Inheritance and Linkage Relationships of Isozyme Variants of Pinus leucodermis Ant.

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Abstract

Inheritance and linkage relationships of 13 enzyme systems were investigated by horizontal starch gel electrophoresis in female gametophytes of *Pinus leucodermis* Ant.. Isozymes observed were under the control of at least 23 loci. In 2 of these loci, GDH and NDH, no variation was detected. The segregation of allozymes in megagametophytes of heterozygous trees revealed simple Mendelian inheritance for most of the enzyme systems analyzed. Distorted segregation ratios were observed at ACO, GOT and LAP loci. Linkages were studied by analyzing 61 2-locus combinations. Significant linkage, as attested by the 95% confidence intervals, was detected for GOT-A:PGI-B, G6PD-A:LAP-B, LAP-B:PGM-A and 6PGD-B:PGI-B. Estimates of recombination frequencies varied among trees. The tightest association was observed between 6PGD-B and PGI-B loci, with a recombination frequency of 8.1%.

Key words: Inheritance, linkage, allozyme, *Pinus leucodermis*.

Introduction

Isozyme analysis is a useful tool to characterize the genetic structure of populations and to assess evolutionary relationships at the species level. The interpretation of measures of variation, gene flow, mutation, and other genetic parameters require that mode of inheritance and linkage relationships among loci are determined. The female gametophyte of gymnosperms allows direct analysis of inheritance and linkage at heterozygous isozyme loci without the need for making controlled crosses.

The number and the subcellular locations of isozymes appear to have been highly conserved during plant evolution (Gottlieb, 1982). However, duplication of structural gene loci in diploid species has increased the number of isozymes. Differences among related species can then provide useful phylogenetic indications.

Pinus leucodermis, a species with a discontinuous and restricted distribution in Italy's Calabria region, grows in extreme xeric and humid habitats with considerable tolerance of temperature fluctuations. This species is considered endangered because of its limited range and the frequent occurrence of fires in these areas; therefore its genetic resources must be conserved. Moreover this species could be used for reforestation of dry areas in southern Italy, as it shows high adaptability to extreme environmental conditions and high colonizing capabilities.

Electrophoretic studies for *Pinus leucodermis* have investigated levels of genetic variatility (Morgante and Vendramin, 1990, 1991) and mating system (Morgante et al., 1991) of different natural populations in Italy. In this paper we report on inheritance and linkage relationships of 23 allozyme loci in *Pinus leucodermis*.

Materials and Methods

Open pollinated seeds were collected from 114 mature trees of 5 different natural *Pinus leucodermis* populations. The seeds were air dried and stored at 4° C until analyzed. Six female gametophytes from each of the 114 trees were first sampled to infer maternal genotypes; then an additional 30 to 280 megagametophytes per tree were examined for 44 trees chosen on the basis of observed heterozygosity at 2 or more loci. The isozymes assayed, their abbreviations, number of scored loci and composition of the electrophoresis buffers are listed in *table 1*. Mega-

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Table 1. — Analyzed enzymatic systems.

ISOZYME SYSTEM	ABBREVIATION	E.C.No.	No. OF SCORED LOCI	BUFFER SYSTEM
ACONITASE	ACO	4.2.1.3	1	A
GLUCOSE-6-PHOSPHATE DEHYDROGENASE	G6PD	1.1.1.49	1	A
GLUTAMATE DEHYDROGENASE	GDH	1.4.1.3	1	A
GLUTAMATE-OXALACETATE-TRANSAMINASE	GOT	2.6.1.1	3	B
ISOCITRATE DEHYDROGENASE	IDH	1.1.1.42	2	A
LEUCINE AMINOPEPTIDASE	LAP	3.4.11.1	2	C
MALATE DEHYDROGENASE	MDH	1.1.1.37	3	A
MENADIONE REDUCTASE	MNR	1.6.99.2	2	A
NADH DEHYDROGENASE	NDH	1.6.99.3	1	A
PHOSPHOGLUCOMUTASE	PGM	2.7.5.1	1	A
6-PHOSPHOGLUCONATE DEHYDROGENASE	6PGD	1.1.1.44	2	A
PHOSPHOGLUCOSE ISOMERASE	PGI	5.3.1.9	2	A
SHIKIMATE DEHYDROGENASE	SKDH	1.1.1.25	2	A

