Nevertheless their fast migration in 10% SDS-PAGE often produces some distortion that makes impossible a systematic analysis; keeping in mind that SP23 may also depend the locus *sp37*, the allelic variation could be a consequence of that described above.

The 2 stands from which seeds were collected showed very similar allelic frequencies with no significant differences in any locus ($\chi^2=6.258$, 4 d.f., N.S.; $\chi^2=4.804$, 2 d.f., N.S.; $\chi^2=0.188$, 1 d.f., N.S. for *sp9**, *sp7** and *sp37* respectively). *Table 1* gives the allelic frequencies among the whole sample of 101 seeds for all loci except *sp22* (for the reason mentioned above). As far as the SDS-PAGE may distinguish, no inter-individual differences were recognized for SP98 and SP74. The other loci showed a strong polymorphism. The mean heterozygosity (H) was H=0.213 and the polymorphic index (PI) as defined by HAMRICK (1979) was PI=0.288. Keeping in mind that we are analysing only a specific type of proteins (seed storage) and that only the variation in MW is detected, those values can be considered high (see the review by HAMRICK, 1979). *S. japonica* is an entomophil pollination species and hence most probably allogamous. The genotypic frequencies for loci *sp9** and *sp37* showed no significant differences with respect to HARDY-WEINBERG expectations, but for locus *sp7** there was a significant increase of homozygotes ($\chi^2=32.617$; d.f.=2; $p<0.001$). A certain degree of selfing could be responsible for this fact, although it is not clear why the heterogeneity of genotypes may affect some loci but not others, such as described in *Robinia pseudoacacia* (SURLES *et al.,* 1990) and in the Douglas-fir (RITLAND and EL-KASSABY, 1985).

European populations of *S. japonica* are quite recent and, in cases of the Spanish populations, data concerning the precise origin and number of seed trees or seed lots imported lack completely (many reforestations are carried out by private companies after an official assignment). Therefore, the diversity in seed protein constitution detected here is important since it implies that these populations of *Sophora* contain a remarkable genetic variability making them an appropriate material for any further selection and breeding programs.

Acknowledgements

The authors are indebted to Ms. VERÓNICA GONZÁLEZ for her excellent and kind assistance in the recollection of the material.

References

CASEY, R., DOMONEY, C. and ELLIS, N.: Legume storage proteins and their genes. Oxford Surv. Plant Mol. Cell Biol. **3**, 1–95 (1986). — DERBYSHIRE, E., WRIGHT, D. J. and BOULTER, D.: Legumin and vicilin, storage proteins of legume seeds. Phytochemistry **15**, 3–24 (1976). — GERBER, S., RODOLPHE, F., BAHRMAN, N. and BARADAT, PH.: Seed-protein variation in maritime pine (*Pinus pinaster* AIT.) revealed by two-dimensional electrophoresis: genetic determinism and construction of a linkage map. Theor. Appl. Genet. **85**, 521–528 (1993). — GIFFORD, D. J.: An electrophoretic analysis of the seed proteins from *Pinus monticola* and eight other species of pine. Can. J. Bot. **66**, 1808–1812 (1988). — GONZÁLEZ, C. and HENRIQUES-GIL, N.: Genetics of seed storage proteins in the love tree *Cercis siliquastrum* L. Theor. Appl. Genet. **89**, 895–899 (1994). — HAMRICK, J. L.: Genetic variation and longevity. In: Topics in plant population biology. O. T. SOLBRIG, S. JANG, G. B. JOHNSON and P. H. RAVEN. Columbia University Press (1979). — HIGGINS, T. J. V.: Synthesis and regulation of major proteins in seeds. Ann. Rev. Plant Physiol. **35**, 191–221 (1984). — JENSEN, U. and BERTHOLD, H.: Legumin-like proteins in gymnosperms. Phytochemistry **28**, 1289–1394 (1989). — JENSEN, U. and LIXUE, C.: *Abies* seed protein profile divergent from other Pinaceae. Taxon **40**, 435–440 (1991). — MATTA, N. K., GATEHOUSE, J. A. and BOULTER, D.: Molecular and subunit heterogeneity of legumin of *Pisum sativum* L. (Garden pea) – a multidimensional gel electrophoretic study. J. Exp. Bot. **32**, 1295–1307 (1981). — PAYNE, P. I., CORFIELD, K. G., HOLT, L. M. and BLACKMAN, J. A.: High-molecular-weight subunits of glutenin and bread making quality in progeny of six crosses of bread wheat. J. Sci. Food Agric. **32**, 51–60 (1981). — RITLAND, K. and EL-KASSABY, Y. A.: The nature of inbreeding in a seed orchard of Douglas-fir as shown by an efficient multilocus model. Theor. Appl. Genet. **71**, 375–384 (1985). — SÁNCHEZ-MONGE, E.: Flora Agrícola. I. M. Agricultura, Pesca y Alimentación. Madrid (1991). — SURLES, S. E., HAMRICK, J. L. and BONGARTEN, B. C.: Mating systems in open-pollinated families of black locust (*Robinia pseudoacacia*). Silvae Genetica **39**, 35–40 (1990). — TUCCI, M., CAPPARELLI, R., COSTA, A. and RAO, R.: Molecular heterogeneity and genetics of *Vicia faba* seed storage proteins. Theor. Appl. Genet. **81**, 50–58 (1991).

Allozyme Variation in Natural Populations of Eurasian Pines

IV. Population Structure and Genetic Variation in Geographically Related and Isolated Populations of *Pinus nigra* ARNOLD on the Crimean Peninsula

By A. E. SILIN and G. G. GONCHARENKO

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

(Received 4th July 1995)

Summary

Eight natural populations of *Pinus nigra* ARN. occurring on the Crimean peninsula were investigated by starch-gel electrophoresis. A total of 70 alleles were observed at 24 loci. Interpopulation genetic diversity was about 2% of the total genetic diversity. NEI' s genetic distance coefficient ranged from 0.005 to 0.022 among populations and averaged 0.012. The level of gene flow was 18.98 migrants per generation. The results of the analysis of gene diversity, genetic differentiation and gene flow demonstrated that the *P. nigra* populations studied had similar genetic structures. Estimated parameters of genetic variation showed that in *P. nigra* more than 66% of loci were polymorphic and the mean expected and observed heterozygosity values were 0.248 and 0.241, respectively, which allowed to regard the *P. nigra* as one of the most highly variable members of the genus *Pinus*.

