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Genetic variation in some isoenzyme loci in Scots pine (*Pinus silvestris* L.) populations*

Majority of genetic studies on Scots pine conducted so far was based on an analysis of quantitative and qualitative characters coded by unknown genetic systems. Using biometrical methods a clinal variation was demonstrated for such characters of Scots pine as seed weight, needle colour, time of bud set etc. Langlet (1959) believes that characters having adaptive value are characterized in Scots pine by a clinal variation conditioned by environmental influences. Other authors, e.g. Wright and Bull (1963) believe that the transcontinental range of Scots pine consists of a large number of various genetically distinct races among which a more or less intense gene flow can take place but between which boundaries are definable.

The electrophoretic method of isozyme separation developed since the early seventies permits an expansion of population studies to include several new characters having a precise number of alleles and loci coding them. This method permits also the determination of such population parameters as their uniformity, degree of heterozygocity, and allows the calculation of genetic distances between individual populations basing on frequencies of genes and genotypes of the studied characters.

In the study reported here two isozyme systems have been investigated L-leucinaminopeptidase (3.4.1.1.) and acid phosphatase (3.1.3.2), in the female gametophyte tissue isolated from seeds in a state of rest.

MATERIALS AND METHODS

The studies covered 19 populations of Scots pine. Fourteen were from Poland (Fig. 1), two from Turkey (Culhali and Catacik), one from West Germany (Göttingen), one from USSR (Novosibirsk) and one from Hungary (Vas). Seeds of the Turkish populations have been collected

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from 50 - 60 year old trees. In each of the population a joint seed sample was collected from at least 25 trees. In the remaining 17 populations the seeds were collected from 25 - 35 single trees aged 100 to 140 years. From the Göttingen and Goleniów population seeds were collected from 10 trees only. For calculations of gene frequencies not only the material



Fig. 1. Map of Poland with location of populations indicated by dots

from single trees was employed but also data from an additional joint sample of 10 trees from each population. The enzymes were extracted from the so called endosperm, that is from the female gametophyte, a haploid tissue, which for this reason is a very convenient material for genetic studies.

The separation of isozymes was performed horizontally on a $12^{0/6}$ starch gel in a discontinuous buffer system (Poulik, 1957). For the homogenization of gametophytes in order to isolate LAP (L-leucinaminopeptidase) isozymes a phosphate homogenization buffer pH 7.5 with 5 mM cistein, 5 mM ascorbic acid and 5 mM saccharose was used. For the acid phosphatase (APH) use was made of Tris-Maleic/NaOH, pH 7.4 buffer without any additions. As a substrate for LAP use was made of L-leucyl-beta-naphtylamide hydrochloride and for APH of sodium-alpha-naphthylphosphate (acid). For the visualisation of isozymes on gels after electrophoresis use was made of diazide salts Fast-Black K salt for LAP and Fast Garnet GBC salt for APH. Details of the studied isozymes have been presented in the studies of Bergmann (1973, 1975) on spruce, Mejnartowicz and Bergmann (1975) on larch, and Mej-

nartowicz (1976) on Douglas fir. A genetic and biochemical evaluation of LAP in pine has been given in the works of Białobok et al. (1976), Mejnartowicz (1976), Krzakowa et al. (1977), Rudin (1977), and Salmia and Mikola (1975). A genetic and biochemical analysis of APH has been presented in the work of Mejnartowicz (1978). The interpretation of coding of APH in the female pine gametophyte given by Mejnartowicz et al. (1978) disagrees with the interpretation contained in the work of Krzakowa et al. (1977).

RESULTS

LEUCINE AMINOPEPTIDASE (LAP)

Visualisation of the products of electrophoresis indicated that isoenzymes of LAP occur on gels in two zones, which on the zymograms are designated as LAP-A and LAP-B (Fig. 2). On the basis of studies conducted so far it was established that both zones are coded by two different loci (Mejnartowicz, 1976a; Mejnartowicz et. al., 1978; Rudin, 1977).

Locus LAP-A is coded by 5 allels among which LAP-A1 occurs most commonly in the studied populations, with frequencies from 0.86 in the Lubiń and Duszniki populations to 1.00 in the Nowy Targ population (Tab. 1). Quite frequently the recessive allele LAP-A4 occurs, attaining a value of 0.14 in the Lubiń population and 0.12 in the Goleniów population. Homozygotic trees for this allele were not found. Least frequent is the allele LAP-A5. It contains a phenotypic pattern in the form of two intensly staining bands. In over 500 studied trees of the European range of Scots pine it was found only twice, and in each of the two Turkish populations it was found at a frequency of 0.03.