A= Gel buffer: 0.14 M Tris—0.04 M citric acid, pH 7.0/electrode buffer: 0.035 M Tris — 0.01 M citric acid, pH 7.0
B= Gel buffer: 0.08 M Tris—0.01 M citric acid, pH 8.7/electrode buffer: 0.06 M NaOH — 0.30 M boric acid, pH 8.2
C= Gel buffer: 0.05 M Tris-0.008 M citric acid, pH 8.1/electrode buffer: 0.19 M boric acid — 0.05 M LiOH, pH 8.1; gels were made using electrode and gel buffer in proportions 1:9

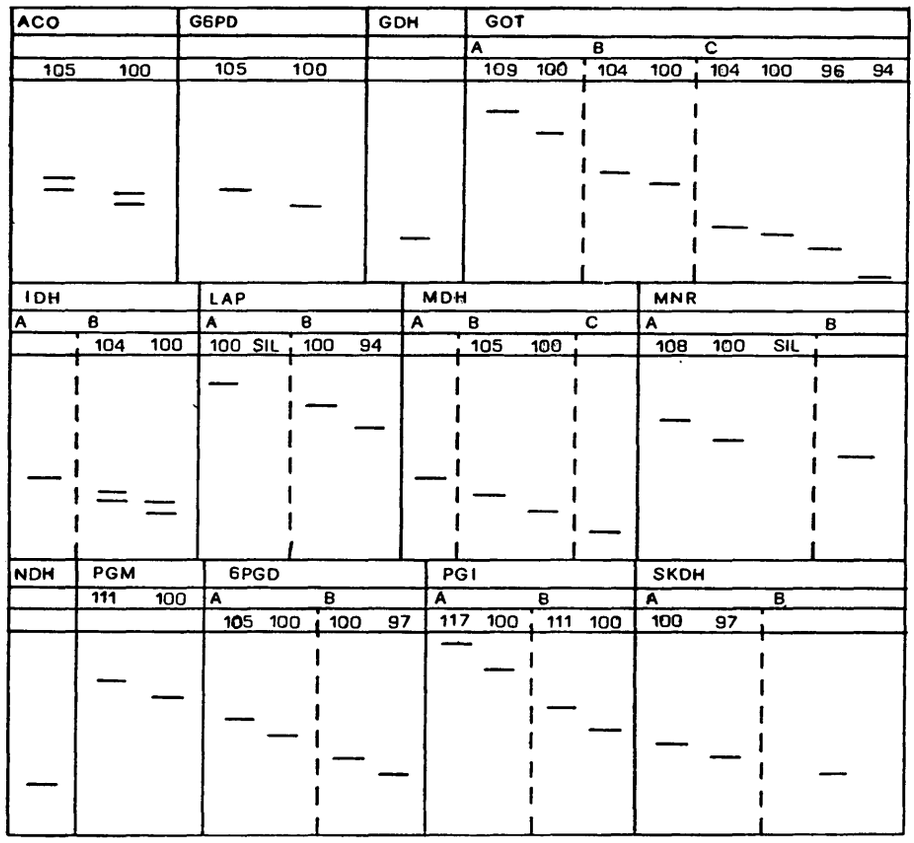

Figure 1. — Enzyme phenotypes found in *Pinus leucodermis*. Sil. = silent.

gametophyte tissue was homogenized in 0.1M Tris-HCl pH 7.3 buffer, with 5% PVP, 1% mercaptoethanol, 0.12% EDTA and 0.1% dithiothreitol. Staining was performed according to CHELIAK and PITEL (1984a).

For each putative locus, deviations from 1:1 segregation ratios were tested using χ^2 tests. When several trees were studied for a specific allelic combination, a χ^2 test was performed to test the heterogeneity of segregation. If deviation was not significant, the data were pooled over trees.