Key words: P. nigra, isozymes, segregation, gene diversity, differentiation, gene flow, genetic variation.

FDC: 165.3; 165.5; 174.7 *Pinus pallasiana.*

Introduction

In our previous studies, we performed genetic and population analysis of 2 5-needle pine species, *Pinus pumila* and *Pinus sibirica* (GONCHARENKO et al., 1993 a and b), and 1 2-needle pine species, *Pinus sylvestris*, in different parts of ranges of these species (GONCHARENKO et al., 1994). All these species occur in Eurasia and have immense distributions. The current study investigates one more 2-needle pine species, *P. nigra* ARN. The range of black pine consists of a network of isolated populations extending from Spain on the west to Turkey and Russia on the east (CRICHFIELD and LITTLE, 1966; MIROV, 1967; VIDAKOVIC, 1991). A part of the eastern populations of this species occurs in the territory of the Crimean peninsula where it has come to be known as Crimean pine. Some authors regard this species as a distinct one, *Pinus pallasiana* LAMB. (KOMAROV, 1934; SUKHACHYOV, 1938; KAPPER, 1954).

The purpose of our study was to analyze genetic diversity, differentiation and the levels of genetic variation in 5 geographically related and 3 isolated populations of *P. nigra* occurring in the Crimea on the basis of the analysis of 24 isozyme loci.

Materials and Methods

Sampling

P. nigra occurring in the Crimea is reported to be characterized by a sufficient reduction of its stands. In consequence of this its present range consists of a single great population extending throughout the southern slope of the major chain of the Crimean mountains and a few small isolated populations (KOMAROV, 1934; SUKHACHYOV, 1938).

Seed material of *P. nigra* was collected from 203 individual trees in 8 natural populations located in the mountain Crimea. Five population samples were collected from a continuous part of this species distribution, 2 of them being obtained from the southern slope of the Ai-Petri Mountain (designated by AP-1 and AP-2), one being collected from the southern slope of the Iograph Mountain range (IG), and the remaining 2 being obtained in the vicinity of the settlement of Nikita (NK-1 and NK-2). Seeds were also collected from 3 populations isolated from the main distribution, one of them being located at the north-east extremity of the Great Canyon (GC), another being located throughout the eastern slope of the Demerdzhi Mountain (DM), and still another being situated in the vicinity of the settlement of Novy Svet (NS) and defined as an extreme eastern isolate of *P. nigra* in the Crimea. The locations of all the populations assayed are shown in *figure 1.*

Isozyme analysis

For the electrophoretic study haploid megagametophytes were used. Individual trees were genotyped using 8 to 20 megagametophytes sampled randomly from each tree set of seeds extracted from 2 to 5 cones. The seed material was collected during the seasons of 1990 to 1993.

The enzymes were electrophorezed on 13% to 14% starch gel. Methods of enzyme extraction and electrophoresis followed CONKLE et al. (1982), CHELIAK and PITEL (1984), and GONCHARENKO et al. (1989). 14 gene-enzyme systems were fractionized in 3 buffer systems: 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; GONCHARENKO et al., 1989) with insignificant modifications. The enzymes assayed, their abbreviations, the buffer systems used, and the number of loci consistently scorable are given in *table 1.*

Alleles were designated as described by PRAKASH et al. (1969) who designated the most common allele within each locus with the arbitrary value 1.00. The other alleles at this locus were numbered according to the electrophoretic migration of allozymes relative to the most common allozyme. Null alleles were designated by symbol 0. In this study alleles of *P. nigra* were

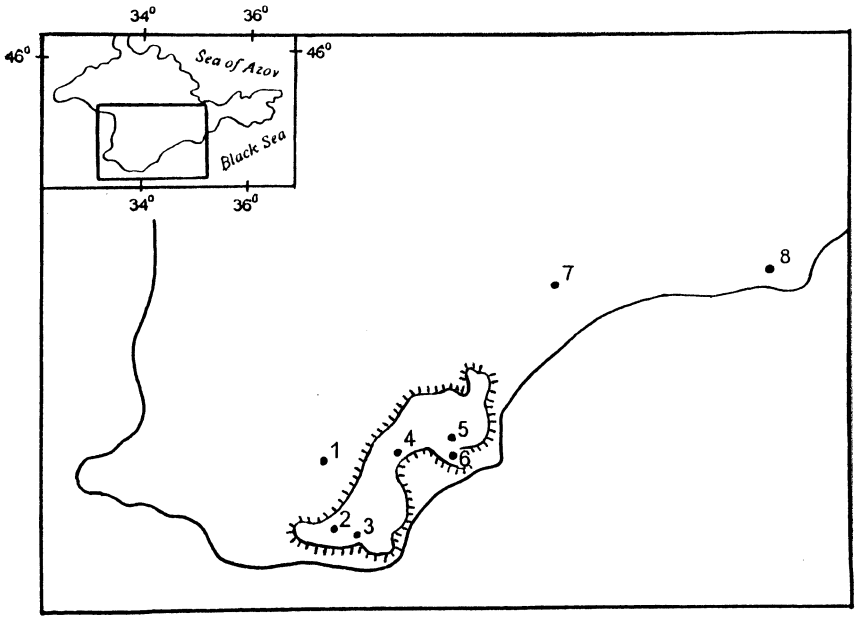

Fig. 1. – P. nigra's continuous distribution on the Crimean peninsula [▭] with locations of 8 sampled populations: 1 - Great Canyon, 2 - Ai-Petri-1, 3 - Ai-Petri-2, 4 - Iograph, 5 -Nikita-1, 6 - Nikita-2, 7 - Demerdzhi, 8 - Novy Svet.