Fig. 2. Schematic drawing of electrophoretic LAP patterns found in extracts of Scots pine macrogametophytes

Locus LAP-B is coded by isozyme molecules of lower electrophoretic mobility. Among 6 observed alleles at this locus the highest frequency of occurrence was found for LAP-B1, attaining 0.97 in the Hajnówka population. The lowest frequency of this allele was found in the Culhali (0.56). Vas (0.63) and Goleniów (0.68) populations in which also a considerable frequency of the allele LAP-B3 was observed (Go - 0.26, Va - 0.17

| himber has been been | then a | | LAP-A | | | LAP-B | | | | | | |
|----------------------|--------|------|-------|------|------|-------|------|------|------|------|-------|--|
| Population name | A1 | A2 | A3 | A4 | A5 | B1 | B2 | B3 | B4 | B5 | B6 | |
| Goleniów (Go) | 0.88 | 0.0 | 0.0 | 0.12 | 0.0 | 0.68 | 0.05 | 0.26 | 0.01 | 0.0 | 0.0 | |
| Babki (Ba) | 0.94 | 0.02 | 0.0 | 0.04 | 0.0 | 0.83 | 0.08 | 0.06 | 0.0 | 0.03 | 0.0 | |
| Bolewice (Bo) | 0.92 | 0.0 | 0.04 | 0.04 | 0.0 | 0.88 | 0.05 | 0.05 | 0.0 | 0.02 | 0.0 | |
| Göttingen (Gö) | 0.89 | 0.02 | 0.0 | 0.09 | 0.0 | 0.95 | 0.0 | 0.05 | 0.0 | 0.0 | 0.0 | |
| Novosybirsk (Ns) | 0.99 | 0.01 | 0.0 | 0.0 | 0.0 | 0.90 | 0.02 | 0.03 | 0.03 | 0.02 | 0.0 | |
| Miłomłyn (Mi) | 0.91 | 0.0 | 0.0 | 0.09 | 0.0 | 0.82 | 0.16 | 0.02 | 0.0 | 0.0 | 0.0 | |
| Strzałowo (St) | 0.91 | 0.0 | 0.02 | 0.07 | 0.0 | 0.83 | 0.11 | 0.06 | 0.0 | 0.0 | 0.0 | |
| Duszniki (Du) | 0.86 | 0.0 | 0.05 | 0.09 | 0.0 | 0.86 | 0.07 | 0.07 | 0.0 | 0.0 | 0.0 | |
| Świerklaniec (Św) | 0.93 | 0.01 | 0.0 | 0.06 | 0.0 | 0.85 | 0.06 | 0.08 | 0.0 | 0.0 | 0.01 | |
| Lubiń (Lu) | 0.86 | 0.0 | 0.0 | 0.14 | 0.0 | 0.78 | 0.19 | 0.03 | 0.0 | 0.0 | 0.0 | |
| Rychtal (Ry) | 0.98 | 0.0 | 0.02 | 0.0 | 0.0 | 0.93 | 0.03 | 0.04 | 0.0 | 0.0 | 0.0 | |
| Maskulińskie (Ma) | 0.79 | 0.0 | 0.10 | 0.11 | 0.0 | 0.88 | 0.01 | 0.07 | 0.02 | 0.02 | 0.0 | |
| Włoszczowa (Wł) | 0.99 | 0.0 | 0.0 | 0.01 | 0.0 | 0.91 | 0.08 | 0.01 | 0.0 | 0.0 | 0.0 | |
| Hajnówka (Ha) | 0.97 | 0.0 | 0.0 | 0.03 | 0.0 | 0.97 | 0.03 | 0.0 | 0.0 | 0.0 | 0.0 | |
| Nowy Targ (NT) | 1.00 | 0.0 | 0.0 | 0.0 | 0.0 | 0.91 | 0.09 | 0.0 | 0.0 | 0.0 | 0.0 | |
| Niepołomice (Ni) | 0.93 | 0.0 | 0.07 | 0.0 | 0.0 | 0.82 | 0.02 | 0.14 | 0.0 | 0.02 | 0.0 | |
| Catacik (Ca) | 0.97 | 0.0 | 0.0 | 0.0 | 0.03 | 0.86 | 0.12 | 0.0 | 0.0 | 0.02 | 0.0 | |
| Culhali (Cu) | 0.97 | 0.0 | 0.0 | 0.0 | 0.03 | 0.56 | 0.0 | 0.16 | 0.0 | 0.0 | 0.28 | |
| Vas (Va) | 0.97 | 0.0 | 0.0 | 0.03 | 0.0 | 0.63 | 0.0 | 0.17 | 0.07 | 0.13 | 0.0 . | |

Allele frequencies of the LAP-A and LAP-B loci in seed samples of 19 populations of Scots pine

and Cu - 0.16) which in the remaining populations occurs at much lower frequences was observed for the allele LAP-B6. It occurs abundantly in the Culhali population atteinin a value of 0.28 and very rarely in Świer-klaniec (0.01). In the remaining populations its occurance was not recorded.

ACID PHOSPHATASE (APH)

Zymogram of the electrophoretic separation of acid phosphatase is presented in Fig. 3. It consists of four zones designated as APH-A, APH-B, APH-C and APH-D. In view of the low activity of enzymes and insufficient reproducibility of results in zone A, C and D the results obtained concerning these zones were disregarded.