Linkage was examined for each possible 2-locus combination, using a test to control the hypothesis of independence between 2 loci (expected segregation ratio 1:1:1:1). This involved the calculation of 3 χ^2 values, one each for the segregation at the 2 loci and 1 for linkage (1 d.f. each) (MATHER, 1966).

The recombination frequencies were estimated by maximum likelihood (Θ_1) and the Bayesian likelihood (Θ_2) methods (GEBUREK and VON WÜHLISCH, 1989). Estimation of homogeneity of Θ over all trees was performed following [1] (RAO, 1973):

$$\chi^2_{n-1} = \Sigma\, N_i\,(\Theta_i-\Theta)^2/\Theta-\Theta^2$$

where n = number of trees, N_i = number of analysed gametes for the i-th tree, Θ_i = estimated recombination frequency for the i-th tree, Θ = overall estimated recombination frequency.

Map distances (MD) were calculated from BAYES estimates (Θ_2) following [2] (KOSAMBI, 1944):

$$MD = 1/4\ \ln\ [(1+2\Theta_2)/(1-2\Theta_2)]$$

The isozyme nomenclature used in this paper follows MORGANTE et al. (1991). The alleles of a locus are designated by a mobility value. The mobility is expressed relative to the common allele for the species, whose mobility is set to 100. Phenotypes marked by the absence of bands are designated silent (S).

Results

A total of 23 putative loci was scored. A single monomorphic locus was observed for both GDH and NDH. Enzyme staining patterns are shown in *figure 1*. Enzyme systems for which polymorphic loci were observed are described below. The inheritance data are presented in *table 2*.

Enzyme description and inheritance

Aconitase (ACO)

One zone of activity was observed on gels stained for ACO, which was represented by 2 different allelic forms among the trees studied here. Segregation was homogeneous among trees, but significant deviation from the Mendelian expectation in pooled data was observed.

Glucose-6-Phosphate Dehydrogenase (G6PD)

One zone of enzyme activity, containing 2 single-band variants, was evident on gels stained for G6PD. No significant heterogeneity of the segregation ratio between the 21 trees we analyzed was detected.

Glutamine Oxalacetate Transaminase (GOT)

Gels stained for GOT developed 3 zones of activity, migrating anodally, each with multiple phenotypes. GOT-A and GOT-B consist of 2 single banded isozyme variants; GOT-C shows 4 bands. For all the 3 putative loci pooled data differed from the 1:1 Mendelian expectation. The comparison of phenotypes from megagametophytes and embryos from the same seed shows that all the 3 GOT loci have a dimeric structure.

Isocitrate Dehydrogenase (IDH)

IDH had 2 zones of activity. IDH-A was single-banded and invariant in our material. In the slower migrating zone, IDH-B, 2 variants were observed and they are inherited as a single locus (*Table 2*).

Leucine Aminopeptidase (LAP)

Two different polymorphic zones of LAP activity were found in the trees studied. For LAP-A 2 variants were observed, 1 of which segregated as a null allele, which seems to be typical of many conifer species for this enzyme (Allendorf et al., 1982; Cheliak and Pitel, 1984b; Kuittinen et al., 1991). The second zone had 2 single-banded variants. At LAP-A 2 trees and at LAP-B 1 tree deviated significantly from 1:1 expectation, but the segregation among the heterozygous trees was homogenous 1:1 at these 2 loci. Segregation distortion at LAP loci in forest trees has previously been observed (Cheliak and Pitel, 1985; Muona et al., 1987; Shiraishi, 1988). The enzyme was functionally monomeric as suggested by the absence of a hybrid band in the heterozygous embryos.

Malate Dehydrogenase (MDH)

Three zones of activity were observed in megagametophytes assayed for MDH. The fastest and the slowest migrating zones (MDH-A and MDH-C) were invariant. At MDH-B 2 variants were observed. The pooled segregation ratio for the 2 trees heterozygous at locus MDH-B fits the Mendelian expectation of a 1:1 ratio of gametes, with no detectable heterogeneity among trees.