Table 1. – Enzyme systems, their Abbreviations (Abbr.), Enzymes Commission numbers (EC), number of loci scored (Scor.), and buffer systems used for electrophoresis.

Enzyme system	Abbr.	EC no.	Scor.loci	Buffer
Acid phosphatase	ACPH	3.1.3.2	1	B
Alcohol dehydrogenase	ADH	1.1.1.1	2	A
Aspartate aminotransferase	AAT	2.6.1.1	3	A,C
Diaphorase	DIA	1.6.4.3	2	C
Fluorescent esterase	FL-EST	3.1.1.2	1	A,C
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	B,C
Phosphoenolpyruvate carboxylase	PC	4.1.1.31	1	A
Phosphoglucomutase	PGM	2.7.5.1	2	A
6-Phosphogluconate dehydrogenase	6-PGD	4.1.1.31	2	A
Sorbitol dehydrogenase	SDH	1.1.1.14	1	A

Table 2. – Segregation of allozyme variants in *P. nigra*.

Locus	Allele	Ratio	χ^2	Locus	Allele	Ratio	χ^2
Adh-1	0.80/1.00	35:31	0.24	**Lap-1**	1.00/0	2:6	2.00
	0.80/0.85	97:101	0.08		0.90/1.00	4:4	0.00
	0.85/1.00	101:97	0.08	**Lap-2**	0.95/1.00	21:21	0.00
	0.70/1.00	9:9	0.00		1.00/1.05	31:35	0.24
	0.85/0.90	45:49	0.17		1.00/1.08	6:6	0.00
	0.80/0.90	4:4	0.00	**Mdh-1**	1.00/1.15	6:2	2.00
	0.70/0.85	12:12	0.00	**Mdh-2**	0.90/1.00	251:225	1.42
	0.90/1.00	12:6	2.00	**Mdh-3**	1.00/1.20	12:6	2.00
Adh-2	1.00/1.60	267:273	0.07		0.70/1.00	210:223	0.39
	1.50/1.60	5:3	0.50		0.90/1.00	7:3	1.60
Aat-1	0.85/1.00	14:12	0.15		0.70/1.20	15:7	2.90
Aat-2	1.00/1.10	303:271	1.78		0.70/0.90	66:48	2.84
	1.00/1.25	11:13	0.17	**Mdh-4**	3.00/6.50	5:7	0.33
	1.10/1.25	10:7	0.53		6.50/0	4:4	0.00
Aat-3	0.70/1.00	6:6	0.00	**Fl-Est**	0.70/1.00	101:102	0.01
	1.00/3.00	123:105	1.42	**Pgm-1**	0.95/1.00	41:31	1.39
	1.00/1.90	4:4	0.00		1.00/1.05	13:11	0.17
Gpi	0.85/1.00	133:146	0.61	**Pgm-2**	0.95/1.00	200:217	0.69
	1.00/1.15	17:19	0.11	**6-Pgd-1**	0.95/1.05	11:25	5.45*
	1.00/1.25	3:5	0.50		1.00/1.05	29:30	0.02
Gdh	1.00/1.30	22:14	1.78		0.85/1.05	37:41	0.21
	1.00/1.40	3:5	0.50		1.05/1.15	25:23	0.08
	0.60/1.00	35:49	2.33		1.05/1.30	26:28	0.07
Dia-1	1.00/1.10	188:160	2.52		0.85/0.95	9:9	0.00
Dia-2	0.95/1.05	126:159	3.82		0.85/1.00	3:5	0.50
	0.95/1.00	5:3	0.50		0.85/1.30	4:4	0.00
Sdh	0.90/1.00	8:4	1.33	**6-Pgd-2**	0.90/1.00	92:9	0.02
Acph	0.90/1.00	8:4	1.33				

*) Level of significance < 0.5

designated relative to those of another 2-needle pine species of Eurasia *P. sylvestris* described earlier (GONCHARENKO et al., 1994).

Statistical analysis

To estimate the levels of genetic diversity, differentiation, and variation in the populations studied we used all the parameters earlier applied to *P. pumila* (GONCHARENKO et al., 1993a): parameters of WRIGHT's F-statistics, NEI's G-statistics, the gene flow parameter (N_em), NEI's genetic distance coefficient (D_N), the mean number of alleles per locus (A), % polymorphic loci (P_{95} and P_{99}), expected heterozygosity (H_e), and observed heterozygosity (H_o).

Results and Discussion

Segregation

An electrophoretic study of 14 enzyme systems revealed 70 different electrophoretic variants. Genetic determination of the variants revealed was established by the analysis for their segregation in heterozygous trees as in our previous investigations of other pine species (GONCHARENKO et al., 1993a and b, 1994). The data on the analysis for segregation in *P. nigra* are presented in *table 2*. On the whole, segregation of the electrophoretic variants corresponds to the expected 1:1 segregation ratio. As seen in *table 2* a case of distortion of the 1:1 segregation ratio occurred only at 6-Pgd-1. For the members of the genus *Pinus,* deviation from the normal segregation ratio is a quite common phenomenon. For instance, distortion of 1:1 segregation at a number of loci was revealed in *Pinus sylvestris* L. (RUDIN and EKBERG, 1978; SZMIDT and MUONA, 1989; GONCHARENKO et al., 1994), *P. taeda* (ADAMS and JOLY, 1980), *P. strobus* (ECKERT et al., 1981), *P. banksiana* (CHELIAK et al., 1984), *P. muricata* (MILLAR, 1985), *P. albicaulis* (FURNIER et al., 1986), *P. attenuata* (STRAUSS and CONKLE, 1986), *P. thunbergii* (SHIRAISHI, 1988), *P. pumila* (GONCHARENKO et al., 1993a), and *P. sibirica* (GONCHARENKO et al., 1993b). In this regard it is notable that in the current study in the case of *P. nigra* distortion of segregation occurred for a single allelic combination, 0.95/1.05, at 6-Pgd-1. But even at this locus for the other allelic combinations the segregation ratio was 1:1 (*Table 2*). On the whole, the data obtained are in good agreement with the supposition that the electrophoretic variants revealed here in *P. nigra* are under gene control. Interestingly, the earlier studies of *P. nigra* natural populations reported that for the AAT (BONNET-MASIMBERT and BIKAY-BIKAY, 1978) as well as EST-B, ACPH, and LAP (NIKOLIC and TUCIC, 1983) enzyme systems segregation ratio of the electrophoretic variants in megagametophytes was also 1:1. However, NIKOLIC and TUCIC (1983) used colorimetric esterase whose genetic origin we failed to determine, while BONNET-MASIMBERT and BIKAY-BIKAY (1978) consider that the AAT enzyme system is encoded by 4 loci rather than 3 ones as in the current study.