Fig. 3. Schematic drawing showing the electrophoretic variants in the one APH-B locus found in extracts of Scots pine gametophytes

Table 2

Allele frequencies of the APH-B locus in seed samples of 19 populations of Scots pine

| TO ON LE-HEA DO MENON | | APH-B | | | | | | | | | | | | | |
|-----------------------|------|-------|------|------|------|------|------|------------|------|------|------|------|------|------|------|
| Population name | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B 8 | B9 | B10 | B11 | B12 | B13 | B14 | B15 |
| Goleniów (Go) | 0.0 | 0.0 | 0.05 | 0.05 | 0.70 | 0.0 | 0.05 | 0.0 | 0.0 | 0.05 | 0.0 | 0.10 | 0.0 | 0.0 | 0.0 |
| Babki (Ba) | 0.08 | 0.0 | 0.06 | 0.08 | 0.62 | 0.02 | 0.02 | 0.0 | 0.04 | 0.0 | 0.0 | 0.04 | 0.02 | 0.02 | 0.0 |
| Bolewice (Bo) | 0.03 | 0.0 | 0.0 | 0.03 | 0.58 | 0.13 | 0.0 | 0.03 | 0.07 | 0.07 | 0.0 | 0.03 | 0.0 | 0.03 | 0.0 |
| Göttingen (Gö) | 0.0 | 0.0 | 0.0 | 0.0 | 0.70 | 0.20 | 0.0 | 0.0 | 0.10 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Novosybirsk (Ns) | 0.0 | 0.0 | 0.0 | 0.10 | 0.50 | 0.06 | 0.0 | 0.0 | 0.0 | 0.06 | 0.0 | 0.26 | 0.02 | 0.0 | 0.0 |
| Miłomłyn (Mi) | 0.0 | 0.0 | 0.0 | 0.0 | 0.58 | 0.10 | 0.0 | 0.03 | 0.13 | 0.05 | 0.03 | 0.05 | 0.0 | 0.0 | 0.03 |
| Strzałowo (St) | 0.0 | 0.0 | 0.0 | 0.02 | 0.57 | 0.04 | 0.02 | 0.02 | 0.11 | 0.10 | 0.04 | 0.02 | 0.02 | 0.0 | 0.0 |
| Duszniki (Du) | 0.0 | 0.0 | 0.02 | 0.0 | 0.68 | 0.0 | 0.0 | 0.16 | 0.02 | 0.05 | 0.0 | 0.0 | 0.05 | 0.0 | 0.02 |
| Świerklaniec (Św) | 0.0 | 0.0 | 0.02 | 0.02 | 0.66 | 0.07 | 0.08 | 0.02 | 0.0 | 0.15 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Lubiń (Lu) | 0.0 | 0.0 | 0.0 | 0.0 | 0.68 | 0.0 | 0.07 | 0.0 | 0.0 | 0.25 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Rychtal (Ry) | 0.0 | 0.0 | 0.0 | 0.0 | 0.77 | 0.0 | 0.0 | 0.0 | 0.04 | 0.19 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Maskulińskie (Ma) | 0.0 | 0.0 | 0.0 | 0.0 | 0.75 | 0.0 | 0.0 | 0.0 | 0.03 | 0.19 | 0.0 | 0.03 | 0.0 | 0.0 | 0.0 |
| Włoszczowa (Wł) | 0.0 | 0.0 | 0.0 | 0.0 | 0.75 | 0.04 | 0.13 | 0.0 | 0.04 | 0.04 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Hajnówka (Ha) | 0.05 | 0.0 | 0.05 | 0.05 | 0.60 | 0.0 | 0.20 | 0.0 | 0.0 | 0.05 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Nowy Targ (NT) | 0.0 | 0.0 | 0.0 | 0.05 | 0.60 | 0.0 | 0.30 | 0.0 | 0.0 | 0.05 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Niepołomice (Ni) | 0.0 | 0.0 | 0.0 | 0.20 | 0.40 | 0.20 | 0.20 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Catacik (Ca) | 0.01 | 0.0 | 0.0 | 0.57 | 0.0 | 0.25 | 0.0 | 0.02 | 0.01 | 0.0 | 0.02 | 0.02 | 0.10 | 0.0 | 0.0 |
| Culhali (Cu) | 0.0 | 0.02 | 0.06 | 0.05 | 0.0 | 0.84 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.01 | 0.02 | 0.0 | 0.0 |
| Vas (Va) | 0.13 | 0.0 | 0.13 | 0.57 | 0.07 | 0.07 | 0.0 | 0.0 | 0.0 | 0.03 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Zone APH-B accumulates the highest quantitities of enzyme, of the highest activity and at the same time the results are best reproducible: As was shown in earlier studies this zone is coded by one locus henceforth referred to as locus APH-B (Mejnartowicz, 1978). This locus is coded by at least 15 electrophoretic alleles having a phenotypic form of one or two bands. Allele APH-B1 is the so called "zero allele" or recessive on which codes an enzymatically inactive protein. It occurs in the studied pine populations rather rarely compared with the frequency of occurrence of recessive alleles coding LAP loci. The highest frequency of 0.13 was observed for APH-B1 in the Vas population than 0.08 in Babki 0.03 in Bolewice and 0.01 in Catacik (Tab. 2). However the least frequent allele is the APH-B2 one the appearance of which was observed only in the Culhali population where it had a frequency of 0.02 (Tab. 2).

