In: HATTEMER, H. H. (Ed.): Erhaltung forstlicher Genressourcen. J. D. Sauerländer's Verlag, Frankfurt am Main. pp. 87—98 (1990). —— MORGANTE, M. and VENDRAMIN, G. G.: Genetic variation in italian populations of *Picea abies* (L.) KARST. and *Pinus leucodermis* ANT.. In: MÜLLER-STARCK, G. and ZIEHE, M. (Eds.): Genetic variation in European populations of forest trees. J. D. Sauerländer's Verlag, Frankfurt am Main. pp. 205—227 (1991). —— MORGANTE, M., VENDRAMIN, G. G. and OLIVIERI, A. M.: Mating system analysis in *Pinus leucodermis* ANT.: detection of self-fertilization in natural populations. Heredity **67**: 197—203 (1991). —— MÜLLER-STARCK, G. and HÜTTERMANN, A.: Aminopeptidase in seeds of *Picea abies* (L.) KARST.: characterization of leucine aminopeptidase by molecular properties and inhibitor. Biochem. Genet. **19**: 1247—1260 (1981). —— MÜLLER-STARCK, G. and LIU, Y. Q.: Genetics of *Cunninghamia lanceolata* HOOK.. 1. Genetic analysis. Silvae Genetica **37**: 236—243 (1988). —— MUONA, O., YAZDANI, R. and LINDQVIST, G.: Analysis of linkage in *Picea abies*. Hereditas **106**: 31—36 (1987). —— NEALE, D. B. and ADAMS, W. T.: Inheritance of isozyme variants in seed tissues of balsam fir (*Abies balsamea*). Can. J. Bot. **59**: 1285—1291 (1981). —— O'MALLEY, D. M., ALLENDORF, F. W. and BLACKE, G. M.: Inheritance of isozyme variation and heterozygosity in *Pinus ponderosa*. Biochem. Genet. **17**: 233—250 (1979). —— PERRY, D. J. and KNOWLES, P.: Inheritance and linkage relationships of allozymes of eastern white cedar (*Thuja occidentalis*) in northwestern Ontario. Genome **32**: 245—250 (1989). —— RAO, C. R.: Linear statistical inference and its application. John Wiley and Sons, New York (1973). —— SHIRAISHI, S.: Inheritance of isozyme variations in Japanese black pine, *Pinus thunbergii* PARL.. Silvae Genetica **37**: 93—100 (1988). —— STEWART, S. C. and SCHOEN, D. J.: Segregation at enzyme loci in megagametophytes of white spruce, *Picea glauca*. Can. J. Genet. Cytol. **28**: 149—153 (1986). —— STRAUSS, S. H. and CONKLE, M. T.: Segregation, linkage, and diversity of allozymes in knobcone pine. Theor. Appl. Genet. **72**: 483—493 (1986). —— XIE, C. Y., DANCIK, B. P. and YEH, F. C.: Inheritance and linkage of isozymes in *Thuja orientalis*. J. of *Hered.* **82**: 329—334 (1991).

Allozyme Variation in Natural Populations of Eurasian Pines

I. Population Structure, Genetic Variation, and Differentiation in Pinus pumila (Pall.) Regel from Chukotsk and Sakhalin

By G. G. GONCHARENKO, V. E. PADUTOV and A. E. SILIN

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

(Received 21st January 1993)

Summary

Five natural populations of *Pinus pumila* were investigated by starch-gel electrophoresis. A total of 56 alleles were observed at 22 loci. More than 68% of the loci were polymorphic and, on average, 28.8% of the loci per tree were heterozygous (observed heterozygosity). Interpopulation genetic diversity was 4.3% of the total genetic diversity and the level of gene flow was 5.56 migrants per generation. NEI's genetic distance coefficient ranged from 0.015 to 0.045 among populations. The data obtained suggest that there are no strong genetic differences between geographically distant populations of *P. pumila* from Chukotsk, on the one hand, and from Sakhalin, on the other.

Key words: *Pinus pumila*, isozymes, inheritance, segregation, population structure, genetic variation, genetic differentiation.

Introduction

In recent years, scientists in several countries successfully conducted populational and genetic analyses for a great number of coniferous species, especially pines. This became possible because of isozyme electrophoresis which came to be widely used in population studies. Using a large set of isozyme loci (more than 14 to 18), the level of variation, gene diversity, and differentiation were quantified in populations of different species of the *Pinus* genus (O'MALLEY et al., 1979; YEH and LAYTON, 1979; CONKLE, 1981; HAMRICK et al., 1981; ALLENDORF et al., 1982; GURIES and LEDIG, 1982; WHEELER and GURIES, 1982; DANCIK and YEH, 1983; LEDIG and CONKLE, 1983; FURNIER and ADAMS, 1986; LEDIG, 1986; PLESSAS and STRAUSS, 1986; MILLAR et al., 1988; MORAN et al., 1988; NIEBLING and CONKLE, 1990). These investigations were devoted to North American pine species. Genetic structure of European populations of *Pinus sylvestris* (MUONA and SZMIDT, 1985; DUKHAREV et al., 1987; PADUTOV et al., 1989; GONCHARENKO et al., 1991) and of Mediterranean populations of the *Pinus halepensis-brutia* species complex (LOUKAS et al., 1983; SCHILLER et al., 1986; CONKLE et al., 1988) were also analyzed.

In the present study, we used 22 isozyme loci to analyze genetic variation and differentiation among populations of *Pinus pumila* (PALL.) REGEL, which has a great continuous distribution in eastern Siberia and the Far East.

Materials and Methods

This study was based on seeds collected in 1989 to 1991 from 63 individual trees in 3 mainland populations of *P. pumila* from the Chukotsk Autonomous Circuit (Dolgy Island in the Velikaya River delta, the Malaya River delta, and Mainets Lake), and 2 populations from the island of Sakhalin (in the vicinity of the towns of Makarov and Nogliki). 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 20 cones from each of the 63 trees. One of the Mdh loci was expressed in the embryo tissues, and in this case, no less than eight embryos from each tree were assayed.