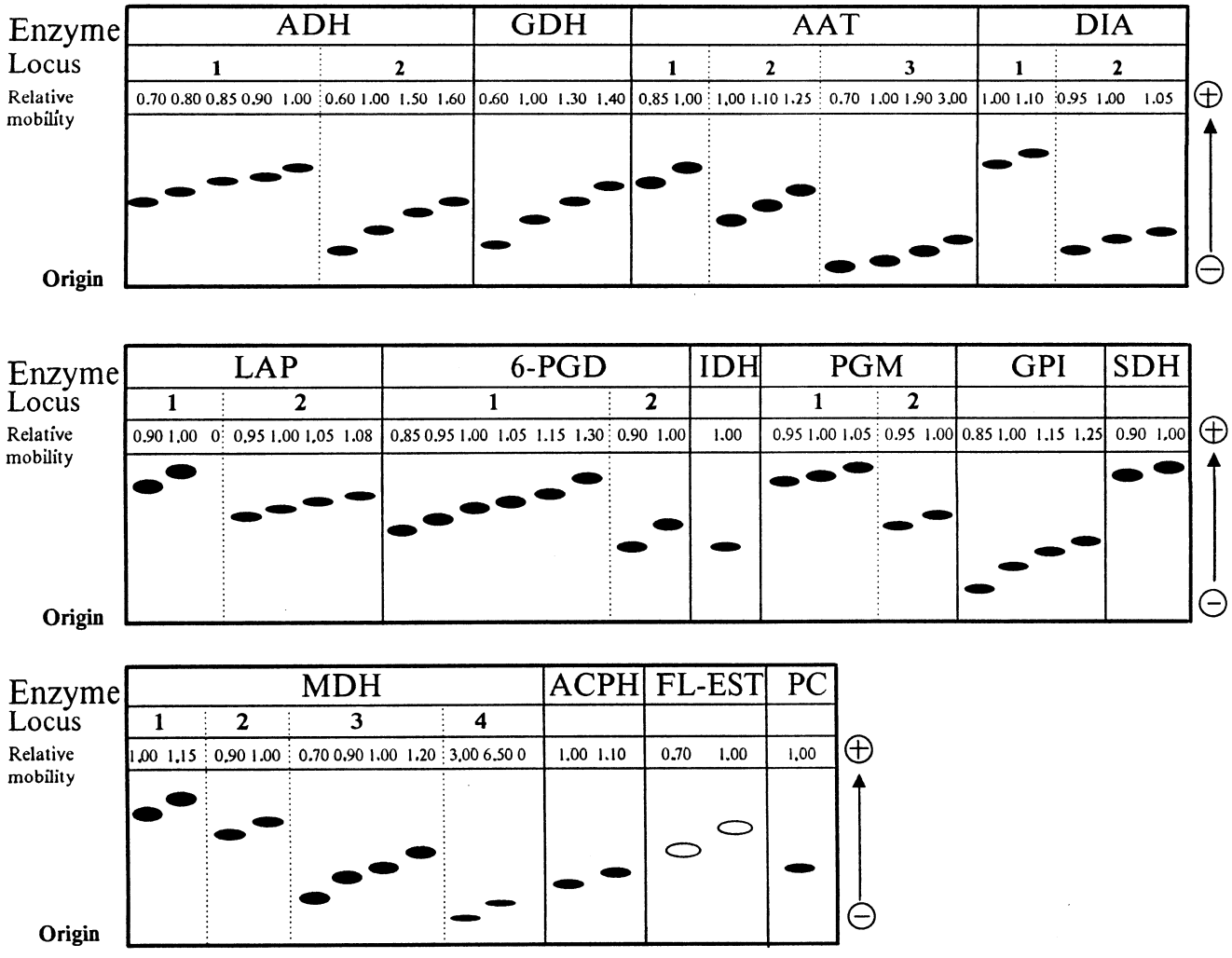

Fig. 2. – Relative mobility and designation of the electrophoretic variants at 24 loci found in *P. nigra*.

Allele frequencies

A total of 70 electrophoretic allele variants was detected by us in the Crimean populations of *P. nigra*. The results of the genetic analysis showed that these variants were under the control of 24 structural loci. All these variants are depicted schematically in *figure 2*.

Allele frequencies for each individual population and the whole of the species are listed in *table 3*. From the table it can be seen that the commonest allele at each locus was predominant practically in all the populations studied. The exceptions were Aat-2, Adh-2, Mdh-2, and Pgm-2 at which alternative alleles, while insignificantly, were predominant in some populations. The most striking allelic frequency differences among the populations exceeding 30% were observed for 8 alleles at Aat-3, Gpi, 6-Pgd-1, and 6-Pgd-2 (*Table 3*). It should be stressed that for 4 alleles such differences were revealed in both the

Table 3. – Allele frequencies for 24 loci in 8 populations of *P. nigra*.