Many of the electrophoretic alleles presented in the zymogram (Fig. 3) have low frequencies of occurrence and this in a low number of populations. Besides the mentioned above APH-B1 and APH-B2 this group includes alleles APH-B11, APH-B13, APH-B14 and APH-B15. The frequency of these alleles does not exceed a value of 0.10. Some others alleles of APH such as APH-B12 appear with large frequencies only in one population (Novosibirsk 0.26) while in other they do not appear at all or with a very low frequency. Similarily APH-B8 in Duszniki attains a frequency of 0.16 while in the remaining five populations in which its presence was recorded the frequency did not exceed 0.03 and in the remaining 13 populations it was nil (Tab. 2).

The greates number of acid phosphatase enzymes in almost all popula-

tions is coded by allele APH-B5 except for the Turkish populations in which this allele was not observed and the Hungarian population Vas in which its frequency was only 0.07. In these populations on the other hand high frequencies of other alleles were observed, namely of APH-B4 (0.57 in populations Catacik and Vas) and APH-B6 having a frequency of 0.84 in the Culhali population (Tab. 2).

HETEROZYGOSITY OF SCOTS PINE POPULATIONS

Making use of the formulae suggested by Nei (1972) it is possible to establish the degree of heterozygosity h at each of studied loci and to calculate the mean heterozygosity H for all the populations jointly:

$$\hat{H} = \sum_{k=1}^{r} h_k / r ,$$

where $h_k = 1 - j_x$, $j_x = \sum x_i^2$,

r — number of loci, to the test to be an interval to the second secon

+0.75 0.04 0.13 9.0 10.04 0.03

 h_k — degree of heterozygosity at locus k,

 x_i — frequency of *i*-th allele in the population.

Among the 19 investigated populations mean heterozygosity lied within the limits from $\hat{H}_{\min}=0.1804$ to $\hat{H}_{\max}=0.4137$. Highest heterozygosity was observed in the Hungarian population Vas ($\hat{H}=0.4137$) and in the Polish population from Goleniów where $\hat{H}=0.3895$ and Niepołomice with a mean heterozygosity $\hat{H}=0.3858$. The high heterozygosity of the Vas population

E sldsT which codes an enzymatically insuite protein. It occurs in the

one or two bands. Allele APH

Heterozygosity at three isoenzyme-loci (LAP-A; LAP-B; APH-B) in nineteen populations of Pinus silvestris

| Population - | LIT (C des | IT (Part Shele Loci : 10 0 here anived a | | | | | | | | | |
|-------------------|------------|---|--------|-------------|--|--|--|--|--|--|--|
| | LAP-A | LAP-B | APH-B | - <i>H</i> | | | | | | | |
| Goleniów (Go) | 0.2112 | 0.4674 | 0.4900 | 0,3895 | | | | | | | |
| Babki (Ba) | 0.1144 | 0.3002 | 0.5944 | 0.3663 | | | | | | | |
| Belewice (Bo) | 0.1504 | 0.2202 | 0.6324 | 0.3343 | | | | | | | |
| Göttingen (Gö) | 0.1994 | 0.0950 | 0.4600 | 0.2515 | | | | | | | |
| Novosybirsk (Ns) | 0.0198 | 0.1874 | 0.6648 | 0.2907 | | | | | | | |
| Miłomłyn (Mi) | 0.1638 | 0.3016 | 0.6290 | 0.3648 | | | | | | | |
| Strzałowo (St) | 0.1666 | 0.2954 | 0.6462 | 0.3694 | | | | | | | |
| Duszniki (Du) | 0.2498 | 0.2506 | 0.5058 | 0.3354 | | | | | | | |
| Świerklaniec (Św) | 0.1314 | 0.2674 | 0.5294 | 0.3094 | | | | | | | |
| Lubiń (Lu) | 0.2408 | 0.3546 | 0.4702 | 0.3552 | | | | | | | |
| Rychtal (Ry) | 0.0392 | 0.1326 | 0.3694 | 0.1804 | | | | | | | |
| Maskulińskie (Ma) | 0.3538 | 0.2198 | 0.3996 | 0.3244 | | | | | | | |
| Włoszczowa (WI) | 0.0198 | 0.1654 | 0.4158 | 0.2003 | | | | | | | |
| Hainówka (Ha) | 0.0582 | 0.0582 | 0.5900 | 0.2355 | | | | | | | |
| Nowy Targ (NT) | 0.000 | 0.1638 | 0.5450 | 0.2363 | | | | | | | |
| Niepolomice (Ni) | 0.1302 | 0.3072 | 0.7200 | 0.3858 | | | | | | | |
| Catacik (Ca) | 0.0582 | 0.2456 | 0.6012 | 0.3017 | | | | | | | |
| Culhali (Cu) | 0.0582 | 0.5824 | 0.2874 | 0.3093 | | | | | | | |
| Vas (Va) | 0.0582 | 0.5524 | 0.6306 | 0.4137 | | | | | | | |

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is the result of a very high heterozygosity of one locus LAP-B while in Niepolomice high heterozygosity results from high heterozygosity in locus APH-B in which four alleles APH-B4, B5, B6, and B7 occur with high frequencies. In the Goleniów populations all three studied loci have a high value of h (Tab. 3).