Methods of enzymes extraction and electrophoresis followed CONKLE et al. (1982), CHELIAK and PITEL (1984), and GONCHARENKO et al. (1989). The enzymes were electropho-

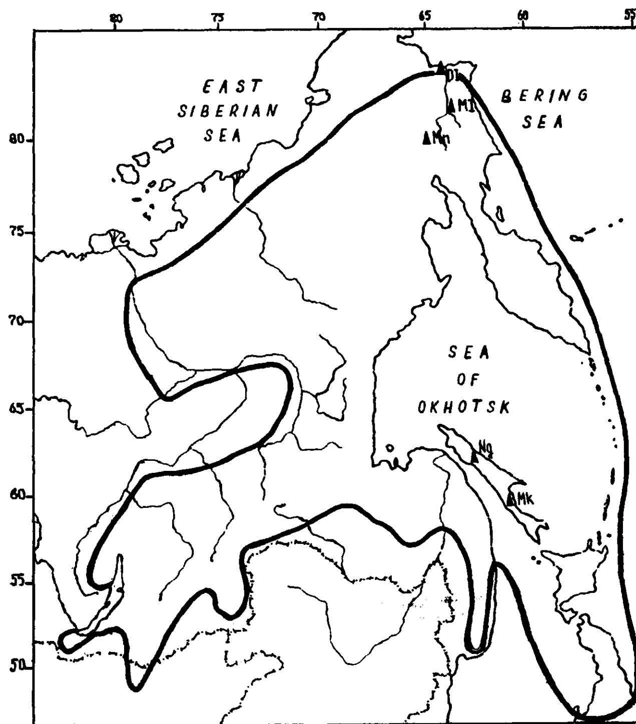

Figure 1. — Location of the 5 populations of *P. pumila* sampled. Dl — Dolgy; Ml — Malaya; Mn — Mainets; Mk — Makarov; Ng — Nogliki.

resed in a vertical chamber on 13% to 14% starch gel. For electrophoresis, 2 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).

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.*

Allozymes were identified by their relative electrophoretic mobilities (PRAKASH et al., 1969). Within each locus, the most common allozyme in *P. sibirica*, a closely related species to *P. pumila*, was designated with the arbitrary value 1.00. Null alleles were designated by symbol 0.

Statistical analysis

To estimate levels of genetic variation and differentiation in the populations studied, a number of parameters were used. Expected heterozygosity (H) for each locus was calculated according to the formula:

$$H_e = 1 - \Sigma x_i^2,$$

where x_i is the frequency of the i-th allele. Observed heterozygosity (H_0) was given by dividing the number of heterozygous trees by the overall number of trees assayed for a particular locus. Both expected and observed mean heterozygosities were estimated as:

$$H = 1/L \, \Sigma H_l,$$

where L is the number of loci surveyed and H_l is heterozygosity for a particular locus. Percent polymorphic loci (P) was calculated by dividing the number of polymorphic loci by the overall number of loci surveyed, and mean number of alleles per locus (A) was obtained by dividing the number of alleles revealed by the overall number of loci surveyed, percent polymorphic loci being estimated at 99% (the frequency of the most common allele was not greater than 99% — P_{99}), at the 95% criteria (P_{95}), and for all the alleles revealed (A). To measure gene diversity in the natural populations, we calculated values of WRIGHT's F-statistics and NEI's G-statistics (NEI, 1975; GURIES and LEDIG, 1982; PASHLEY, 1986). The F_{ST} values were utilized to obtain the gene flow parameter, $N_e m$ (number of migrants per generation), as follows:

$$N_e m = (1/F_{ST} - 1)/4,$$

after SLATKIN (1985). Genetic distance coefficient (D_N) among the populations was estimated by NEI's method (NEI, 1972).

Results and Discussion

Enzyme phenotypes

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	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	2	B

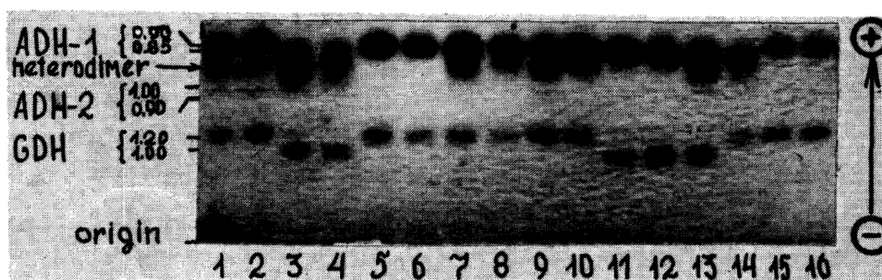

Figure 2. — Zymogram of ADH and GDH allozymes of megagametophytes of *P. pumila.* ADH: slots 1 to 4, 8, 10, 13, 14 — Adh-$1^{0.85}$ Adh-$2^{1.00}$; slots 5, 6, 11, 12 — Adh-$1^{0.85}$ AdH-2^{0}; slots 7,9 — Adh-$1^{0.85}$ Adh-$2^{\cdot 0.90}$; slots 15,16 — Adh-$1^{0.90}$ Adh-2^{0}. GDH: slots 1,2, 5 to 10, 14 to 16 — $Gdh^{1.20}$; slots 3, 4, 11 to 13 — $Gdh^{1.00}$.