Loci	Allele	Populations								
		AP-1	AP-2	IG	NK-1	NK-2	GC	DM	NS	Mean
1	2	3	4	5	6	7	8	9	10	11
		n=23	n=21	n=34	n=24	n=46	n=10	n=24	n=21	n=203
Aat-1	0.85	.022	.0	.0	.021	.0	.0	.021	.024	.010
	1.00	.978	1.0	1.0	.979	1.0	1.0	.979	.976	.990
Aat-2	1.00	.543	.476	.441	.354	.413	.600	.458	.476	.453
	1.10	.413	.452	.544	.583	.576	.400	.542	.524	.522
	1.25	.043	.071	.015	.063	.011	.0	.0	.0	.025
Aat-3	0.70	.0	.0	.015	.0	.011	.0	.0	.024	.008
	1.00	.913	.964	.853	.937	.902	.937	.889	.619	.872
	1.90	.0	.0	.0	.0	.0	.0	.028	.0	.003
	3.00	.087	.036	.132	.063	.087	.063	.083	.357	.117
Adh-1	0.70	.043	.024	.029	.0	.054	.0	.042	.0	.029
	0.80	.174	.190	.250	.313	.098	.200	.125	.143	.180
	0.85	.522	.571	.456	.521	.522	.550	.520	.500	.515
	0.90	.130	.048	.088	.021	.022	.100	.188	.095	.079
	1.00	.130	.167	.176	.146	.304	.150	.125	.262	.197
Adh-2	0.60	.0	.0	.0	.0	.0	.0	.042	.0	.005
	1.00	.478	.738	.588	.571	.457	.500	.479	.500	.531
	1.50	.0	.0	.0	.0	.0	.0	.0	.024	.003
	1.60	.523	.262	.412	.429	.543	.500	.479	.476	.461
Gdh	0.60	.022	.0	.0	.063	.022	.0	.021	.0	.017
	1.00	.956	.976	.956	.895	.967	1.0	.958	1.0	.960
	1.30	.022	.0	.044	.042	.011	.0	.021	.0	.020
	1.40	.0	.024	.0	.0	.0	.0	.0	.0	.002
Gpi	0.85	.391	.167	.206	.146	.098	.050	.104	.143	.165
	1.00	.609	.833	.735	.812	.880	.950	.896	.833	.813
	1.15	.0	.0	.059	.021	.022	.0	.0	.024	.020
	1.25	.0	.0	.0	.021	.0	.0	.0	.0	.002
Dia-1	1.00	.652	.643	.633	.687	.685	.550	.771	.667	.670
	1.10	.348	.357	.367	.313	.315	.450	.229	.333	.330
Dia-2	0.95	.543	.690	.574	.729	.663	.800	.646	.690	.653
	1.00	.0	.0	.0	.0	.011	.0	.0	.0	.002
	1.05	.457	.310	.426	.271	.326	.200	.354	.310	.345
Idh	1.00	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Lap-1	0	.0	.0	.0	.0	.0	.0	.021	.0	.002
	0.90	.0	.024	.0	.0	.0	.0	.0	.0	.003
	1.00	1.0	.976	1.0	1.0	1.0	1.0	.979	1.0	.995
Lap-2	0.95	.0	.071	.0	.042	.011	.071	.021	.0	.021
	1.00	.957	.833	1.0	.958	.946	.929	.875	.905	.931
	1.05	.043	.071	.0	.0	.043	.0	.083	.095	.043
	1.08	.0	.024	.0	.0	.0	.0	.021	.0	.005

1	2	3 n=23	4 n=21	5 n=34	6 n=24	7 n=46	8 n=10	9 n=24	10 n=21	11 n=203
Mdh-1	1.00	1.0	1.0	1.0	.979	1.0	1.0	1.0	1.0	.998
	1.15	.0	.0	.0	.021	.0	.0	.0	.0	.002
Mdh-2	0.90	.432	.625	.620	.521	.620	.429	.409	.361	.535
	1.00	.568	.375	.380	.479	.380	.571	.591	.639	.465
Mdh-3	0.70	.630	.548	.632	.604	.685	.650	.455	.667	.617
	0.90	.109	.190	.044	.167	.076	.250	.227	.095	.124
	1.00	.239	.167	.265	.229	.228	.0	.273	.190	.219
	1.20	.022	.095	.059	.0	.011	.100	.045	.048	.040
Mdh-4	0	.0	.0	.0	.021	.0	.0	.0	.0	.002
	3.00	.0	.0	.015	.021	.011	.0	.0	.0	.007
	6.50	1.0	1.0	.985	.958	.989	1.0	1.0	1.0	.990
Fl-Est	0.70	.043	.214	.074	.146	.080	.100	.042	.119	.097
	1.00	.957	.786	.926	.854	.920	.900	.958	.881	.903
Pgm-1	0.95	.062	.024	.029	.0	.022	.0	.0	.0	.019
	1.00	.938	.952	.956	1.0	.967	1.0	1.0	1.0	.973
	1.05	.0	.024	.015	.0	.011	.0	.0	.0	.007
Pgm-2	0.95	.391	.476	.485	.521	.415	.429	.479	.476	.459
	1.00	.609	.524	.515	.479	.585	.571	.521	.524	.541
6-Pgd-1	0.85	.109	.024	.074	.083	.033	.071	.042	.048	.058
	0.95	.022	.0	.0	.042	.011	.0	.0	.024	.013
	1.00	.152	.381	.030	.104	.011	.214	.166	.0	.105
	1.05	.652	.571	.896	.667	.848	.714	.750	.881	.770
	1.15	.022	.0	.0	.042	.043	.0	.0	.024	.020
	1.30	.043	.024	.0	.063	.054	.0	.042	.024	.035
6-Pgd-2	0.90	.261	.214	.294	.354	.196	.500	.354	.214	.272
	1.00	.739	.786	.706	.646	.804	.500	.646	.786	.728
Sdh	0.90	.0	.0	.015	.021	.0	.0	.0	.0	.005
	1.00	1.0	1.0	.985	.979	1.0	1.0	1.0	1.0	.995
Acph	1.00	1.0	1.0	.985	1.0	1.0	1.0	1.0	1.0	.997
	1.10	.0	.0	.015	.0	.0	.0	.0	.0	.003
Pc	1.00	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Ai-Petri-2 population from the continuous Crimean pine distribution and the isolated Great Canyon population. It is interesting to point out that in the most distant Novy Svet population allelic frequency differences between this and the other *P. nigra* populations exceeding 30% were observed for 3 alleles only. As a whole, allelic frequencies in the 5 geographically related populations had not any distinct differencies from those in isolated ones.