Menadione Reductase (MNR)

MNR gels showed 2 zones of activity. MNR-B was monomorphic; 2 variants and a null allele were observed in the fastest migrating zone (MNR-A) which showed 1:1 segregation.

Table 2. — Segregation at single loci in *Pinus leucodermis*.

LOCUS	ALLELES	N. OF TREES	RATIO	DEVIATION χ^2	HETEROGENETY χ^2
ACO-A	105/100	5	285: 158	36.41**	N.S.
G6PD-A	105/100	21	962:1036	N.S.	N.S.
GOT-A	109/100	8	565: 746	24.99**	N.S.
GOT-B	104/100	2	160: 200	4.44*	N.S.
GOT-C	104/100	6	400: 502	11.53**	N.S.
	100/ 96	6	510: 412	10.42**	N.S.
	100/ 94	3	192: 234	4.14*	N.S.
IDH-B	104/100	5	337: 340	N.S.	N.S.
LAP-A	100/ S	7	466: 388	7.12*	N.S.
LAP-B	100/ 94	7	340: 327	N.S.	N.S.
MDH-B	105/100	2	97: 95	N.S.	N.S.
MNR-A	108/100	7	417: 434	N.S.	N.S.
	100/ S	3	123: 144	N.S.	N.S.
PGMA	111/100	12	531: 563	N.S.	N.S.
6PGD-A	105/100	1	139: 126	N.S.	N.S.
6PGD-B	100/ 97	12	593: 557	N.S.	N.S.
PGI-A	117/100	2	122: 129	N.S.	N.S.
PGI-B	111/100	13	629: 626	N.S.	N.S.
SKDH-A	100/ 97	16	1138:1063	N.S.	N.S.

**) $P<0.01$ *) $P<0.05$

Phosphoglucomutase (PGM)

A single zone of activity was observed when gels were stained for PGM, with 2 alleles. This locus showed 1:1 segregation. The heterozygous phenotype indicats that PGM is functionally monomeric as has been inferred for numerous other conifers (CHELIAK and PITEL, 1985; HARRY, 1986; EL-KASSABY et al., 1987; ERNST et al., 1987; PERRY and KNOWLES, 1989; GEBUREK et al., 1990; XIE et al., 1991). Only 1 tree deviates significantly from 1:1 but no heterogeneity was observed in the segregation ratio among the 12 trees surveyed.

6 Phosphogluconate Dehydrogenase (6PGD)

Gels stained for 6PGD had 2 zones of activity. 6PGD-A was variable in only 1 tree that showed a 1:1 segregation. At 6PGD-B 2 variants were observed: the average segregation ratio of the 12 trees heterozygous for this gene locus fits the expected 1:1 ratio and no heterogeneity among trees was detected. The heterozygous embryos showed a three-banded phenotype, indicating that the functional form of 6PGD is a dimer.

Phosphoglucose isomerase (PGI)

Phosphoglucose isomerase had 2 zones of activity. Only 2 heterozygous trees were available to study the genetic control at PGI-A: both of them segregated according to a 1:1 ratio. 13 heterozygous trees at PGI-B locus were studied: segregation was 1:1 and homogenous over trees.

Shikimate dehydrogenase (SKDH)

For SKDH 2 zones were observed, 1 of which (SKDH-B) did not show variation in the trees we studied. For SKDH-A 2 variants were found and segregation of putative heterozygotes different from 1:1 Mendelian expectation in only 1 tree. Segregation ratios were homogenous among single-tree data. Analysis of female gametophytes and corresponding embryos showed that SKDH-A is monomeric.

In summary, when the data for all heterozygous trees were pooled, most loci did not deviate significantly from the 1:1 expected ratio and all ratios appeared consistent among trees: there was no significant heterogeneity among trees for the same loci. ACO, GOT (at all the 3 loci) and LAP-A showed significant deviations. Analyses of individual trees revealed 18 cases of significant segregation distortion, of which 8 involved GOT, 3 involved ACO and LAP, 2 G6PD and the other 2 PGM and SKDH. It is interesting to note that at GOT loci, 7 of the 8 ratios show an excess of the phenotype that is most frequent in the population sample. In 16 cases the deviations from 1:1 expected ratio were determined by an excess of the most common allele.