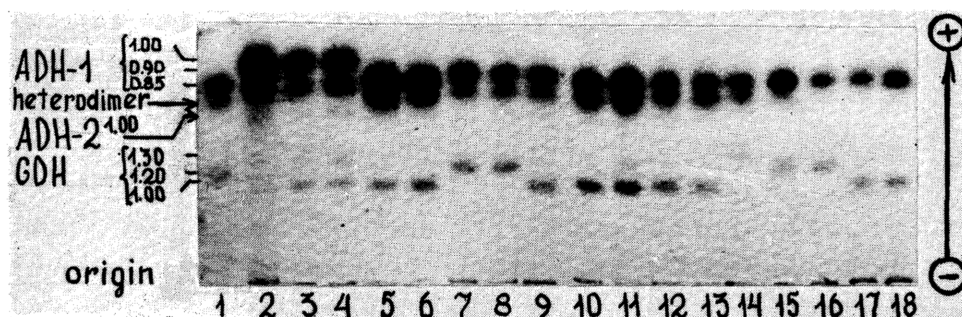

Figure 3. — Zymogram of ADH and GDH allozymes of megagametophytes of *P. pumila.* ADH: slots 1, 5, 6, 10 to 15 — Adh-$1^{0.85}$ Adh-$2^{1.00}$; slots 2 to 4 — Adh-$1^{1.00}$ Adh-$2^{1.00}$; slots 7 to 9 — Adh-$1^{0.90}$ Adh-$2^{1.00}$; slots 16 to 18 — Adh-$1^{0.85}$ Adh-2^{0}. GDH: slots 1, 7, 8, 15, 16 — $Gdh^{1.20}$; slots 2 to 6, 9 to 13, 17, 18 — $Gdh^{1.00}$; slot 14 — $Gdh^{1.30}$.

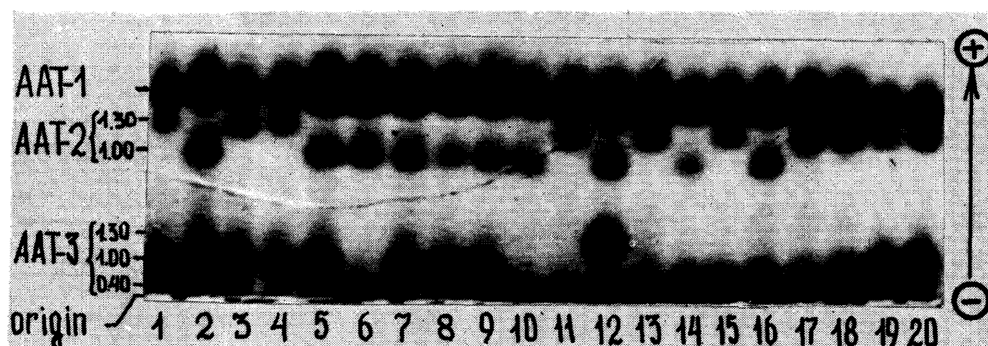

Figure 4. — Zymogram of AAT allozymes of megagametophytes of *P. pumila:* slots 1, 3, 4, 19, 20 — Aat—$1^{1.00}$ Aat-$2^{1.30}$ Aat-$3^{1.00}$; slots 2,5, 7 to 9 — Aat-$1^{1.00}$ Aat-$2^{1.00}$ Aat-$3^{1.00}$; slots 6, 10, 14, 16 — Aat-$1^{1.00}$ Aat-$2^{1.00}$ Aat-$3^{0.40}$; slots 11, 13, 15, 17, 18 — Aat-$1^{1.00}$ Aat-$2^{1.30}$ Aat-$3^{0.40}$; slot 12 — Aat-$1^{1.00}$ Aat-$2^{1.00}$ Aat-$3^{1.30}$.

The number of loci consistently scorable in *P. pumila* is given in *table 1.*

Alcohol dehydrogenase (ADH)

Gels stained for ADH show up to 3 zones of activity, the most anodal zone being ADH-1 and the most cathodal being ADH-2 (*Fig. 2, 3*). The third zone located midway between ADH-1 and ADH-2 represents a heterodimer between these 2 loci. Variation in ADH-1 or ADH-2 results in a change of position of the band representing the heterodimer, so that it is always midway between the ADH-1 and ADH-2. ADH-2 was weakly staining. No heterodimer is observed when there is a null allele at ADH-2.

Aspartate aminotransferase (AAT)

Four zones of activity occurred on gels stained for AAT, but gene control was established only for the 3 faster-migrating zones. The upper zone (AAT-1) was monomorphic (*Fig. 4*). A single locus with 2 alleles controlled the isozymes at AAT-2, and a single locus with four alleles controlled the isozymes at AAT-3 (*Table 2*).

Diaphorase (DIA)

Three zones of activity were detected on gels stained for DIA. The faster migrating zone (DIA-1) had 2 variants, which segregate as alleles at a single locus (*Table 2*). The other zones were monomorphic. Since the middle zone in *P. sibirica* (GONCHARENKO et al., 1989), a species closely related to *P. pumila,* was variable, we presume that three loci are present.

Fluorescent esterase (FL-EST)

One polymorphic zone of activity indicated a Fl-Est locus with three alleles (*Table 2*). Some other zones of

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

Locus	Allele	Ratio	χ^2	Locus	Allele	Ratio	χ^2
Adh-1	0.85/0.90	21:23	0.09	Mdh-3	1.00/1.05	69:57	1.14
	0.85/1.00	12:14	0.15	Mdh-4	0.80/1.00	78:78	0.00
Adh-2	0.90/1.00	20:21	0.02		0.80/1.20	14:17	0.29
	0.90/0	4:4	0.00		0.80/1.80	14:16	0.13
	1.00/0	87:54	7.72**		0.80/0	14:13	0.04
Aat-2	1.00/1.30	71:84	1.09		1.00/1.20	111:60	15.21***
Aat-3	0.40/1.00	123:104	1.59		1.00/0	17:18	0.03
	0.40/1.30	14:13	0.04	Gdh	1.00/1.20	163:164	0.00
	0.40/0	52:67	1.89		1.00/1.30	7:4	0.82
	1.00/1.30	6:2	2.00		1.20/1.30	14:10	0.67
	1.00/0	10:7	0.53	Fl-Est	1.00/1.30	7:17	4.17*
Gpi	1.00/1.25	127:98	3.74		1.00/1.50	120:124	0.07
Dia-1	0.80/1.00	177:155	1.46		1.30/1.50	5:7	0.33
Lap-1	0.90/1.00	120:168	8.00**	Pgm-1	0.90/0.95	16:12	0.57
	1.00/1.05	4:8	1.33		0.90/1.00	36:29	0.75
	1.00/0	31:18	3.45		0.95/1.00	113:99	0.92
Lap-2	0.70/0.90	20:20	0.00		1.00/1.05	8:8	0.00
	0.70/1.00	14:10	0.67	Pgm-2	1.00/1.10	68:51	2.43
	0.90/1.00	91:63	5.09*	Skdh-1	0.90/1.00	4:8	1.33
Mdh-2	0.70/0.90	34:38	0.22		0.95/1.00	25:31	0.64
	0.70/1.00	32:26	0.62		1.00/1.10	32:31	0.02
	0.90/1.00	164:159	0.08	Skdh-2	1.00/1.10	20:26	0.78
	1.00/1.30	6:2	2.00				

*) level of significance < 0.05
**) level of significance < 0.01
***) level of significance < 0.001

fluorescent activity were diffuse and inconsistently resolved.