The population from Rychtal in central Poland has a low heterozygosity of \hat{H} =0.1804. In this population in the majority of studied trees all three loci are to a large degree monomorphic. Similar is the case with genetic variability within the population from Włoszczowa, where \hat{H} = =0.2003.

Some of the studied populations have only one monomorphic locus as for example Novosibirsk, where h in locus LAP-A is 0.0198 while at another locus APH-B it is high h=0.6648 indicating a high polymorphism of that locus.

INTER-POPULATION VARIABILITY

While the genetic variability within populations may be evaluated by the mean heterozygosity per locus, differences between two populations are evaluated on the basis of genetic distances D, which were calculated here according to N e i (1972) by the formula:

$$D = -\log_{e} I$$

and where I is the normalized identity of genes computed by

$$I = \frac{J_{XY}}{\sqrt{J_X J_Y}}$$

in which J_X and J_Y are the average homozygosity per locus in populations X and Y respectively and J_{XY} is the average identity of genes between X and Y. J_X is calulated as:

$$J_{X} = \sum_{k=1}^{r} \sum x_{i}^{2},$$
$$J_{Y} = \sum_{k=1}^{r} \sum y_{i}^{2},$$
$$J_{XY} = \frac{\sum_{k=1}^{r} \sum x_{i} y_{i}}{r}.$$

It is a measure of similarity between populations and it served to draw a phenogram presented in Fig. 4 on which all the Polish populations except Niepolomice and Goleniów constitue a homogenous group of populations which are closely related to the Lower Saxony population from

| Coeffi | cients o | of ge | nic identi | ty (I) abo | ove | the diagonal | an | d Nei | 's gene | etic distance (1 | D-value, Ne | i, |
|--------|----------|-------|------------|------------|------|----------------|------|--------|---------|------------------|-------------|----|
| 1972) | below | the | diagonal | between | 19 | populations | of | Scots | pine. | Abbreviations | identifying | |
| nain | | | ol beils | amples an | re a | s indicated on | 1 th | e map, | Fig. 1 | s. In the G | Signs in ri | |

| Population | Go | Bo | Mi | Ma | St | Lu | Du | Św | Wł |
|-------------------|--------------|----------|--------------------|------|------|------|------|-------|--------|
| Goleniów (Go) | Ling average | .968 | .962 | .974 | .971 | .960 | .971 | .977 | .969 |
| Bolewice (Bo) | .032 | 0117 111 | .992 | .982 | .992 | .969 | .981 | .989 | .986 |
| Milomlyn (Mi) | .038 | .008 | D R C 1 | .975 | .994 | .974 | .979 | .980 | .983 |
| Maskulińskie (Ma) | .026 | .018 | .025 | - | .987 | .984 | .983 | .991 | .981 |
| Strzałowo (St) | .029 | .008 | .006 | .013 | m_m | .979 | .985 | .990 | .988 |
| Lubiń (Lu) | .040 | .031 | .026 | .016 | .021 | - | .971 | .988 | .976 |
| Duszniki (Du) | .029 | .019 | .021 | .017 | .015 | 0.29 | | .984 | .982 |
| Świerklaniec (Św) | .023 | .012 | .020 | .009 | .010 | .012 | .016 | 10_3m | .992 |
| Włoszczowa (Wł) | .031 | .014 | .017 | .019 | .012 | .024 | .018 | .008 | -07-5e |
| Niepołomice (Ni) | .082 | .064 | .088 | .099 | .083 | .107 | .099 | .065 | .065 |
| Hajnówka (Ha) | .038 | .030 | .039 | .030 | .028 | .034 | .032 | .016 | .008 |
| Nowy Targ (NT) | .058 | .048 | .053 | .049 | .042 | .042 | .049 | .027 | .016 |
| Rychtal (Ry) | .031 | .014 | .021 | .006 | .010 | .015 | .017 | .006 | .012 |
| Vas (Va) | .328 | .331 | .375 | .368 | .355 | .383 | .371 | .345 | .426 |
| Culhali (Cu) | .459 | .389 | .397 | .475 | .435 | .489 | .468 | .409 | .443 |
| Catacik (Ca) | .411 | .344 | .372 | .419 | .372 | .415 | .402 | .377 | .396 |
| Novosybirsk (Ns) | .046 | .037 | .046 | .047 | .046 | .067 | .057 | .047 | .049 |
| Babki (Ba) | .022 | .013 | .019 | .024 | .015 | .035 | .019 | .017 | .013 |
| Göttingen (Gö) | .039 | .007 | .013 | .028 | .018 | .048 | .028 | .028 | .018 |

