0.014	0.017	0.083	0.000	0.000	0.017	0.000	0.050	0.000	0.000	0.000	0.017	0.000	0.000
Ho	0.022	0.047	0.035	0.033	0.027	0.033	0.100	0.000	0.000	0.033	0.000	0.100	0.000	0.000	0.000	0.033	0.091	0.000
Aat-2																		
0	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.059	0.000	0.033	0.150	0.000	0.020	0.017	0.033	0.000	0.038
1.00	0.567	0.487	0.570	0.500	0.583	0.550	0.733	0.629	0.691	0.583	0.667	0.750	0.625	0.580	0.667	0.567	0.727	0.692
1.10	0.344	0.368	0.377	0.478	0.278	0.433	0.233	0.339	0.250	0.317	0.300	0.100	0.333	0.360	0.317	0.383	0.273	0.231
1.25	0.077	0.145	0.053	0.022	0.139	0.017	0.033	0.032	0.000	0.083	0.000	0.000	0.042	0.040	0.000	0.017	0.000	0.038
1.40	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ho	0.556	0.632	0.439	0.391	0.528	0.500	0.467	0.258	0.500	0.500	0.533	0.300	0.417	0.520	0.267	0.633	0.364	0.462
Aat-3																		
1.00	0.644	0.576	0.684	0.600	0.579	0.583	0.667	0.435	0.662	0.650	0.650	0.554	0.625	0.680	0.583	0.750	0.477	0.615
1.90	0.011	0.098	0.009	0.000	0.132	0.000	0.000	0.000	0.000	0.000	0.033	0.071	0.000	0.040	0.000	0.000	0.068	0.000
3.00	0.344	0.326	0.307	0.400	0.289	0.417	0.333	0.565	0.338	0.350	0.317	0.375	0.375	0.240	0.417	0.250	0.455	0.385
5.50	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.000	0.000
Ho	0.578	0.630	0.351	0.267	0.658	0.500	0.333	0.355	0.471	0.367	0.367	0.500	0.417	0.320	0.233	0.423	0.409	0.308
Idh																		
1.00	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ho	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Dia-1																		
0	0.000	0.000	0.044	0.067	0.000	0.000	0.000	0.000	0.000	0.033	0.017	0.000	0.042	0.000	0.000	0.000	0.000	0.000
0.85	0.178	0.317	0.175	0.133	0.359	0.233	0.250	0.016	0.088	0.400	0.267	0.250	0.250	0.220	0.161	0.300	0.250	0.115
0.90	0.044	0.012	0.097	0.067	0.026	0.067	0.067	0.081	0.044	0.117	0.033	0.000	0.000	0.100	0.071	0.083	0.000	0.000
1.00	0.778	0.671	0.675	0.733	0.615	0.700	0.683	0.903	0.868	0.450	0.683	0.750	0.708	0.680	0.750	0.583	0.750	0.846
1.20	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.033	0.000	0.038
Ho	0.356	0.463	0.439	0.433	0.718	0.533	0.467	0.194	0.265	0.600	0.467	0.500	0.417	0.520	0.393	0.367	0.318	0.308
Dia-2																		
0.80	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.000
1.00	1.000	1.000	1.000	0.983	0.968	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.982	0.967	1.000	1.000
1.15	0.000	0.000	0.000	0.017	0.032	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000
Ho	0.000	0.000	0.000	0.033	0.065	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.067	0.000	0.000
Lap-1																		
0	0.000	0.065	0.096	0.017	0.017	0.000	0.000	0.065	0.000	0.033	0.017	0.017	0.000	0.000	0.017	0.017	0.000	0.038
0.95	0.011	0.016	0.044	0.033	0.033	0.050	0.017	0.000	0.015	0.050	0.050	0.150	0.000	0.020	0.000	0.067	0.000	0.000
1.00	0.989	0.903	0.825	0.900	0.900	0.950	0.967	0.935	0.985	0.917	0.933	0.833	1.000	0.980	0.983	0.917	1.000	0.962
1.05	0.000	0.016	0.035	0.050	0.050	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ho	0.022	0.194	0.228	0.200	0.200	0.100	0.067	0.129	0.029	0.167	0.133	0.300	0.000	0.040	0.033	0.167	0.000	0.077
Lap-2																		
0	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.95	0.056	0.000	0.061	0.033	0.129	0.017	0.000	0.000	0.088	0.017	0.017	0.000	0.083	0.100	0.017	0.017	0.068	0.077
1.00	0.900	1.000	0.930	0.867	0.839	0.967	0.917	0.984	0.882	0.983	0.950	0.917	0.917	0.820	0.950	0.983	0.909	0.923
1.05	0.044	0.000	0.000	0.083	0.032	0.017	0.083	0.016	0.029	0.000	0.033	0.083	0.000	0.080	0.033	0.000	0.023	0.000
1.10	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ho	0.200	0.000	0.140	0.267	0.323	0.067	0.167	0.032	0.235	0.033	0.100	0.100	0.167	0.360	0.100	0.033	0.182	0.154
6-Pgd-1																		
0.85	0.000	0.000	0.044	0.000	0.000	0.017	0.000	0.000	0.020	0.000	0.000	0.056	0.000	0.040	0.034	0.022	0.000	0.000
0.95	0.420	0.403	0.447	0.400	0.547	0.414	0.433	0.274	0.200	0.362	0.233	0.333	0.542	0.380	0.414	0.326	0.500	0.615
1.00	0.580	0.597	0.491	0.600	0.453	0.534	0.550	0.726	0.780	0.621	0.750	0.611	0.417	0.580	0.552	0.652	0.500	0.385
1.05	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000
1.30	0.000	0.000	0.009	0.000	0.000	0.034	0.017	0.000	0.000	0.017	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ho	0.432	0.694	0.474	0.533	0.594	0.724	0.567	0.419	0.400	0.517	0.500	0.741	0.333	0.440	0.586	0.696	0.636	0.308
6-Pgd-2																		
0	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.80	0.034	0.030	0.018	0.000	0.050	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000
0.90	0.193	0.303	0.316	0.200	0.300	0.431	0.333	0.435	0.400	0.397	0.283	0.463	0.417	0.240	0.276	0.391	0.500	0.308
1.00	0.761	0.667	0.667	0.800	0.650	0.569	0.633	0.565	0.600	0.603	0.717	0.537	0.542	0.760	0.724	0.609	0.500	0.692
Ho	0.364	0.485	0.491	0.400	0.633	0.517	0.567	0.484	0.280	0.517	0.367	0.704	0.333	0.480	0.414	0.435	0.455	0.308