Glutamate dehydrogenase (GDH)

The single zone of activity on gels stained for GDH is apparently controlled by one locus with three alleles (*Table 2*). The allozymes of GDH are presented in *figures 2* and *3*.

Isocitrate dehydrogenase (IDH)

Gels stained for IDH had one zone of activity. Isocitrate dehydrogenase showed no variability in *P. pumila,* but it was variable in *P. albicaulis* (Furnier et al., 1986), a species in the same subsection of the genus, subsection Cembrae Loud.

Leucine aminopeptidase (LAP)

Gels stained for LAP showed two polymorphic zones of activity. Four variants were found in LAP-1, one of them being 0. The lower zone of activity (LAP-2) had 3 variants.

Malate dehydrogenase (MDH)

Four zones of activity occurred on gels stained for MDH, but the fastest migrating zone (MDH-1) was visible only in embryos. The other zones were active both in the megagametophytes (*Fig. 5*) and in the embryos. MDH-1 was monomorphic. MDH-2, MDH-3 and MDH-4 were polymorphic with 3, 2 and 5 variants, respectively. The lower zone (MDH-4) had a 0 variant. A total of 4 loci were observed.

Phosphoglucomutase (PGM)

Two zones of activity were evident on gels stained for PGM. Both zones (PGM-1 and PGM-2) were polymorphic with 4 and 2 variants, respectively. Isozymes of the upper zone (PGM-1) stained much more intensely than those of the lower zone (PGM-2).

Phosphoglucose 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. We interpret the slow migrating zone as a single, polymorphic locus.

Shikimate dehydrogenase (SKDH)

Two zones of activity occurred on gels stained for

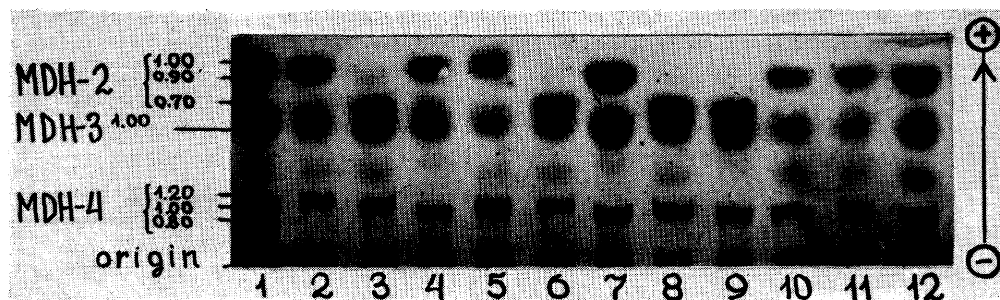

Figure 5. — Zymogram of MDH allozymes of megagametophytes of P. pumila: slots 1,5 — Mdh-2$^{1.00}$ Mdh-3$^{1.00}$ Mdh-4$^{1.00}$; slot 2 — Mdh-2$^{1.00}$ Mdh-3$^{1.00}$ Mdh-4$^{1.20}$; slots 3,6 — Mdh-2$^{0.70}$ Mdh-3$^{1.00}$ Mdh-4$^{1.00}$; slot 4 — Mdh-2$^{1.00}$ Mdh-3$^{1.00}$ Mdh-4$^{0.80}$; slots 7, 10, 12 — Mdh-2$^{0.90}$ Mdh-3$^{1.00}$ Mdh-4$^{0.80}$; slots 8,9 — Mdh-2$^{0.70}$ Mdh-3$^{1.00}$ Mdh-4$^{0.80}$; slot 11 — Mdh-2$^{0.90}$ Mdh-3$^{1.00}$ Mdh-4$^{1.00}$.

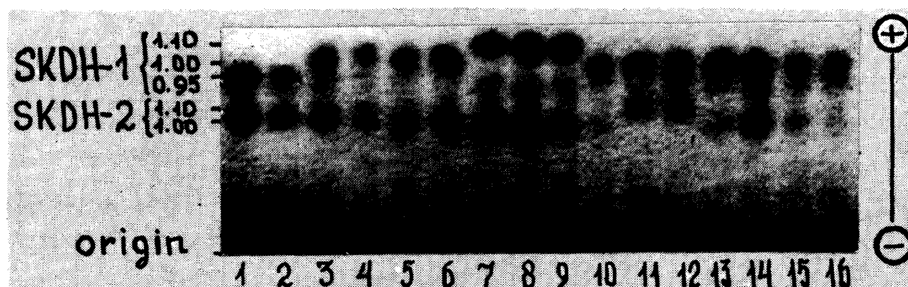

Figure 6. — Zymogram of SKDH allozymes of megagametophytes of *P. pumila*: slots 1, 2 — Skdh-1$^{0.95}$ Skdh-2$^{1.00}$; slots 3 to 6, 10, 13 to 16 — Skdh-1$^{1.00}$ Skdh-2$^{1.00}$; slots 7 to 9 — Skdh-1$^{1.10}$ Skdh-2$^{1.00}$; slots 11 to 12 — Skdh-1$^{1.00}$ Skdh-2$^{1.10}$.