beunitroo 4 elder on the basis of genetic distances D, which were calculated

| | | | | | | | | | 10.000 | S BTEN |
|-------------------|------|--------|------|------|------|------|------|------|--------|--------|
| Population | Ni | Ha | NT | Ry | Va | Cu | Ca | Ns | Ba | Gö |
| Goleniów (Go) | .921 | .962 | .946 | .969 | .720 | .632 | .663 | .955 | .978 | .961 |
| Bolewice (Bo) | .938 | .970 | .953 | .986 | .718 | .678 | .709 | .963 | .987 | .993 |
| Miłomłyn (Mi) | .916 | .961 | .948 | .979 | .687 | .672 | .689 | .955 | .981 | .987 |
| Maskulińskie (Ma) | .906 | .970 | .952 | .994 | .692 | .622 | .658 | .954 | .976 | .972 |
| Strzałowo (St) | .920 | .972 | .959 | .990 | .701 | .647 | .689 | .955 | .985 | .982 |
| Lubiń (Lu) | .898 | .966 | .959 | .985 | .682 | .613 | .660 | .935 | .965 | .953 |
| Duszniki (Du) | .906 | .968 | .952 | .983 | .690 | .626 | .669 | .945 | .981 | .972 |
| Świerklaniec (Św) | .937 | .984 | .973 | .994 | .708 | .664 | .686 | .954 | .983 | .972 |
| Włoszczowa (Wł) | .936 | .992 | .984 | .988 | .653 | .639 | .673 | .952 | .987 | .982 |
| Niepołomice (Ni) | 1 | .946 | .950 | .904 | .819 | .763 | .822 | .915 | .932 | .936 |
| Hajnówka (Ha) | .055 | anirab | .994 | .975 | .720 | .625 | .688 | .943 | .981 | .963 |
| Nowy Targ (NT) | .051 | .006 | - | .959 | .704 | .625 | .689 | .928 | .962 | .945 |
| Rychtal (Ry) | .101 | .025 | .042 | - | .691 | .625 | .664 | .954 | .980 | .975 |
| Vas (Va) | .200 | .328 | .351 | .370 | | .688 | .945 | .769 | .747 | .697 |
| Culhali (Cu) | .270 | .470 | .470 | .470 | .374 | - | .770 | .567 | .612 | .714 |
| Catacik (Ca) | .196 | .374 | .372 | .409 | .057 | .261 | - | .736 | .710 | .698 |
| Novosybirsk (Ns) | .090 | .059 | .075 | .047 | .263 | .567 | .306 | - | .941 | .948 |
| Babki (Ba) | .070 | .019 | .038 | .020 | .292 | .491 | .342 | .061 | - | .979 |
| Göttingen (Gö) | .066 | .037 | .057 | .025 | .361 | .337 | .360 | .053 | .021 | - |

Göttingen (which is very similar to Bolewice). The Turkish populations are clearly distinct. They have a high genetic distance between each other and with all the other populations (Fig. 4). They are related to the Hungarian Vas population. Of intermediate nature between the Turkish-Hungarian group and the Polish group is the Niepołomice population from southern Poland. The population from Goleniów is a pine seed stand with a high degree of intrapopulation variability, having also on the phenogram a somewhat independent position (Fig. 4).

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DISCUSSION

The results of studies presented here on the frequency of occurrence of alleles in pine populations indicates that there are considerable differences in the heterozygosity of populations depending on the studied locus. Much more important is the information about the heterozygosity of populations than about the frequency of occurrence of any particular allele. Wills (1973) suggests that the greater adaptability of populations



is associated with a greater heterozygosity of enzymes per individual regardless of the individual enzymatic alleles present in the functioning polymorphism. An evaluation of the known part of heterozygosity in populations of Scots pine in Europe encounters difficulties resulting from

the fact that in the majority of existing stands the populations are of artificial orgin.

In introduced spruce populations in Sweden a high degree of heterozygosity was observed (Lundkvist and Rudin, 1977). Similarily the undoubtedly introduced Vas population in Hungary has had a high degree of heterozygosity. On the other hand the certainly indigenous populations from Miłomłyn (the famous Tabórz pine race), Maskulińskie and Strzałowo are also highly heterozygotic. It is possible that the well known adaptability of pine from this region ($\Pr z y b y l s k i$, 1972; T r o e g e r, 1960) results from this high heterozygosity. It is also possible that human activity is to some extent responsible for this high heterozygosity since it is known that even in this region where phenotypically magnificent pine races grow there have been introductions of seed from far away places in Germany and France which differ substancially from the local races (B i a l o b o k, 1973).