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Gpi																		
0.95	0.044	0.056	0.018	0.033	0.035	0.033	0.017	0.183	0.063	0.033	0.125	0.000	0.042	0.020	0.017	0.033	0.000	0.038
1.00	0.944	0.844	0.982	0.917	0.884	0.967	0.933	0.817	0.937	0.917	0.828	0.833	0.875	0.960	0.917	0.967	1.000	0.962
1.15	0.000	0.078	0.000	0.000	0.023	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000
1.25	0.011	0.022	0.000	0.050	0.058	0.000	0.017	0.000	0.000	0.050	0.047	0.167	0.083	0.020	0.050	0.000	0.000	0.000
Ho	0.111	0.244	0.035	0.167	0.233	0.067	0.067	0.300	0.125	0.167	0.344	0.267	0.250	0.080	0.167	0.067	0.000	0.077
Gdh																		
1.00	0.567	0.685	0.561	0.583	0.707	0.667	0.767	0.419	0.515	0.650	0.766	0.633	0.542	0.700	0.650	0.700	0.682	0.885
1.30	0.433	0.315	0.439	0.400	0.293	0.333	0.233	0.581	0.485	0.350	0.234	0.367	0.458	0.300	0.350	0.300	0.318	0.115
1.40	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ho	0.511	0.519	0.421	0.300	0.439	0.433	0.467	0.516	0.500	0.500	0.406	0.600	0.750	0.520	0.300	0.333	0.636	0.231
Mdh-1																		
0.85	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.00	0.989	0.927	0.877	0.950	0.943	0.983	0.967	0.935	1.000	0.883	0.922	1.000	1.000	0.960	0.983	0.983	0.932	1.000
1.15	0.011	0.073	0.123	0.050	0.057	0.017	0.033	0.065	0.000	0.100	0.078	0.000	0.000	0.040	0.017	0.017	0.068	0.000
Ho	0.022	0.104	0.246	0.100	0.114	0.033	0.067	0.065	0.000	0.167	0.156	0.000	0.000	0.080	0.033	0.033	0.136	0.000
Mdh-2																		
0'	0.000	0.010	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0	0.000	0.010	0.053	0.000	0.011	0.000	0.000	0.000	0.000	0.017	0.016	0.000	0.042	0.000	0.017	0.033	0.091	0.038
0.80	0.000	0.000	0.000	0.000	0.000	0.000	0.117	0.000	0.000	0.000	0.047	0.000	0.042	0.020	0.033	0.000	0.000	0.038
1.00	0.989	0.979	0.947	1.000	0.989	1.000	0.867	1.000	1.000	0.983	0.938	1.000	0.917	0.980	0.933	0.967	0.909	0.923
1.10	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000
Ho	0.022	0.042	0.070	0.000	0.023	0.000	0.267	0.000	0.000	0.033	0.125	0.000	0.167	0.040	0.133	0.067	0.182	0.154
Mdh-3																		
0'	0.011	0.020	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.017	0.000	0.000
0.70	0.278	0.230	0.201	0.383	0.273	0.167	0.117	0.145	0.044	0.400	0.328	0.183	0.250	0.180	0.133	0.200	0.205	0.154
0.90	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.00	0.678	0.750	0.781	0.617	0.727	0.833	0.883	0.823	0.941	0.600	0.672	0.817	0.708	0.820	0.850	0.783	0.795	0.846
1.20	0.011	0.000	0.009	0.000	0.000	0.000	0.000	0.016	0.015	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000
Ho	0.489	0.460	0.298	0.367	0.500	0.267	0.167	0.355	0.118	0.533	0.531	0.167	0.583	0.360	0.300	0.267	0.409	0.308
Mdh-4																		
0	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.00	0.678	0.426	0.561	0.450	0.471	0.517	0.567	0.726	0.640	0.550	0.406	0.667	0.667	0.680	0.433	0.533	0.364	0.462
3.00	0.000	0.000	0.018	0.017	0.015	0.034	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6.50	0.244	0.426	0.360	0.400	0.338	0.241	0.383	0.194	0.340	0.350	0.453	0.283	0.292	0.260	0.467	0.367	0.636	0.423
8.00	0.078	0.147	0.061	0.133	0.162	0.207	0.050	0.081	0.020	0.100	0.141	0.050	0.042	0.060	0.100	0.100	0.000	0.115
Ho	0.489	0.735	0.544	0.600	0.794	0.552	0.500	0.516	0.480	0.500	0.406	0.633	0.250	0.560	0.600	0.667	0.545	0.692