SKDH. Both zones were polymorphic (*Fig. 6*). SKDH-1 consisted of 4 isozyme variants. The second more cathodal zone, consisted of 2 variants.

Segregation

In the electrophoretic analysis of the 11 enzyme systems, 56 different electrophoretic variants were revealed in the populations of *P. pumila*. The analysis for segregation of haploid megagametophytes in heterozygous trees enabled us to test whether these variants were under gene control. A parent tree heterozygous for any locus produces haploid megagametophytes in a 1:1 allelic segregation. Total data on segregation of variants are presented in *table 2*. As seen in this table, cases of distortion of the expected 1:1 segregation occurred for some allelic combinations at Adh-2, Lap-1, Lap-2, Mdh-4, and Fl-Est. Segregation distortion has been observed in a number of other pine species (Adams and Joly, 1980; Eckert et al., 1981; Cheliak et al., 1984; Millar, 1985; Furnier et al., 1986; Strauss and Conkle, 1986; Shiraishi, 1988). For example, in *P. albicaulis* (Furnier et al., 1986) segregation distortion was revealed for three loci that correspond to Mdh-4, Fl-Est, and Gpi of the current study.

It should be stressed that we observed cases of segregation distortion only for particular allelic combinations. The same alleles in other combinations gave a 1:1 segregation of alleles (*Table 2*). Similar cases of segregation were earlier revealed in the course of analyses for *P. muricata* (Millar, 1985). Interestingly, segregation distortion varies substantially in various individuals as well as in one and the same individual in different years. This was shown in investigations of *P. attenuata, P. banksiana,* and *P. thunbergii* (Cheliak et al., 1984; Strauss and Conkle, 1986; Shiraishi, 1988).

As a whole, our data support the allelic nature of the electrophoretic variants revealed in *P. pumila.*

Genetic variation

Frequencies of the 56 alleles at 22 loci in *P. pumila* are listed in *table 3*. From this table it is obvious that in practically all the populations of *P. pumila* surveyed, 8 loci (Aat-3, Gdh, Dia-1, Mdh-2, Mdh-4, Pgm-1, Lap-1, Fl-Est) appeared to be highly polymorphic. Three other loci (Lap-2, Gpi, and Adh-2) were highly variable in some populations and weakly polymorphic in the others. In some populations of *P. pumila* no variation was observed at Aat-2, Mdh-3, Pgm-2, Skdh-1, Adh-1, and Skdh-2. With regard to the latter two loci, even in variable populations, frequency of the most common allele approximated to 95%. Aat-1, Dia-2, Dia-3, Idh, and Mdh-1 appeared to be entirely monomorphic (*Table 3*).

On the basis of all 22 loci, we computed the proportion polymorphic in the 5 natural populations of *P. pumila* (*Table 4*). The summary in table 4 indicates that these populations had very similar levels of genetic variation. It should be noted that the Nogliki population differs greatly from the others. The proportion of polymorphic loci and mean number of alleles per locus are highest in Nogliki, but mean observed heterozygosity, on the contrary, is lowest. Moreover, this population is the only one in which observed heterozygosity (H_0) was similar to the theoretically expected heterozygosity (H_e) under Hardy-Weinberg equilibrium (*Table 4*). In the other populations of *P. pumila,* an excess of heterzygotes was observed. Differences revealed for the Nogliki population are apparently the result of the larger sample size (*Table 3*). The proportion of polymorphic loci and mean number of alleles per locus are strongly dependent on sample size.

Although, we analyzed only 63 trees it is evident that more than 68% of the loci in this species are polymorphic and the mean number of alleles per loci is greater than 2.5. The observed heterozygosity (H_0) for 22 loci is 0.288.

According to our data, *P. pumila* is one of the most polymorphic species in the *Pinus* genus. Only a few pine species analyzed for 18 or more genes demonstrated similar or higher levels of genetic variation. For instance, CONKLE (1981) showed that expected heterozygosities in the North American species, *P. lambertiana* and *P. jeffreyi*, exceeded 26% and 27%, respectively. Populations of *P. sylvestris* from Byelorussia have slightly higher observed heterozygosity (0.314) and expected heterozygosity (0.286) based on 18 loci (PADUTOV et al., 1989). However, the highest heterozygosities recorded are for *P. longaeva* (Ho=0.302 and He=0.327) and *P. taeda* (Ho=0.377 and He=0.362) (HIEBERT and HAMRICK, 1983; CONKLE, 1981). It should be noted, however, that the values for *P. longaeva* and *P. taeda* are based on only 14 and 10 loci, respectively, and are perhaps slightly overestimated.

Analysis of genetic structure

We made an attempt to identify population structure of *P. pumila* using NEI's G- and WRIGHT's F-statistics. On

Table 3. — Allele frequencies for 22 loci in 5 populations of *P. pumila*..

Locus	Allele	Populations*				
		Dl	Ml	Mn	Mk	Ng
Adh-1	n**	10	10	10	8	25
	0.85	1.000	1.000	1.000	1.000	0.940
	0.90	0.000	0.000	0.000	0.000	0.040
	1.00	0.000	0.000	0.000	0.000	0.020
Adh-2	n	10	10	10	7	25
	0	0.100	0.150	0.100	0.286	0.100
	0.90	0.000	0.000	0.200	0.286	0.000
	1.00	0.900	0.850	0.700	0.429	0.900
Aat-1	n	10	10	10	8	25
	1.00	1.000	1.000	1.000	1.000	1.000
Aat-2	n	10	10	10	8	25
	1.00	0.950	1.000	1.000	0.875	0.900
	1.30	0.050	0.000	0.000	0.125	0.100
Aat-3	n	10	10	10	8	25
	0	0.000	0.000	0.100	0.125	0.080
	0.40	0.700	0.700	0.650	0.563	0.480
	1.00	0.300	0.300	0.250	0.313	0.400
	1.30	0.000	0.000	0.000	0.000	0.040
Gdh	n	10	10	10	8	25
	1.00	0.400	0.450	0.500	0.563	0.380
	1.20	0.600	0.550	0.500	0.375	0.600
	1.30	0.000	0.000	0.000	0.063	0.020
Gpi	n	10	10	10	8	25
	1.00	0.850	0.700	0.850	0.813	0.900
	1.25	0.150	0.300	0.150	0.188	0.100
Dia-1	n	10	10	10	8	25
	0.80	0.550	0.650	0.700	0.563	0.520
	1.00	0.450	0.350	0.300	0.438	0.480
Dia-2	n	10	10	10	8	25
	1.00	1.000	1.000	1.000	1.000	1.000
Dia-3	n	10	10	10	8	25
	1.00	1.000	1.000	1.000	1.000	1.000