APH has had a greater influence on the measure of heterozygosity than LAP in the studied pine populations. In spruce populations a distinct correlation was observed between ecological conditions and the frequency of occurrence of certain APH alleles. This would indicate that this enzyme is of adaptive nature (Bergmann, 1978). However it does not appear that in pine the acid phosphatase isozymes are in any way associated with morphological differences in pine populations. This can be judged on the example of *Pinus silvestris* f. conglomerata. In this form which

Table 5

Standard estimates (D) of net codon differences between race, subspecies, sibling species, and non sibling species in various organisms (after Nei, 1973)

| Taxa | D | |
|-----------------|--------------|--|
| Local races | | |
| Man | 0.005 - 0.02 | |
| Mouse | 0.002 - 0.03 | |
| Horsehoe | 0.002 - 0.04 | |
| D. pseudobscura | 0.003 - 0.13 | |
| Subspecies | | |
| Mouse | 0.13 - 0.20 | |
| Species | | |
| Drosophila | | |
| Sibling | 0.18 - 1.54 | |
| Nonsibling | 1.3 - 2.54 | |

has considerable deviations in morphology from the type in having a broom like habit, cones in abundant clusters etc. the pattern of APH and LAP isozymes was quite normal (Mejnartowicz, 1979).

Though on the basis of studies conducted so far it is not possible to draw far reaching conclusions, it is perhaps worth noting that the value

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of the genetic distance between populations in the north-south direction is much greater than in the east-west direction. There is an exceptionally great distance between the Turkish and Hungarian populations on the one hand and the remaining European populations on the other, the southern Polish population from Niepołomice being intermediate. There are suggestions that the Niepołomice stand are of Austrian origin. The value of the genetic distances between European populations and the southern ones are 0.196 - 0.415 for Catacik, 0.270 - 0.489 for Culhali and 0.222 - 0.426 for Vas. These values indicate distances greater than those usually observed between races within one species (Tab. 5).

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CONCLUSIONS

1. In the studied populations of Scots pine occurrence of five alleles coding locus LAP-A and six alleles coding locus LAP-B were observed. The greatest frequencies were observed for LAP-A1 and LAP-B1 and the majority of studied genotypes were of the type LAP-A1A1B1B1.

2. Much greater genetic polymorphism was observed in the studied locus APH-B coding acid phosphatase. This locus is coded by 15 alleles. In European populations the allele APH-B5 was most common.

3. In all populations except Culhali from Turkey the heterozygosity of trees in locus APH-B is much greater than in the LAP-A and LAP-B loci.

4. A large number of heterozygotes was found with the recessive allele LAP-A4 while heterozygotes having the recessive alleles LAP-B4 and APH-B1 are very rare in populations of Scots pine.

5. Considerable differences were found in the genetic structure of populations between the southern part of range, represented by pine from Turkey and from Hungary (and to some extent the southern Polish population from Niepołomice) and the other populations studied.

6. The rather low genetic distance between the Polish populations and the populations from Göttingen and Novosibirsk indicate that there is a much lower variability in Scots pine along the East-West axis than along the North-South one.

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Female gametophytes from seeds of 19 Scots pine (*Pinus silvestris*) from Poland, FRG, USSR, Turkey and Hungary were analysed for the content of leucine aminopeptidase (LAP) and acid phosphatase (APH)

isozymes. A considerable variability of APH enzyme was observed, with 15 alleles coding the APH-B locus. There were two LAP loci, LAP-A coded by 5 alleles and LAP-B coded by 6 alleles. A considerable genetic distance was observed between the Turkish and Hungarian populations. These populations from the southern part of the range differ substancially from the other European ones, the Niepolomice population from southern Poland being intermediate. The variability of Scots pine proved to be much greater along the N-S axis than along the W-E axis. The populations from the Mazury region known for they great adaptability are characterized by considerable heterozygosity.