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Fl-Est																		
0.30	0.122	0.177	0.158	0.200	0.210	0.067	0.083	0.306	0.088	0.174	0.268	0.500	0.000	0.160	0.117	0.152	0.182	0.154
0.40	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000
0.70	0.078	0.081	0.053	0.150	0.113	0.150	0.083	0.258	0.074	0.217	0.071	0.000	0.375	0.120	0.183	0.283	0.182	0.077
0.95	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.038
1.00	0.767	0.742	0.772	0.650	0.677	0.783	0.833	0.435	0.838	0.609	0.661	0.500	0.625	0.700	0.667	0.565	0.636	0.731
1.05	0.022	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000
Ho	0.444	0.387	0.316	0.433	0.581	0.300	0.333	0.613	0.235	0.739	0.321	0.481	0.583	0.520	0.533	0.696	0.545	0.385
Pgm-1																		
0.95	0.044	0.041	0.053	0.017	0.056	0.083	0.050	0.033	0.029	0.017	0.031	0.000	0.042	0.000	0.000	0.033	0.000	0.000
1.00	0.922	0.918	0.904	0.933	0.889	0.900	0.900	0.917	0.926	0.917	0.953	1.000	0.875	0.980	0.950	0.917	1.000	1.000
1.05	0.033	0.041	0.044	0.050	0.056	0.017	0.050	0.050	0.044	0.067	0.016	0.000	0.083	0.020	0.050	0.050	0.000	0.000
Ho	0.111	0.164	0.193	0.067	0.222	0.200	0.200	0.167	0.147	0.167	0.094	0.000	0.250	0.040	0.100	0.167	0.000	0.000
Pgm-2																		
0	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.95	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000
1.00	1.000	1.000	0.982	0.967	1.000	1.000	0.950	1.000	1.000	0.983	0.984	1.000	0.958	0.980	1.000	1.000	1.000	1.000
1.10	0.000	0.000	0.000	0.033	0.000	0.000	0.050	0.000	0.000	0.000	0.016	0.000	0.042	0.000	0.000	0.000	0.000	0.000
Ho	0.000	0.000	0.035	0.067	0.000	0.000	0.100	0.000	0.000	0.033	0.031	0.000	0.083	0.040	0.000	0.000	0.000	0.000

*) Populations: 1 — Tukumus, 2 — Albertin, 3 — Podsvilye, 4 — Polesye, 5 — Markovichi, 6 — Vygodskaya, 7 — Mizunskaya, 8 — Zelenskaya, 9 — Gorganskaya, 10 — Novomoskovsk, 11 — Slavyansk, 12 — Shebekino, 13 — Kursk, 14 — Usmansk, 15 — Khvalynsk, 16 — Zhygulyovsk, 17 — Karaulnoye, 18 — Uyarskoye.
**) n — number of trees analysed
***) Ho — observed heterozygosity

6-Pgd-1, and 6-Pgd-2 were the most variable loci; their expected heterozygosities were greater than 35 %. Over 6 other loci (Gpi, Lap-1, Lap-2, Mdh-1,Mdh-2, and Pgm-1) heterozygosity values ranged from 5 % to 20 %. Aat-1, Dia-2, and Pgm-2 appeared to be the least variable; their heterozygosities were not more than 5%. In most of the populations at Dia-2 and Pgm-2 there was no genetic variation. Only Idh was monomorphic over all the populations assayed.

Table 4 lists genetic variation values calculated for the *P. sylvestris* populations studied. As seen in *table 4*, percent polymorphic loci at 99 % criterion in the East-European populations varies from 71.4 % to 90.5 %. It is interesting to note that central and isolated populations do not demonstrate pronounced differencies in this parameter as far as the highest proportion of polymorphic loci, 0.905, was observed both in 3 populations from the main distribution (Podsvilye, Polesye, and Markovichi) and 3 isolated ones (Mizunskaya, Zhygulyovsk and Novomoskovsk). A similar situation was observed at the 95 % criterion, because the 2 isolated populations had values for percent polymorphic loci that equaled or exceeded values for any central population. Nevertheless, in the central populations, P_{99} values vary from 81 % to 90.5 % and in the isolated populations from 71.4 % to 90.5 %. This suggests that populations from the main segment of the *P. sylvestris* distribution are characterized by higher levels of genetic variation than the isolated populations. Other genetic parameters also demonstrate a similar tendency (*Table 4*). For example, expected and observed heterozygosities for the central populations range from 26.5 % to 31.5 % and from 26.5 % to 36.8 %, respectively, while in the isolated populations, H_e and H_o values vary from 22.7 % to 30.5 % and from 23.2 % to 30.5 %, respectively. With regard to the average number of alleles per locus, the central populations had values in excess of 2.47 while for the isolated populations it was generally lower (*Table 4*). This emphasizes once again that genetic variation is somewhat higher in the central populations than in the isolated populations. This low variation in isolated East-European *P. sylvestris* populations might not be casual and, perhaps, is the result of inbreeding and drift in small populations. Further studies are necessary to provide deeper insights.