In addition to the frequent alleles, the analysis of the Crimean pine revealed 16 rare alleles (frequency for the whole of the species was less than 1%). Most of rare alleles were found only within a particular population, which permit to regard them as "private" alleles. Of the 11 "private" alleles detected in the 6 populations assayed only 4 were found in isolated ones. However, in all cases frequencies of "private" alleles were insignificant and were not greater than 4.2%, which consequently excluded any sufficient contribution of these alleles to genetic differentiation.

Thus, on the basis of the analysis of allelic frequencies we can infer that neither geographically related nor isolated populations studied have distinct differences in their genetic structures.

Genetic diversity and differentiation

On the basis of allelic frequencies we computed parameters of WRIGHT's F-statistics (GURIES and LEDIG, 1982) and NEI's G-statistics (NEI, 1975) which permited to judge the level of genetic diversity in the populations investigated. 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}) are compiled in *table 4*. As seen from the table, the F_{IS} values calculated on the basis of analysis for 24 loci ranged from –0.087 at Aat-2 to 0.089 at Gdh and averaged –0.004 for *P. nigra*. A negative value indicates a slight excess of heterozygotes within each *P. nigra* population. At the same time, F_{IT} values ranged from –0.074 at Aat-2 to 0.101 at Gdh and averaged 0.009, indicating a minor heterozygote deficiency in the whole of Crimean pine. These small F_{IS} and F_{IT} values show that the *P. nigra* populations studied are in good agreement

with HARDY-WEINBERG expectation. F_{ST} and G_{ST} values which estimate the level of interpopulation diversity were 0.013 and 0.024, respectively (*Table 4*). Thus about 98% of genetic variation resides within each population. To date, sufficient genetic diversity, from 12% to 22%, has been revealed in a few North American pine species such as *P. ponderosa, P. muricata, P. attenuata, P. radiata, P. monticola, P. jeffrey,* and *P. pungens* (O'MALLEY et al., 1979; BROWN and MORAN, 1981; MILLAR, 1983; STEINHOFF et al., 1983; FURNIER and ADAMS, 1986; MORAN at al., 1988; MILLAR et al., 1988; GIBSON and HAMRICK, 1991). The highest value of diversity, 100%, occured in *P. torreyana* (LEDIG and CONKLE, 1983). The 2 currently existing populations which constitute this species have qualitative differences at 2 of the 59 genes analyzed with the absence of polymorphic loci within each of them. In conformity with HAMRICK et al.'s review (1992) the mean level of diversity estimated for the genus *Pinus* using G_{ST} is 6.5%. Thus, the F_{ST} and G_{ST} values obtained support the speculation of similarity of the genetic structures of the populations assayed and enable us to infer that there is no meaningful diversity in *P. nigra* occurring on the Crimean peninsula regardless of the fact that at present three of the populations studied are geographically isolated and most of them have "private" alleles.

Using NEI's genetic distance coefficient (NEI, 1972) we estimated the level of genetic differentiation (D_N) among the *P. nigra* populations studied. The D_N values are given in *table 5*. As seen in the table, values of D_N among the populations ranged from 0.005 (Iograph and Nikita-2) to 0.022 (Novy Svet and Ai-Petri-2), averaging 0.012. The average D_N value computed for *P. nigra* was similar to the genetic distance values in other pine species with no sufficient geographical isolation between their populations (GURIES and LEDIG, 1982; DANCIK and YEH, 1983; WOODS et al., 1983; GULLBERG et al., 1985; ROSS and HAWKINS, 1986; WHEELER and GURIES, 1987; GONCHARENKO et al., 1993a and b; KIM et al., 1994).

Table 4. – Estimates of F_{IS}, F_{IT}, F_{ST}, and G_{ST} for 24 loci in *P. nigra*.

Locus	F_{IS}	F_{IT}	F_{ST}	G_{ST}
Aat-1	-.006	.0	.006	.012
Aat-2	-.087	-.074	.011	.016
Aat-3	-.036	.003	.038	.076
Adh-1	-.010	.003	.014	.022
Adh-2	.005	.021	.017	.029
Gdh	.089	.101	.013	.019
Gpi	-.063	-.030	.031	.055
Dia-1	.055	.058	.004	.011
Dia-2	.043	.053	.010	.020
Idh	.0	.0	.0	.0
Lap-1	-.003	.006	.010	.019
Lap-2	-.046	-.026	.019	.034
Mdh-1	-.002	.011	.012	.019
Mdh-2	-.042	-.028	.013	.040
Mdh-3	-.016	.002	.018	.025
Mdh-4	-.002	.007	.009	.014
Fl-Est	.008	.015	.007	.030
Pgm-1	-.026	-.014	.012	.018
Pgm-2	-.007	-.004	.003	.007
6-Pgd-1	.024	.062	.039	.071
6-Pgd-2	.025	.036	.011	.028
Sdh	-.001	.008	.008	.013
Acph	.001	.013	.012	.012
Pc	.0	.0	.0	.0
Mean	-.004	.009	.013	.024

Table 5. – Estimates of NEI's genetic distance coefficient based upon data from 24 loci.

Populations	AP-2	GC	IG	NK-1	NK-2	DM	NS
AP-1	.016	.017	.009	.012	.012	.010	.014
AP-2	—	.016	.014	.010	.015	.014	.022
GC		—	.018	.011	.017	.011	.018
IG			—	.007	.005	.011	.010
NK-1				—	.008	.007	.013
NK-2					—	.010	.009
DM						—	.011

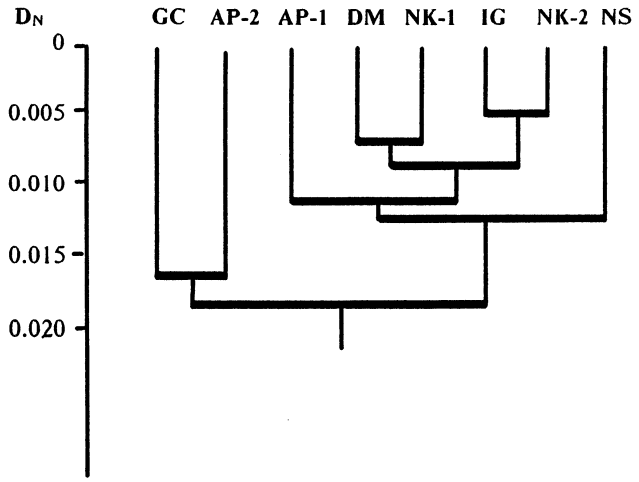

Fig. 3. – Dendogram showing the clustering of the 8 natural populations of *P. nigra* based on NEI's genetic distance coefficient.