Locus	Allele	Populations				
		Dl	Ml	Mn	Mk	Ng
Idh	n	10	10	10	8	25
	1.00	1.000	1.000	1.000	1.000	1.000
Mdh-1	n	10	10	10	8	25
	1.00	1.000	1.000	1.000	1.000	1.000
Mdh-2	n	10	10	10	8	25
	0.70	0.000	0.050	0.200	0.000	0.020
	0.90	0.550	0.350	0.350	0.563	0.640
	1.00	0.450	0.600	0.450	0.438	0.340
Mdh-3	n	10	10	10	8	25
	1.00	0.950	0.700	0.800	1.000	0.960
	1.05	0.050	0.300	0.200	0.000	0.040
Mdh-4	n	10	10	10	8	25
	0	0.100	0.050	0.000	0.000	0.060
	0.80	0.150	0.200	0.050	0.250	0.320
	1.00	0.650	0.500	0.600	0.438	0.520
	1.20	0.100	0.250	0.350	0.250	0.100
	1.60	0.000	0.000	0.000	0.063	0.000
Pgm-1	n	10	10	10	8	25
	0.90	0.200	0.100	0.000	0.313	0.140
	0.95	0.350	0.400	0.250	0.063	0.260
	1.00	0.450	0.400	0.750	0.563	0.600
	1.05	0.000	0.100	0.000	0.063	0.000
Pgm-2	n	10	10	10	8	25
	1.00	1.000	0.900	0.850	0.813	0.880
	1.10	0.000	0.100	0.150	0.188	0.120
Lap-1	n	10	10	10	8	25
	0	0.200	0.000	0.000	0.000	0.020
	0.90	0.250	0.350	0.450	0.063	0.160
	1.00	0.550	0.600	0.550	0.875	0.820
	1.10	0.000	0.050	0.000	0.063	0.000
Lap-2	n	10	10	10	8	25
	0.70	0.000	0.100	0.000	0.188	0.000
	0.90	0.950	0.750	0.900	0.500	0.860
	1.00	0.050	0.150	0.100	0.313	0.140

the basis of all 22 loci (*Table 5*), the genetic structure of *P. pumila* populations 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}).

In *P. pumila*, F_{IS} varied from —0.322 at Gpi to 0.134 at Lap-2. The weighted mean over all loci was —0.076; i.e., there was a 7.6% excess of heterozygotes relative to HARDY-WEINBERG expectations averaged over all loci and populations. F_{IT} averaged —0.030; i. e., heterozygosity was in excess by 3% in *P. pumila*.

F_{ST} for multiple alleles was obtained as a weighted mean of F_{ST} for all the populations investigated. It ranged from 0.013 (Skdh-2) to 0.123 (Adh-2). Mean F_{ST} was equal to 0.043 (4.3%). G_{ST} was also 0.043. This means that 95.7%

Locus	Allele	Populations				
		Dl	Ml	Mn	Mk	Ng
Fl-Est	n	10	10	10	8	25
	1.00	0.500	0.600	0.950	0.813	0.740
	1.30	0.000	0.000	0.000	0.000	0.060
	1.50	0.500	0.400	0.050	0.188	0.200
Skdh-1	n	10	10	10	7	25
	0.90	0.000	0.000	0.000	0.000	0.020
	0.95	0.000	0.000	0.000	0.071	0.120
	1.00	0.950	1.000	0.700	0.857	0.800
	1.10	0.050	0.000	0.300	0.071	0.060
Skdh-2	n	10	10	10	7	25
	1.00	0.950	0.950	1.000	1.000	0.960
	1.10	0.050	0.050	0.000	0.000	0.040

*) Dl — Dolgy, Ml — Malaya, Mn — Mainets, Mk — Makarov, Ng — Nogliki
**) n — number of trees analysed for a locus

of the total variation is contained within each *P. pumila* population while only 4.3% is due to interpopulation variation.

The F_{ST} and G_{ST} values (*Table 5*) indicate that there is no significant genetic diversity among the Chukotsk and the Sakhalin populations of *P. pumila*.

On the basis of F_{ST} values it is possible to calculate the amount of gene flow, N_em (SLATKIN, 1985). According to our data,

$$N_em = (1/F_{ST}-1)/4 = 5.56.$$

This means that the number of migrants exchanged between *P. pumila* populations is over 5 per generation. Gene flow can be mediated both by seed and pollen migration. The established fact of gene exchange among the populations studied shows that the 11 kilometer strait that separates the island of Sakhalin from the continent does not apparently isolate island populations of *P. pumila* from mainland ones.

Genetic distance

Using NEI's genetic distance coefficient (NEI, 1972) we estimated the level of genetic differentiation among the populations. D_N values based on the 22 loci are listed in *table 6*. Distance values ranged from 0.015 (Dolgy — Malaya) to 0.045 (Dolgy — Makarov).

To better visualize the results, a dendrogram was constructed (*Fig. 7*). It shows the relationships among the populations. The D_N values were clustered using the

Table 4. -- Genetic variation in natural populations of *P. pumila*.