The Asian populations appeared to exhibit the lowest level of genetic variation among the 18 populations studied. However, our analysis of the Asian segment of *P. sylvestris*' distribution was weak because only two Siberian populations of Scotch pine were assayed.

Table 4. — Genetic variation in natural populations of *P. sylvestris*.

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)
Tukumus	c	0.667	0.857	2.762	0.265	0.285
Albertin	c	0.714	0.810	2.476	0.296	0.332
Podsvilye	c	0.762	0.905	2.952	0.297	0.276
Polesye	c	0.762	0.905	2.619	0.297	0.265
Markovichi	c	0.762	0.905	2.667	0.315	0.368
Vygodskaya	i	0.619	0.810	2.333	0.271	0.284
Mizunskaya	i	0.810	0.905	2.524	0.262	0.273
Zelenskaya	i	0.714	0.762	2.143	0.254	0.236
Gorgamskaya	i	0.667	0.714	2.143	0.227	0.232
Novomoskovsk	i	0.714	0.905	2.571	0.305	0.305
Slavyansk	i	0.762	0.857	2.524	0.279	0.271
Shebekino	i	0.667	0.714	2.000	0.256	0.282
Kursk	i	0.714	0.762	2.286	0.295	0.282
Usmansk	i	0.571	0.857	2.429	0.261	0.282
Khvalynsk	i	0.714	0.857	2.667	0.270	0.239
Zhygulyovsk	i	0.619	0.905	2.524	0.267	0.280
Karaulnoye	s	0.667	0.714	1.952	0.261	0.271
Uyarskoye	s	0.619	0.714	2.048	0.236	0.227
In total in the species		0.714	0.905	4.190	0.286	0.282

*) C — central population, i — isolated population,
S — Siberian population

On the whole, our investigation shows that in the segments of *P. sylvestris* distribution studied more than 90 % of the loci are polymorphic, 28 % to 29 % of genes within each tree being heterozygous (*Table 4*). These data suggest that *P. sylvestris* is the most polymorphic pine species of all the pine species studied to date on the basis of analysis for 20 and more loci (Goncharenko et al., 1993b).

Other studies of genetic variation in *P. sylvestris* populations were based on analyses for: 10 Polish populations at 12 and 9 loci (Krzakowa et al., 1977; Mejnartowicz and Bergmann, 1985), 6 Swedish populations at 9 and 13 loci (Gullberg et al., 1982; Szmidt, 1984), 2 Czechoslovakian and 1 Ural populations at 10 loci (Starova et al., 1990; Filppula et al., 1992). Samples of trees from the Urals and a number of Swedish populations were stratified by age. For a number of Polish and Swedish populations, a mixture of seeds or plus trees were examined. Because of these reasons and also because of differences in the numbers and composition of the loci analyzed, we experienced some difficulties in comparing our results with those obtained by the aforementioned authors.

KINLOCH et al. (1986) carried out a more thorough analysis for *P. sylvestris* populations. They studied genetic structures and estimated genetic parameters for 14 Scotch populations at 16 allozyme loci. Averaged expected (0.31) and observed (0.30) heterozygosities obtained by these researchers are practically in agreement with ours.

In only 1 study was a low level of variation reported in *P. sylvestris*. This was revealed in populations from the Kalinin Region of the former USSR on the basis of an analysis of 39 loci (DUKHAREV et al., 1987). However, in that study no data were reported on allelic frequencies, proportion of polymorphic loci, mean number of alleles per locus, and expected heterozygosity that would allow us to make a valid comparison.

Analysis of genetic structure

We used WRIGHT's F-statistics and NEI's G-statistics to analyze population structure of *P. sylvestris*. Data on the correlation between uniting gametes within populations (F_{IS}), among populations (F_{ST}), for the species as a whole (F_{IT}), and the ratio of diversity among populations to the total diversity (G_{ST}) calculated on the basis of analysis for 21 loci are compiled in *table 5*.

The F_{IS} values ranged from —0.117 at 6-Pgd-1 to 0.111 at Aat-3 and averaged —0.014. A negative value indicates an excess of heterozygotes within *P. sylvestris* populations. At the same time, the F_{IT} values averaged 0.015, indicating a 1.5 % heterozygote deficiency in Scotch pine as a whole. F_{IS} and F_{IT} values show that the *P. sylvestris* populations studied are in general agreement with HARDY-WEINBERG expectation. This was obvious from a comparison of observed and expected heterozygosities, which were almost identical (*Table 4*). F_{ST} and G_{ST} values were not high, 0.028 and 0.033, respectively. Thus about 97% of genetic variation resides within population and about 3% among populations. Hence, regardless of the geographical isolation of most of the populations studied, their genetic structures are quite similar. It should be noted that in the course of the previous analyses for Swedish, German, and Scotch populations of *P. sylvestris* subdivision was also weak; G_{ST} and F_{ST} values ranged from 1 % to 2.8 % (GULLBERG et al., 1982 and 1985; MÜLLER-STARCK and GREGORIUS, 1986; KINLOCH et al., 1986).