Linkage

Twenty-three loci were available to evaluate linkage relationships. We examined 61 of the 253 possible combinations of loci. The number of trees employed for linkage analysis and results of statistical tests are given in *table 3*. Maximum likelihood and BAYES estimates of Θ were very similar (*Table 4*). In *table 4*, estimates for a pair of isozyme loci are indicated only when the upper limit of the confidence interval excludes 0.5. Compared to the maximum likelihood estimation, the BAYES estimates result in lower values when the recombination rate is high and vice versa. The same trend was found by GEBUREK and VON WUEHLISCH (1989) in *Picea abies*. The closest linkage we noted was between 6GPD-B and PGI-B with recombination frequency, pooled over the trees (BAYES estimates), of 8.1%. GOT-A was linked moderately to PGI-B. This combination showed moderate linkage in 2 trees with a Θ_2 of 0.432 and 0.380, whereas the other 2 trees we analyzed showed no linkage. Other possible linkages were between G6PD-A and LAP-B, with 39.3 Θ_2, based on pooled data from 5 trees, but only 3 trees showed significant linkage. LAP-B and PGM-A showed a BAYES estimate, pooled over 3 trees, of 42%, with only 1 significant case of linkage.

The rest of the linkages found were less close. G6PD-A was linked to GOT-C, with 45% Θ_2, in the overall data from 3 trees. The Θ_2 value in single trees ranged from 36.4% to 47.0%, with 2 significant ($P<0.05$) cases. G6PD-A

Table 3. — Two-locus combinations and number of single tree progenies employed for the linkage analysis (upper right half and results of statistical testing (lower half).

	LOCUS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	ACO-A		1	1	–	–	–	–	1	2	1	1	–	–	1	3
2	G6PD-A	n.s.		2	2	3	2	3	5	7	–	1	7	1	4	8
3	GOT-A	n.s.	n.s.		–	1	–	–	1	–	3	–	4	–	4	2
4	GOT-B	–	n.s.	–		1	–	–	–	1	1	–	1	–	–	–
5	GOT-C	–	*	n.s	n.s.		1	–	–	–	4	1	–	1	1	–
6	IDH-A	–	n.s.	–	–	n.s.		1	–	1	3	–	–	–	–	2
7	LAP-A	–	*	–	–	–	n.s.		1	1	1	–	–	–	–	2
8	LAP-B	n.s.	*	n.s.	–	–	–	n.s.		2	3	–	1	–	1	4
9	MNR-A	n.s.	*	–	n.s.	–	n.s.	n.s.	n.s.		3	–	3	–	2	4
10	PGM-A	n.s.	–	n.s.	*	n.s.	n.s.	n.s.	*	*		–	3	–	2	3
11	6PGD-A	n.s.	n.s.	–	–	n.s.	–	–	–	–	–		–	1	–	1
12	6PGD-B	–	n.s.	n.s.	n.s.	–	–	–	n.s.	n.s.	n.s.	–		–	9	5
13	PGI-A	–	n.s.	–	–	n.s.	–	–	–	–	–	n.s.	–		–	1
14	PGI-B	n.s.	*	*	–	n.s.	–	–	n.s.	n.s.	n.s.	–	*	–		5
15	SKDH-A	n.s.	n.s.	n.s.	–	–	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	

– = 2 locus combination not tested. n. s. = not significant
*) = significant linkage at 5% level in at least 1 tree

Table 4. — Estimates of the recombination frequencies and measures for significance.