Using the D_N values and an unweighted pair-group method (UPGMA), we constructed a dendrogram visualizing genetic differentiation in the *P. nigra* populations (*Fig. 3*). As evident from the dendrogram, clustering resulted in formation of 2 groups of the populations studied, genetic distance between them being 0.018. From the dendrogram it can be seen that clustering did not depend on geographical distance or isolation. For instance, the Nikita-1 population from the continuous Crimean pine distribution appeared to be closer to the Demerdzhi isolated population (D_N is 0.007) than to the closely-spaced Nikita-2 population. A similar situation held for the 2 adjacent Ai-Petri-1 and Ai-Petri-2 populations which appeared in different groups as a result of clustering. With regard to the most divergent population Novy Svet, contrary to our expectations, it did not separate from the remaining populations and related quite closely (D_N is 0.012) to the populations of the main cluster (*Fig. 3*).

The low level of genetic differentiation among the *P. nigra* populations studied which was estimated using the F_{ST}, G_{ST} and D_N values was apparently the result of intensive gene exchange among these populations. Gene flow (N_em) can be expressed as migrants per generation and its amount can be calculated using the mean F_{ST} value as follows: $F_{ST} = 1/(1+4N_em)$ (SLATKIN, 1985). In the current study we calculated N_em as 18.98. Such a high amount of gene flow which is equivalent to 19 migrants per generation is in good agreement with the data which indicate that comparatively recently the range of *P. nigra* was more extended and its stands distributed throughout the whole of the mountain Crimea (KOMAROV, 1934; SUKACHYOV, 1938). On this basis isolation and shrinkage of some present Crimean pine populations in size did not affect their genetic structures because they occurred as long ago as in historic times, and may be regarded as a result of influence of man-made factors.

Genetic variation

To estimate genetic resources of natural *P. nigra* populations occurring in the Crimea we calculated parameters of genetic variation in each population and the whole of the species. The values of these parameters are tabulated in *table 6*. As is seen from the table, the proportion of polymorphic loci (P_{95} and P_{99}), mean number of alleles per locus (A) as well as expected (H_e) and observed (H_o) heterozygosity values are virtually similar in all the populations. This fact corroborates once again the inference about the absence of any sufficient genetic distinctions between the populations assayed which was made on the basis of analysis of allelic frequencies and the F_{ST}, G_{ST}, D_N and N_em values. On the basis of the values of genetic variation computed for the whole of the species it is resonable to say that in the *P. nigra* occurring in the Crimea 67% (P_{99}) of the genes are polymorphic and the mean number of alleles per locus is 2.9 (*Table 6*). The average H_e and H_o values were 0.248 and 0.241, respectively. Interestingly, a similar level of genetic variation was earlier revealed on the basis of analysis of 13 allozyme loci in the Crimean pine populations (SHURKHAL et al., 1988) as well as on the strength of analysis of 4 loci in *P. nigra* populations of the Balkan-Mediterranean origin (NIKOLIC and TUCIC, 1983).

Regardless of the fact that only a small segment of its range was assayed, even now the data obtained enable us to regard *P. nigra* as one of the most polymorphic pine species. When its genetic variation is compared with those of the pine species studied to date on the basis of analysis for 20 and more loci, it is apparent that in the H_e value *P. nigra* ranks below only *P. oocarpa* (MILLAR et al., 1988), *P. pumila* (GONCHARENKO et al., 1993a), and *P. sylvestris* (GONCHARENKO et al., 1994).

Acknowledgements

The authors would like to thank warmly the academicians Dr. L.V. KHOTYLJEVA and Dr. V. A. IPATYEV for their support of the current genetic research. We wish to thank V. V. BURKOVSKAYA for her assistance in carrying out the electrophoretic analysis as well as Dr. I. A. RUGUZOV and Dr. V. P. KOBA for their help in collection of the seed material. We gratefully acknowledge Prof. Dr. T. LEDIG, Dr. V. E. PADUTOV and the anonymous reviewer for their helpful suggestions on this paper. We also thank E. GUSEVA for her assistance in translation of our manuscript into English. These investigations were supported by a grant add from ISF N RW2000 and RW2300.

References

ADAMS, W. T. and JOLY, R. J.: Genetics of allozyme variants in loblolly pine. Heredity **71**: 33–40 (1980). — BONNET-MASIMBERT, M. and BIKAY-BIKAY, V.: Variabilite intraspecifique des isozymes de la glutamate-oxaloacetate-transaminase chez *Pinus nigra* ARNOLD interet pour la taxonomie des sous especes. Silvae Genet. **27**: 71–79 (1978). — BROWN, A. H. D. and MORAN, G. F.: Isozymes and the genetic resources of forest trees. In: CONKLE, M. T. (ed): Proc. Symp. on Isozymes of North American Forest Trees and Forest Insects. Berkeley, Ca., (1979). USDA Gen. Tech. Rep. PSW-48. p. 1–10 (1981). — 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. Theor. Appl. Genet. **69**: 145–151 (1984). — 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). — 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). — 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,

Table 6. – Genetic variation in natural populations of *P. nigra*.