F_{ST} enables us to estimate the level of gene flow, N_em, as follows: $F_{ST}=1/(1+4N_em)$. Using the F_{ST} values obtained (*Table 4*) we calculated N_em as 8.68. This means that gene exchange among the *P. sylvestris* populations studied is high, its rate being 8 migrants per generation. Gene flow can occur owing to transfer of pollen and seeds. In any case, our data show that *P. sylvestris* populations exchange genetic material at present or exchanged it not long ago quite intensively, and as a result they have similar gene pools, regardless of the fact that most of them are isolated and disjunct today (*Fig. 1*).

Genetic distance

NEI's genetic distance coefficient (D_N) was used (NEI, 1972) to estimate genetic differentiation amongst all the 18 *P. sylvestris* populations assayed, including East-European and West-Siberian populations. D_N values obtained for the 21 genes are given in *table 6*. As seen in the table, the Tukumuu population (central) and the Usmansk pine forest (isolated) have the most similar genetic structures; the genetic distance coefficient between them was 0.005. Major differencies in genetic structure occurred between the Zelenskaya and Uyarskaya populations, D_N values between them being 0.046. For all possible pairs of populations, D_N values averaged 0.017. Such a small value of D_N shows that the populations are weakly differentiated. It should be noted that we have revealed a great number of unique alleles, i. e. alleles that are specific only for a concrete population (*Table 3*). 14 unique alleles did not contribute significantly to the genetic distance coefficient value (*Table 6*), but they apparently reflect microevolutionary processes occurring in *P. sylvestris* populations.

Using an unweighted pair-group method (UPGMA) and the D_N values, we constructed a dendrogram visualizing genetic differentiation in the *P. sylvestris* populations (*Fig. 4*).

Table 5. — Estimates of F_{IS}, F_{IT}, F_{ST} and G_{ST} for 21 loci in *P. sylvestris*.

Locus	F_{IS}	F_{IT}	F_{ST}	G_{ST}
Adh-1	-0.095	-0.068	0.024	0.039
Adh-2	-0.016	0.017	0.032	0.035
Aat-1	0.012	0.042	0.031	0.025
Aat-2	0.075	0.109	0.037	0.030
Aat-3	0.111	0.144	0.036	0.025
Gpi	-0.005	0.041	0.046	0.044
Gdh	-0.050	-0.017	0.031	0.039
Dia-1	-0.038	-0.000	0.037	0.047
Dia-2	-0.005	0.020	0.025	0.023
Idh	0.000	0.000	0.000	0.000
Lap-1	-0.012	0.026	0.038	0.041
Lap-2	-0.044	-0.019	0.025	0.033
Mdh-1	0.021	0.048	0.027	0.033
Mdh-2	0.002	0.033	0.032	0.041
Mdh-3	-0.036	-0.011	0.025	0.043
Mdh-4	-0.043	-0.013	0.029	0.040
Fl-Est	0.007	0.045	0.039	0.059
Pgm-1	-0.010	0.004	0.014	0.014
Pgm-2	-0.005	0.014	0.019	0.024
6-Pgd-1	-0.117	-0.086	0.028	0.036
6-Pgd-2	-0.046	-0.021	0.024	0.031
Mean	-0.014	0.015	0.028	0.033

Table 6. — Estimates of Nei's genetic distance coefficient based upon data from 21 loci.

Populations	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1) Tukumus	-	0.009	0.006	0.007	0.010	0.010	0.013	0.022	0.012	0.013	0.013	0.026	0.011	0.005	0.009	0.015	0.023	0.018
2) Albertin		-	0.008	0.011	0.006	0.010	0.014	0.032	0.018	0.011	0.009	0.027	0.017	0.012	0.007	0.014	0.014	0.015
3) Podsvilye			-	0.009	0.008	0.008	0.012	0.025	0.012	0.012	0.014	0.025	0.012	0.007	0.008	0.012	0.017	0.016
4) Polesye				-	0.012	0.012	0.020	0.025	0.022	0.012	0.010	0.032	0.017	0.010	0.010	0.014	0.025	0.024
5) Markovichi					-	0.011	0.014	0.036	0.023	0.010	0.012	0.025	0.013	0.009	0.010	0.014	0.015	0.012
6) Vygodskaya						-	0.012	0.024	0.015	0.013	0.016	0.031	0.011	0.010	0.009	0.011	0.021	0.018
7) Mizunskaya							-	0.032	0.017	0.017	0.013	0.021	0.017	0.009	0.011	0.011	0.018	0.017
8) Zelenskaya								-	0.021	0.029	0.029	0.022	0.023	0.025	0.025	0.024	0.036	0.046
9) Gorganskaya									-	0.026	0.020	0.026	0.020	0.013	0.011	0.021	0.022	0.024
10) Novomoskovsk										-	0.010	0.025	0.013	0.013	0.015	0.008	0.021	0.029
11) Slavyansk											-	0.021	0.024	0.011	0.011	0.010	0.019	0.021
12) Shebekino												-	0.031	0.022	0.026	0.021	0.026	0.034
13) Kursk													-	0.014	0.012	0.015	0.019	0.023
14) Usmansk														-	0.009	0.009	0.023	0.017
15) Khvalynsk															-	0.014	0.010	0.009
16) Zhygulyovsk																-	0.022	0.027
17) Karaulnoye																	-	0.013
18) Uyarskoye																		-