TREE	OBSERVED NO. 11 12 21 22	CHI-SQUARE (1 d.f.) SEGREGATION LOCUS1	LOCUS2	LINKAGE	MAXIMUM LIKELIHOODS ESTIMATED (Θ_1)	BAYES ESTIMATES (Θ_2)	MAXIMUM LIKELIHOODS CONFIDENCE (CI 95%)	KOSAMBI MAP DISTANCE (CM)
GOT-A:PGI-B								
3 POLL	23 22 21 18	0.43	0.19	0.06	0.500	0.456	0.392-0.500	77.03
4 POLL B	44 27 28 45	0.03	0.00	8.03**	0.382	0.384	0.305-0.463	50.65
8 POLL C	48 37 44 50	0.45	0.14	1.66	0.458	0.450	0.381-0.500	73.30
18 POLL C	28 22 12 28	1.11	1.11	5.93*	0.378	0.380	0.282-0.488	49.83
	HETEROGENEITY (3 d.f.)= 4.60 N.S.		*2.60 N.S.*		*0.426*	*0.428*	*0.383-0.473*	*54.51*
G6PD-A:LAP-B								
30 POLL	29 17 17 27	0.04	0.04	5.36*	0.378	0.380	0.282-0.488	49.83
10 POLL A	20 20 15 29	0.27	0.27	5.82*	0.377	0.378	0.282-0.481	49.49
15 POLL A	17 3 6 26	2.77	0.69	20.46**	0.173	0.185	0.087-0.290	19.44
7 POLL C	18 16 28 26	4.55*	0.18	0.01	0.500	0.458	0.397-0.500	78.22
13 POLL C	14 13 12 15	0.00	0.07	0.30	0.500	0.440	0.350-0.500	68.56
	*HETEROGENEITY (4 d.f.)= 17.59**		*11.59**		*0.392*	*0.392*	*0.343-0.442*	*32.21*
LAP-B:PGM-A								
16 POLL A	19 21 25 25	1.11	0.04	0.05	0.500	0.458	0.396-0.500	77.94
7 POLL C	21 25 26 16	0.18	0.41	2.31	0.422	0.420	0.321-0.500	60.73
13 POLL C	23 36 41 20	0.03	0.53	9.56**	0.358	0.360	0.276-0.446	45.51
	HETEROGENEITY (2 d.f.)= 4.25 N.S.		*2.28 N.S.*		*0.419*	*0.420*	*0.363-0.480*	*50.45*
6PGD-B:PGI-B								
3 POLL	41 3 1 39	0.19	0.00	68.76**	0.048	0.058	0.015-0.107	5.84
22 POLL	43 2 0 38	0.59	0.11	74.71**	0.024	0.035	0.004-0.073	3.54
29 POLL	38 4 3 42	0.10	0.29	60.96**	0.081	0.090	0.036-0.150	6.49
30 POLL	35 2 1 22	3.27	2.40	43.69**	0.050	0.065	0.013-0.125	6.49
10 POLL B	30 2 4 24	0.27	1.07	37.55**	0.100	0.113	0.041-0.192	11.49
8 POLL C	45 2 5 38	0.18	1.11	63.43**	0.078	0.087	0.034-0.145	8.78
13 POLL C	27 1 0 29	0.02	0.16	52.97**	0.018	0.034	0.001-0.075	3.40
17 POLL C	32 12 11 33	0.00	0.05	20.05**	0.262	0.267	0.177-0.359	29.74
18 POLL C	37 0 3 50	2.84	1.11	75.12**	0.034	0.043	0.008-0.085	4.36
	*HETEROGENEITY (8 d.f.)=50.77**		*48.02**		*0.080*	*0.081*	*0.062-0.101*	*6.07*

**) $P<0.01$ *) $P<0.05$ N.S.= not significant
The first heterogeneity value refers to Θ_1, the second to Θ_2.

and LAP-A showed 44.3% Θ_2 in the total data, but linkage was significant ($P<0.05$) in only 1 tree ($\Theta_2=0.309$). G6PD-A and PGI-B, LAP-B and SKDH-A, and SKDH-A and MNR-A seem to be also moderately linked in 1 of the 4 investigated trees, and PGM-A and MNR-A in 1 of the 3 analyzed trees. Pooled over the 7 trees recombination frequency (BAYES estimate) for MNR-A and G6PD-A was 0.466: only 1 tree showed significant linkage. GOT-B and

PGM-A showed 36% recombination, but this result was based on 1 tree only.