Populations	Percentage of loci polymorphic		Averege number of alleles per locus	Percentage of heterozygous loci per individual	
	P_{95}	P_{99}	A	H_e	H_o
Ai-Petri-1	.542	.708	2.167	.255	.245
Ai-Petri-2	.542	.708	2.167	.253	.242
Iograph	.542	.750	2.167	.242	.247
Nikita-1	.583	.792	2.292	.257	.255
Nikita-2	.583	.708	2.375	.222	.240
Great Canion	.583	.583	1.750	.224	.222
Demerdzhi	.542	.708	2.208	.245	.214
Novy Svet	.583	.625	2.042	.241	.253
In total in the species	.583	.667	2.917	.248	.241

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). — GIBSON, J. P. and HAMRICK, J. L.: Genetic diversity and structure in *Pinus pungens* (Table Mountain pine) populations. Can. J. For. Res. **21**: 635–642 (1991). — GONCHARENKO, G. G., PADUTOV, V. E. and POTENKO, V. V.: Guide to conifer species research by isozyme methods. Gomel (in Russian) (1989). — GONCHARENKO, G. G., PADUTOV, V. E. and SILIN, A. E.: Population structure, gene diversity, and differentiation in 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). — GONCHARENKO, G. G., SILIN, A. E. and PADUTOV, V. E.: 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. Silvae Genet. **43**: 119–131 (1994). — 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). — 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. and GODT, M. J. W.: Allozyme diversity in plant species. In: BROWN, A. H. D., CLEGG, M. T., KAHLER, A. L. and WEIR, B. S. (eds.): Plant Population Genetics, Breeding and Genetic Resources. Sinauer Associates, Inc.. Chapter 3, Sunderland, MA. p. 43–63 (1989). — HAMRICK, J. L., GODT, M. J. W. and SHERMAN-BROYLES, S. L: Factors influencing levels of genetic diversity in woody plant species. New Forests **6**: 95–124 (1992). — KAPPER, O. G.: Coniferous species. Goslesbumizdat, Moscow and Leningrad (in Russian) (1954). — KIM, Z.-S., LEE, S.-W., LIM, J.-H., HWANG, J.-W. and KWON, K.-W.: Genetic diversity and stucture of natural populations of *Pinus koraiensis* (SIEB. et ZUCC.) in Korea. Forest Genetics **1**: 41–49 (1994). — KOMAROV, V. L. (Editor): Flora of the USSR. Edition of the USSR's Academy of Sciences, Leningrad (in Russian) (1934). — 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). — MILLAR, C. I.: A steep cline in *Pinus muricata*. Evolution **37**: 311–319 (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). — MIROV, N. T.: The genus *Pinus*. New York (1967). — 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). — NEI, M.: Genetic distance between populations. Amer. Nat. **106**: 283–292 (1972). — NEI, M.: Molecular population genetics and evolution. In: NEUBERGER, A. and TATUM, E. L. (eds): Frontiers of Biology **40**: Am. Elsevier, New York (1975). — NICOLIC, D. and TUCIC, N.: Isoenzyme variation within and among populations of European black pine (*Pinus nigra* ARNOLD). Silvae Genet. **32**: 80–89 (1983). — 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). — 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). — RUDIN, D. and EKBERG, I.: Linkage studies in *Pinus sylvestris* L. using macro-gametophyte allozymes. Silvae Genet. **27**: 1–12 (1978). — SHIRAISHI, S.: Inheritance of isozyme variations in Japanese black pine, *Pinus thunbergii* PARL. Silvae Genet. **37**: 93–100 (1988). — SHURKHAL, A. V., PODOGAS, A. V., ZHIVOTOVSKY, L. A. and PODGORNY, YU. K.: Study of genetic variability of Crimean pine (*Pinus pallasiana* ASCH., GRAEBN.). Genetika **24**: 311–315 (in Russian) (1988). — SLATKIN, M.: Gene flow in natural populations. Ann. Rev. Ecol. Syst. **16**: 393–430 (1985). — STEINHOFF, R. J., JOYCE, D. G. and FINS, L.: Isozyme variation in *Pinus monticola*. Can. J. For. Res. **13**: 1122–1131 (1983). — STRAUSS, S. H. and CONKLE, M. T.: Segregation, linkage, and diversity of allozymes in knobcone pine. Theor. Appl. Genet. **72**: 483–493 (1986). — SUKHACHYOV, V. N.: Dendrology with foundations of forest geobotany. Goslestekhizdat, Leningrad (in Russian) (1938). — SZMIDT, A. E. and MUONA, O.: Linkage relationship of allozyme loci in *Pinus sylvestris*. Hereditas **111**: 91–97 (1989). — VIDAKOVIC, M.: Conifers. Morphology and variation. Grafićki Zavod Hrvatske (1991). — WHEELER, N. C. and GURIES, R. P.: A quantitative measure of introgression between lodgepole and jack pines. Can. J. Bot. **65**: 1876–1885 (1987). — 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).

Heritability and Gain of Reduced Spotting vs. Blister Rust on Western White Pine in British Columbia, Canada

By M. D. MEAGHER and R. S. HUNT[1])

(Received 3rd August 1995)

Abstract

Analyses of rust spots per seedling and cankering percentages of 215 families of western white pine in British Columbia, inoculated under controlled conditions in variable numbers in 4 inoculation years, indicate that spot frequency per seedling is under genetic control. Family heritability estimates ranged from 18.2% to 86.6%, averaging 77.3% for the years for which adequate families for selection were screened using 2-year-old seedlings. Confidence limits ranged from –12% to +163%. Repeated seed collections from parent trees often showed similar needle-spot-frequency performance against rust following inoculation under artificial conditions. Correlation between low spots per seedling and low percentage of cankering in many families indicates that fairly simple screening can produce tangible reductions in rust susceptibility. Mean spots per seedling from all parent trees tested vs. the mean for stock from unselected parents indicates gain of about 24.5% in reduced spotting by phenotypic selection of rust-free seed parents. Seed collections from the "selected" candidates *in situ* should reduce mean spots per seedling by 52% vs. unselected parents; cloning the selected candidates into a seed

[1]) Canadian Forest Service, Pacific Forestry Centre, 506 W. Burnside Rd., Victoria, B. C., V8Z 1M5, Canada