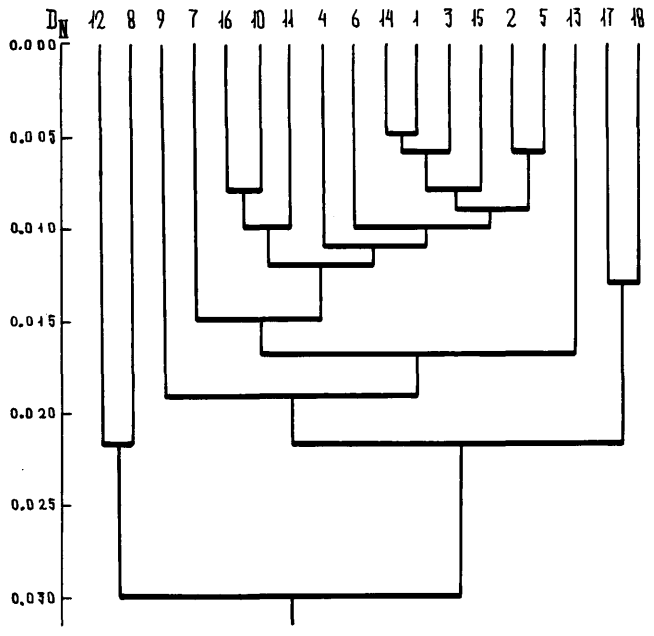

Figure 4. — Dendrogram showing the clustering of the eighteen natural populations of *P. sylvestris* based on Nei's genetic distance coefficient.

From the dendrogram it can be seen that clustering did not depend on geographical proximity or distance. For example, populations from the continuous *P. sylvestris* distribution did not form a common cluster. The 2 distant Siberian populations were united in a separate cluster, but genetic distance between them and the main set of populations is small. The most divergent populations appeared to be the Shebekino population from the forest-steppe zone of Russia and the Zelenskaya population from the Carpathians (*Fig. 4*). Judging from their geographical locations, the 4 Carpathians populations (Vygodskaya, Mizunskaya, Zelenskaya, and Gorganskaya) were expected to be united into an independent group because they have a common history of formation and are located far from the remaining East-European populations we studied. However, from *figure 4* it is seen that the Carpathian populations are dispersed all over the dendrogram.

A number of isolated populations (Slavyansk, Shebekino, Khvalynsk, and Zhygulyovsk) consist of pines that can grown on cretaceous soils. Some scientists regard them as a distinct species, *Pinus cretacea* Kalen (Lypa, 1955; Mashkin, 1971; Artamonov, 1989), whereas others consider that it would be more precise to name them *P. sylvestris* L. var *cretacea* Kalen (Sukachyov, 1938; Pravdin, 1964). If *P. sylvestris* and *P. cretacea* are distinct species, it might be reasonable to expect significant differences between them in allelic frequencies, which must be reflected in Nei's genetic distance coefficient and formation of distinct clusters in the dendrogram. But the dendrogram contradicts these expectations. The 4 populations of *Pinus cretacea* (Slavyansk, Shebekino, Khvalynsk, and Zhygulyovsk) are evenly dispersed all over the dendrogram (*Fig. 4*).

In addition, the genetic distance coefficient between *P. sylvestris* and *P. cretacea* was 0.017, this D_N value being even smaller than genetic distances between some populations within the groups, *P. cretacea* and *P. sylvestris* (*Table 6*). The data demonstrate that *P. cretacea,* which occurs on ancient carbonate rock outcrops, can be regarded simply as a race of *P. sylvestris.*

On the whole, our results show that in *P. sylvestris* sufficient genetic variation exists to explain its great ecological plasticity and evolutionary potential. Estimated parameters of gene diversity, genetic distance, and gene flow show that in the segment of the *P. sylvestris* distribution that we studied, a common gene pool is shared.

Acknowledgements

We should like to thank warmly academician, Dr. L. V. Khotyljeva for every kind of assistance in carrying out of the genetic research. We gratefully acknowledge Prof. Dr. L. I. Korochkin and Prof. Dr. F. T. Ledig for review and comments on an earlier draft of this paper. We also thank E. Guseva for her assistance in translation of our manuscripts into English.