Discussion

The 13 enzyme systems studied revealed 16 variable gene loci. Segregation of isozyme variants showed direct evidence of Mendelian inheritance; although most heterozygotes segregated as expected, segregation distortion was observed for some trees and/or pooled distribution of megagametophytes. These types of segregation distortion have commomly been observed in forest tree species (Eckert et al., 1981; Cheliak et al., 1984). Some enzymes seem to exhibit more frequent segregation distortion than others. Several studies indicate that in conifers GOT, LAP, MDH, 6PGD and PGI often do not segregate in the expected 1:1 Mendelian ratio (Strauss and Conkle, 1986). In this study segregation distortions were observed at ACO-A, GOT (all 3 loci) and LAP-A pooled over all trees. Several factors could cause segregation distortion, as reported by Strauss and Conkle (1966). Significant departures from expected segregation ratios were found at LAP locus for a silent allele. With respect to alleles classified as silents, apparent unequal segregation ratios may be the results of differences in staining (Guries and Ledig, 1978; Müller-Starck and Hüttermann, 1981; Harry, 1986). It is interesting to note that there is no presence of homozygotes for silent alleles and there is no tendency for a higher frequency of silent alleles for enzymes coded by multiple loci (LAP, MNR). The multiple loci are apparently not redundant in function, but it is likely that the products of the different loci coding for the same enzyme are somehow specialized in metabolic function or are located in different sub-cellular components (e. g. chloroplast, mitochondria, cytoplasm) (Gottlieb 1982).

There are few notable exceptions in the isozyme pattern between *Pinus leucodermis* and numerous other conifers. We did not detect a GOT zone having multiple bands: a double- or triple-banded GOT locus has been often reported in *Pinus* and other genera in the *Pinaceae*. Our results indicate a lack of an interlocus heterodimer band in MDH, contrasting with observations in other conifer species.

Linkages in *Pinus leucodermis* are similar to those found in other members of the *Pinaceae*. We have found linkage between loci encoding GOT and PGI, but not as close as reported for other conifers. Close linkage of GOT and PGI has been reported for several conifers (Guries et al., 1978; O'Malley et al., 1979; Conkle, 1981; Eckert et al., 1981; Neale and Adams, 1981; El-Kassaby et al., 1982; King and Dancik, 1983; Strauss and Conkle, 1986; Harry, 1986; Muona et al., 1987; Geburek and von Wühlisch, 1989). This evidence suggests that this gene block is 1 of the most highly conserved in conifers. The observed differences in the recombination frequencies between the GOT-A/PGI-B loci in *Pinus leucodermis* and other conifers may be the results of a chromosomal rearrangement or mutation. Barrett et al. (1987) reported differences in the recombination fraction between the GOT-A/PGI-B loci in a seed orchard population and a marginal eastern population of *Picea mariana*. Müller-Starck and Liu (1988) have reported in 2 clones of *Cunninghamia lanceolata* recombination frequencies between the GOT and PGI loci of 0.263 and 0.222, respectively, and Xie et al. (1991) of 0.406 in natural populations of *Thuja orientalis*.

Of the pairs of linked loci detected in *Pinus leucodermis*, the 6PGD-B/GI-B combination has the lowest recombination frequency (*Table 4*). To our knowledge, linkage between these 2 loci has not been reported for any other conifer. Only Müller-Starck and Liu (1988) find a close linkage between 6PGD-A and PGI-A loci in *Cunninghamia lanceolata* Hook.. Our data indicate that the gene loci GOT-A, PGI-B and 6PGD-B are probably located on the same chromosome. The rate of recombination varied between single tree progenies with respect to the same pair of loci. This variation may be environmental or genetic, as reported by Moran et al. (1983).

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Allozyme Variation in Natural Populations of Eurasian Pines

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

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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-