Populations	Percentage of loci polymorhic P_{95}	Percentage of loci polymorhic P_{99}	Average number of alleles per locus A	Percentage of heterozygous loci per individual: Expected (H_e)	Percentage of heterozygous loci per individual: Observed (H_o)
Dolgy	0.682	0.682	1.864	0.230	0.305
Malaya	0.636	0.636	1.955	0.271	0.318
Mainets	0.636	0.636	1.818	0.247	0.300
Makarov	0.636	0.636	2.091	0.283	0.322
Nogliki	0.682	0.773	2.318	0.252	0.253
In total in the species	0.682	0.773	2.545	0.255	0.288

Table 5. — Estimates of F_{IS}, F_{IT}, F_{ST} and G_{ST} for 22 loci in *P. pumila.*

Locus	F_{IS}	F_{IT}	F_{ST}	G_{ST}
Adh-1	-0.019	0.006	0.025	0.029
Adh-2	0.060	0.176	0.123	0.116
Aat-1	0.000	0.000	0.000	0.000
Aat-2	-0.071	-0.029	0.039	0.039
Aat-3	-0.171	-0.138	0.028	0.028
Gdh	0.048	0.069	0.022	0.020
Gpi	-0.322	-0.276	0.035	0.035
Dia-1	-0.296	-0.272	0.019	0.019
Dia-2	0.000	0.000	0.000	0.000
Dia-3	0.000	0.000	0.000	0.000
Idh	0.000	0.000	0.000	0.000
Mdh-1	0.000	0.000	0.000	0.000
Mdh-2	-0.129	-0.055	0.065	0.052
Mdh-3	-0.133	0.002	0.119	0.119
Mdh-4	-0.219	-0.166	0.043	0.040
Pgm-1	-0.063	-0.003	0.056	0.052
Pgm-2	-0.130	-0.095	0.030	0.030
Lap-1	-0.024	0.064	0.086	0.089
Lap-2	0.134	0.216	0.094	0.090
Fl-Est	-0.242	-0.140	0.082	0.101
Skdh-1	-0.065	0.004	0.065	0.082
Skdh-2	-0.034	-0.020	0.013	0.013
Mean	-0.076	-0.030	0.043	0.043

unweighted pair group method (UPGMA). Contrary to our expectations, the Chukotsk mainland populations and the Sakhalin Island populations did not form independent groups, but formed one mixed cluster. The Dolgy and the Malaya populations appeared closer to the north-eastern coastal populations from the vicinity of the town of Nogliki than to the third Chukotsk population sampled

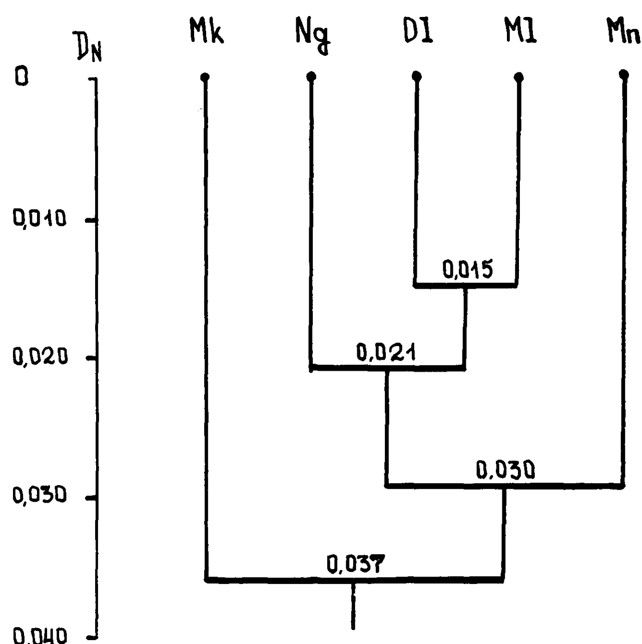

Figure 7. — Dendrogram showing the clustering of the five natural populations of *P. pimula* based on NEI's genetic distance coefficient.

near the Mainets Lake. Nevertheless, as shown in the dendrogram, genetic distance between the three Chukotsk and the Nogliki (sampled on the island of Sakhalin) populations did not exceed 0.030, a value characteristic of geographically related populations of a single pine 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 a low D_N value indicates a high level of genetic similarity among the sampled populations of *P. pumila.*

We conclude that populations of *P. pumila* have a very high level of genetic variation, and share a common gene pool in the north-eastern and eastern parts of the species' range.

Acknowledgements

The authors wish to thank 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 technical assistance.

References

ADAMS, W. T. and JOLY, R. J.: Genetics of allozyme variants in loblolly pine. Heredity 71: 33—40 (1980). —— ALLENDORF, F. W., KNUDSEN, K. L. and BLAKE, G. M.: Frequencies of null alleles at

Table 6. — Estimates of NEI's genetic distance coefficient, D_N, based upon data from 22 loci.

Populations	Dl	Ml	Mn	Mk	Ng
Dolgy	–	0.015	0.036	0.045	0.016
Malaya		–	0.028	0.041	0.026
Mainets			–	0.040	0.029
Makarov				–	0.026
Nogliki					–