References

Artamonov, V. I.: Rare and disappearing plants. Agropromizdat, Moscow (in Russian) (1989). — Bobrov, E. G.: Forest-forming conifers of the USSR. Nauka, Leningrad (in Russian) (1978). — Cheliak, W. M. and Pitel, J. A.: Techniques for starch gel electrophoresis of enzymes from forest tree species. Petawawa National Forestry Institute (1984). — Conkle, M. T., Hodgskiss, P. D., Nunnally, L. B. and Hunter, S. C.: Starch gel electrophoresis of conifer seeds: a laboratory manual. USDA For. Serv. Tech. Rep. PSW-64 (1982). — Critchfield, W. B. and Little, E. L.: Geographic distribution of the Pinus of the world. Miscellaneous Publication. U. S. Dept. Agricult. 991, Washington (1966). — Dukharev, V. A., Romanovsky, H. G. and Ryabokon, S. M.: Heterozygosity and seed productivity of *Pinus sylvestris* L. individuals. Lesovedenie **2**: 87—90 (in Russian) (1987). — Filppula, S., Szmidt, A. E. and Savolainen, O.: Genetic comparison between *Pinus sylvestris* and *P. mugo* using isozymes and chloroplast DNA. Nord. J. Bot. **12**: 381—386 (1992). — Goncharenko, G. G., Padutov, V. E. and Potenko, V. V.: Guide to conifer species research by isozymes methods. Gomel (in Russian). (1989). — Goncharenko, G. G., Padutov, V. E. and Silin, A. E.: Population structure, gene diversity, and differentiation in the natural populations of Cedar Pines (*Pinus subsect.* Cembrae, Pinaceae) in the USSR. Plant Syst. Evol. **182**: 121—134 (1992). — Goncharenko, G. G., Padutov, V. E. and Silin, A. E.: Allozyme variation in natural populations of Eurasian pines. I. Population structure, genetic variation, and differentiation in *Pinus pumila* (Pall.) Regel from Chukotsk and Sakhalin. Silvae Genet. **42**: 237—246 (1993a). — Goncharenko, G. G., Padutov, V. E. and Silin, A. E.: 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. Silvae Genet. **42**: 246—253 (1993b). — Gullberg, U., Yazdani, R. and Rudin, D.: Genetic differentiation between adjacent populations of *Pinus sylvestris.* Silva Fennica **16**: 205—214 (1982). — Gullberg, U., Yazdani, R., Rudin, D. and Ryman, N.: Allozyme variation in Scots pine (*Pinus sylvestris* L.) in Sweden. Silvae Genet. **34**: 193—201 (1985). — Hamrick, J. L., Mitton, J. B. and Linhart, Y. B.: Levels of genetic variation in trees: influence of life history characteristics. In: M. T. Conkle (ed): Proc. Symp. on Isozymes of North American Forest Trees and Forest Insects. Berkeley, Ca. (1979). USDA Gen. Tech. Rep. PSW-48, p. 35—41 (1981). — Kapper, O. G.: Coniferous species. Goslesbumizdat, Moscow, Leningrad. (In Russian). (1954). — Kinloch, B. B., Westfall, R. D. and Forrest, G. I.: Caledonian Scotch pine: origins and genetic structure. New Phytol. **104**: 703—729 (1986). — Komarov, V. L. (Editor): Flora of the USSR. Edition of the USSR's Academy of Sciences, Leningrad. (In Russian). (1934). — Kozubov, G. M. and Muratova, E. N.: Modern gymnosperms. Nauka, Leningrad (1986). — Krzakowa, M.: Genetic differentiation of Scotch pine populations. I. Genotypes. Silva Fennica **16**: 200—205 (1982). — Krzakowa, M., Szweykowski, J. and Korczyk, A.: Population genetics of Scotch pine (*Pinus sylvestris* L.) forests. Genetic structure of plus-trees in Bolewice near Poznan (West Poland). Bulletin De L'Academie Polonaise des Sciences. Series des sciences biologiques **25**: 583—589 (1977). — Ledig, F. T.: Heterozygosity, heterosis, and fitness in outbreeding plants. In: M. E. Soule (ed). Conservation biology: the science of scarcity and diversity. Sinauer Associates, Sunderland, Massachusetts. p. 77—104 (1986). — Lypa, A. L.: Identifier of trees and bushes. V. 1. Kiev. (In Russian). (1955). — Mashkin, C. I.: Dendrology of Central Chernozemye. V. 1. Voronezh. (In Russian). (1971). — Mejnartowicz, L. and Bergmann, F.: Genetic differentiation among Scotch pine populations from the lowland and the mountains in Poland. In: H.-R. Gregorius (ed). Population Genetics