enzyme loci in natural populations of ponderosa and red pine. Genetics 100: 497—504 (1982). —— CHELIAK, W. M., MORGAN, K.. DANCIK, B. P., STROBECK, K. C. and YEH, F. C.: Segregation of allozymes in megagametophytes of viable seed from a natural population of jack pine, *Pinus banksiana* LAMB. TAG 69: 145—151 (1984). —— CHELIAK, W. M. and PITEL, J. A.: Techniques for starch gel electrophoresis of enzymes from forest tree species. Petawawa Natural Forestry Institute (1984). —— CONKLE, M. T.: Isozyme variation and linkage in six conifer species. 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. 11—17 (1981). —— 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). —— CONKLE, M. T., SCHILLER, G. and GRUNWALD, C.: Electrophoretic analysis of diversity and phylogeny of *Pinus brutia* TEN. and closely related taxa. Systematic Botany 13: 411—424 (1988). —— DANCIK, B. P. and YEH, F. C.: Allozyme variability and evolution of lodgepole pine (*Pinus contorta* var. *latifolia*) and jack pine (*P. banksiana*) in Alberta. Can. J. Genet. Cytol. 25: 57—64 (1983). —— 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). —— ECKERT, R. T., JOLY, R. J. and NEALE, D. B.: Genetics of isozyme variants and linkage relationships among allozyme loci in 35 eastern white pines clones. Can. J. For. Res. 11: 573—579 (1981). —— FURNIER, G. R. and ADAMS, W. T.: Geographic patterns of allozyme variation in Jeffrey pine. Am. J. Bot. 73: 1009—1015 (1986). —— FURNIER, G. R., KNOWLES, P., ALEKSIUK, M. A. and DANCIK, B. P.: Inheritance and linkage of allozymes in seed tissue of whitebark pine. Can. J. Genet. Cytol. 28: 601—604 (1986). —— GONCHARENKO, G. G., PADUTOV, V. E. and POTENKO, V. V.: Guide to conifer species research by isozymes methods. Gomel (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., SILIN, A. E., CHERNODUBOV, A. N., ISAKOV, YU. N. and KAMALOVA, I. I.: Genetic structure of *Pinus sylvestris* L. and *Pinus cretacea* KALEN. populations and their taxonomic relationship. Doklady Akademii Nauk USSR 319: 1230—1234 (in Russian) (1991). —— GURIES, R. P. and LEDIG, F. T.: Genetic diversity and population structure in Pitch pine (*Pinus rigida* MILL.). Evolution 36: 387—402 (1982). —— 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). —— HIEBERT, R. D. and HAMRICK, J. L.: Patterns and levels of genetic variation in Great Basin bristlecone pine, *Pinus longaeva*. Evolution 37: 302—310 (1983). —— 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). —— LEDIG, F. T. and CONKLE, M. T.: Gene diversity and genetic structure in a narrow endemic, Torrey pine (*Pinus torreyana* PARRY ex CARR.). Evolution 37: 79—85 (1983). —— LOUKAS, M., VERGINI, Y. and KRIMBAS, C. B.: Isozyme variation and heterozygosity in *Pinus halepensis* L. Biochem. Genet. 21: 497—507 (1983). —— MILLAR, C. I.: Inheritance of allozyme variants in bishop pine (*Pinus muricata* D. DON.). Biochem. Genet. 23: 933—946 (1985). —— MILLAR, C. I., STRAUSS, S. H., CONKLE, M. T. and WESTFALL, R. P.: Allozyme differentiation and biosystematics of the Californian closed cone pines (*Pinus subsect.* Oocarpae). Systematic Botany 13: 351—370 (1988). —— MORAN, G. F., BELL, I. C. and ELDRIDGE, K. G.: The genetic structure and the conservation of the five natural populations of *Pinus radiata*. Can. J. For. Res. 18: 506—514 (1988). —— MUONA, O. and SZMIDT, A. E.: A multilocus study of natural populations of *Pinus sylvestris* L. In: H.-R. GREGORIUS (ed).: Population Genetics in Forestry. Lecture Notes in Biomathematics 60. Springer-Verlag, Berlin, Heidelberg, New York. p. 226—240 (1985). —— NEI, M.: Genetic distance between populations. Amer. Nat. 106: 283—292 (1972). —— NEI, M.: Molecular population genetics and evolution. In: A. NEUBERGER and E. L. TATUM (eds): Frontiers of Biology 40. Am. Elsevier, New York (1975). —— NIEBLING, C. R. and CONKLE, M. T.: Diversity of Washoe pine and comparison with allozymes of ponderosa pine races. Can. J. For. Res. 20: 298—308 (1990). —— O'MALLEY, D. M., ALLENDORF, F. W. and BLAKE, G. M.: Inheritance of isozyme variation and heterozygosity in *Pinus ponderosa*. Biochem. Genet. 17: 233—250 (1979). —— PADUTOV, V. E., GONCHARENKO, G. G. and PODSZHAROVA, Z. S.: Genetic variability in *Pinus sylvestris* L. Doklady Acad. Sci. BSSR 33: 1039—1042 (in Russian) (1989). —— PASHLEY, D. P.: The use of population genetics in migration studies: a comparison of three Noctuid species. In: D. R. MACHENRIC et al. (eds): The movement and dispersal of agriculturally important biotic agents. Claitor's Publishing Division, Baton Rouge. p. 305—324 (1986). —— PLESSAS, M. E. and STRAUSS, S. H.: Allozyme differentiation among populations stands and cohorts on Monterey pine. Can. J. For. Res. 16: 1155—1164 (1986). —— 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). —— ROSS, H. A. and HAWKINS, J. L.: Genetic variation among local populations of Jack pine (*Pinus banksiana*). Can. J. Genet. Cytol. 28: 453—458 (1986). —— SCHILLER, G., CONKLE, M. T. and GRUNWALD, C.: Local differentation among Mediterranean of Aleppo pine in their isoenzymes. Silvae Genet. 35: 11—19 (1986). —— SHIRAISHI, S.: Inheritance of isozyme variations in Japanese black pine, *Pinus thunbergii* PARL.. Silvae Genet. 37: 93—100 (1988). —— SLATKIN, M.: Gene flow in natural populations. Ann. Rev. Ecol. Syst. 16: 393—430 (1985). —— STRAUSS, S. H. and CONKLE, M. T.: Segregation, linkage, and diversity of allozymes in knobcone pine. TAG 72: 483—493 (1986). —— WHEELER, N. C. and GURIES, R. P.: Population structure, genic diversity, and morphological variation *Pinus contorta* DOUGL. Can. J. For. Res. 12: 595—606 (1982). —— WOODS, J. H., BLAKE, G. M. and ALLENDORF, F. W.: Amount and distribution of isozyme variation in ponderosa pine from Eastern Montana. Silvae Genet. 32: 151—157 (1983). —— YEH, F. C. and LAYTON, C.: The organization of genetic variability in marginal populations of lodgepole pine *Pinus contorta* spp. latifolia. Can. J. Genet. Cytol. 21: 487—503 (1979).

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

By G. G. GONCHARENKO, V. E. PADUTOV and A. E. SILIN

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

(Received 21st January 1993)

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.