in Forestry. Lecture Notes in Biomathematics 60. Springer-Verlag, Berlin, Heidelberg, New York. p. 253—266 (1985). —— Müller-Starck, G. and Gregorius, H. R.: Monitoring genetic variation in forest tree population. Proceedings 18th World Congress IUFRO, Yugoslavia. September 7 to 21. Division 2. Vol. II. p. 589—599 (1986). —— Padutov, V. E., Goncharenko, G. G. and Podzharova, Z. S.: Genetic variability in *Pinus sylvestris*. Doklady Acad. Sci. of Byelorussia 33: 1039—1042 (in Russian) (1989). —— Prakash, S., Lewontin, R. C. and Hubby, J. L.: A molecular approach to the study of genic heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal and isolated populations of *Drosophila pseudoobscura*. Genetics 61: 841—858 (1969). —— Pravdin, L. F.: Scotch pine. Variability, intraspecies taxonomy and breeding. Nauka, Moscow (in Russiae) (1964). —— Rudin, D. and Ekberg, I.: Linkage studies in *Pinus sylvestris* L. — using macro-gametophyte allozymes. Silvae Genet. 27: 1—12 (1978). —— Starova, N. V., Yanbayev, Yu. A., Yumadilov, N. Kh., Adler, E. N., Dukharev, V. A. and Shigapov, Z. Kh.: Genetic variability of *Pinus sylvestris* in age classes. Genetika 26: 498—505 (in Russian) (1990). —— Sukhachyov, V. N.: Dendrology with foundations of forest geobotany. Goslestekhizdat, Leningrad (in Russian) (1938). —— Szmidt, A. E.: Genetic studies of Scotch pine (*Pinus sylvestris* L.) domestication by means of isozyme analysis. Ph. D. diss., Swedish University of Agricultural Sciences, Umea (1984). —— Szmidt, A. E. and Muona, O.: Linkage relationship of allozyme loci in *Pinus sylvestris*. Hereditas 111: 91—97 (1989).

Allozyme Variation in Populations of Pinus sylvestris L. from a 1912 Provenance Trial in Pulawy (Poland)

By W. Prus-Glowacki and E. Bernard

Adam Mickiewicz University, Faculty of Biology,
Department of Genetics, ul. Dąbrowskiego 165,
60—594 Poznań Poland

(Received 8th December 1993)

Summary

Study of the genetic variation and genetic structure of 13 populations of *Pinus sylvestris* from Eastern Europe and Turkey has shown significant genetic differentiation of the populations. The average genetic differentiation (index GST) is high (0.076), but it is about 50 % lower in populations from the area covered by ice during the last glaciation (GST = 0.035). The mean multilocus heterozygosity for 8 loci (Ho = 0.357) and the average number of alleles per locus are similar to those from the other parts of Europe. The genetic similarities of populations (Nei) are not correlated with the geographical localities of the studied stands.

Key words: Pinus sylvestris, genetic structure, isoenzymes, provenance.

FDC: 165.3; 165.5; 232.12; 174.7 *Pinus sylvestris*; (438).

Introduction

The experiment in Pulawy was established in the year 1912 and is the oldest provenance trial in Poland. This experiment was discovered in 1980 and described in detail by Oleksyn ad Giertych (1982, 1984). The trial comprises seed lots from 13 provinces of imperial pre-revolutionary Russia. The seed sample origins ranged from Latvia and Ukraine to Western Siberia and Turkey, and so, it covered a relatively large natural range of *Pinus sylvestris* in Eastern Europe. This experiment has also some additional advantages. Seeds collected in the beginning of 20th century were more likely to be from autochtonous stands, because there was no major practice in pre-revolutionary Russia to transfer seeds from one region to another for reforestation proposes. However, in relation to the IUFRO 1938 provenance trials, the trial in Pulawy has some disadvantages. The seeds did not originate from single stands but the material was collected from different localities and pooled together, because at that time, the province was regarded as the basic forest unit (Giertych and Oleksyn, 1981). On the other hand this kind of material could be useful for investigation of geographic races of the species because seeds originated from different geographical regions. This work aimed at describing genetic structure and level of genetic variation as well as geographical differentiation of *Pinus sylvestris* in Eastern Europe. The knowledge concerning this matter remains still largely incomplete (Prus-Glowacki, 1991; Müller-Stark et al., 1992).

Material and Methods

Dormant winter buds were used for isoenzymatic analyses. Plant material was collected from 12 to 30 randomly choosen trees from each province. In cases when less than 30 individuals were available, the samples from all living trees were collected. The Tobolsk province was excluded from the isoenzymatic analyses to avoid the error of a small sample, because it was represented by only 7 trees. Detailed data on the geographical origin of the samples are given in *table 1* and *figure 1*. Variation of the following enzymatic loci was studied: fluorescent esterase FEST (E.C.3.1.1.1.), glutamate oxalo-acetate transaminase GOT 2 loci (E.C. 2.6.11.), diaforase DIA (E.C. 1.6.4.3.), glutamate dehydrogenase GDH (E.C. 1.4.1.3.), alcohol dehydrogenase ADH (E.C. 1.1.1.1.), shikimate dehydrogenase ShDH (E.C. 1.1.1.25.) and NADH — dependent dehydrogenase (NDH). The separation of isozymes on starch gels and the genetic interpretation of the results were performed as described in Muona and Szmidt (1985) and Prus-Glowacki (1986). The following genetic parameters for the studied groups of trees were calculated: the frequency of alleles and genotypes, number of alleles (A/L) and genotypes (G/L) per locus, number of effective alleles (ne), heterozygosity (Ho, He — observed and expected respectively), total (HT) and average (Hs) gene diversity between populations, genotypic polymorphism indices (Pg), fixation indices (F), genetic differentiation of populations DST = HT-Hs and GST = DST/HT and genetic similarity coefficients according to Nei and Hedrick (SN and SH). Based on genetic distances (DN = 1 — SN, DH = 1 — SH), dendrites and dendrograms illustrating mutual systematic positions (cluster analysis UPGMA) of the populations were con-