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- 1. Bergmann F. 1973. Genetische Untersuchungen bei *Picea abies* mit Hilfe der Isoenzym-Identifizierung. II. Genetische Kontrolle von Esterase- und Leucinaminopeptidase-Isoenzymen im haploiden Endosperm ruhender Samen. Theoret. Appl. Genet. 43: 222 - 225.
- 2. Bergmann F. 1975. Adaptive acid phosphatase polymorphism in conifer seeds. Silvae Genetica 24: 175 177.
- 3. Bergmann F. 1978. The allelic distribution at an acid phosphatase locus in Norway spruce (*Picea abies*) along similar climatic gradients. Theor. Appl. Genet. 52: 57 - 74.
- 4. Białobok S., Mejnartowicz L., Karolewski P. 1976. Genetic characteristic of the Scots pine specimens resistant and susceptible to SO_2 . Second Ann. Rep. FG-Po-326 "Studies on the effect of Sulphur Dioxide and Ozone
- on respiration and assimilation of trees and shrubs in order to select individuals resistant to the action of these gases", 39 - 50.
- Krzakowa M., Szweykowski J., Korczyk A. 1977. Population genetics of Scots pine (*Pinus silvestris* L.) forests. Genetic structure of plus-trees in Bolewice near Poznań (West Poland). Bull. de l'Academie Pol. des Scie. Serie des scien. biol. Cl. II. 25 (9): 583 590.
- 6. Langlet O. 1959. A cline or not a cline- a question of Scot pine. Silvae Genetica 8: 1-36.
- 7. Lundkvist K., Rudin D. 1977. Genetic variation in eleven populations of *Picea abies* as determined by isozyme analisis. Hereditas 85: 67 - 74.
- Mejnartowicz L. 1976. Genetic investigation on Douglas fir (Pseudotsuga menziesii (Mirb.) Franco) populations. Arboretum Kórnickie 21: 126-179.
- Mejnartowicz L. 1976a. Analiza genetycznej zmienności roślin znajdujących się pod wpływem emisji przemysłowych. Ann. Report 10.2.10.: 1-7.
- Mejnartowicz L. 1978. Genetic characteristic of some Scots pine trees susceptible or somewhat resistant to the action of SO₂. Frounth Ann. Rep.
- FG-Po-326 "Studies on the effect of Sulphur Dioxide and Ozone on the respiration and assimilation on trees and shrubs in order to select individuals resistant to the action of these gases". Ed. S. Białobok: 26-33.
- 11. Mejnartowicz L., Białobok S., Karolewski P. 1978. Genetic

102

characteristics of Scots pine specimens resistant and susceptible to SO_2 action. Arboretum Kórnickie 23: 233 - 238.

- Mejnartowicz L. 1979. Pinus silvestris L. forma conglomerata Carriere w Polsce. Roczn. Sekcji Dendr. PTB. 32 in press.
- Mejnartowicz L., Bergmann F. 1975. Genetic studies on European larch (*Larix decidua* Mill.) employing isoenzyme polymorphisms. Genet. Polonica 16 (1): 295 - 35.
- Nei M. 1972. Genetic distance between populations. The American Naturalist (106) 949: 283 - 292.
- Nei N. 1973. The theory and estimation of genetic distance. in "Genetic structure of populations". Ed., N. E. Morton. Univ. Press of Hawaii, Honolulu: 45-54.
- Poulik M. D. 1957. Starch gel electrophoresis in discontinous system of buffers. Nature 180: 1477 - 1479.
- Przybylski T. 1972. Variability of Scots pine (*Pinus silvestris* L.) of Polish provenances. Arboretum Kórnickie 17: 121-167.
- Rudin D. 1977. Leucine-amino-peptidases (LAP) from needles and macrogametophytes of *Pinus silvestris* L. Inheritance of allozymes. Hereditas 85: 219-226.
- Salmia M., Mikola J. J. 1975. Activities of two peptidases in resting and germinating seeds of Scots Pine (*Pinus silvestris*). Physiol. Plant. (33): 261-265.
- Troeger R. 1960. Kiefernprovenienzversuche. All. Forst. u. Jagd. Ztg. (131): 49 - 59, 81 - 93.
- Wills C. 1973. In defense of naive pan-selectionism. Amer. Natur. 107: 23-34.
- 22. Wright J. W., Bull W. I. 1963, Geographic variation in Scots pine. Silvae Genetica 12: 1-40.

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Zmienność genetyczna izoenzymów w niektórych loci w populacjach sosny zwyczajnej (Pinus silvestris L.)

Streszczenie

Badania izoenzymów leucyloaminopeptydazy (LAP) i kwaśnej fosfatazy wykonano na endospermach nasion zebranych w 19 populacjach sosny zwyczajnej z Polski, RFN, ZSRR oraz Turcji i Węgier. Stwierdzono dużą zmienność enzymu APH, który w badanym locus APH-B kodowany był w postaci 15 alleli. LAP kodowana była w 2 loci, z których LAP-A zawierał 5 alleli, a ALP-B sześć alleli. Stwierdzono znaczny dystans genetyczny między populacjami tureckimi i populacją węgierską. Populacje te, z południowej części zasięgu znacznie różnią się od pozostałych populacji europejskich, łącząc się z nimi przez południowopolską populację Niepołomice. Znacznie większa okazała się zmienność sosny zwyczajnej w kierunku południkowym niż równoleżnikowym.

Znane ze swej zdolności adaptacyjnej populacje sosen mazurskich charakteryzują się dużą zmiennością wewnątrzpopulacyjną, wyrażającą się wysokim stopniem heterozygotyczności tych populacji.

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Генетическая изменчивость некоторых изоферментных локусов в популяциях сосны обыкновенной (Pinus silvestris L.)

Резюме

В работе представлены исследования генетической изменчивости изоферментов лейцинаминопептидазы (LAP) и кислой фосфатазы (APH) в эндоспермах семян сосны обыкновенной из 19 популяций с территории Польши, ФРГ, СССР, Турции и Венгрии. В результате исследований была установлена большая изменчивость фермента АРН, который в исследуемом локусе АРН-В кодировался 15 аллелями. LAP кодировался 2 локусами, из которых LAP-А содержал 5 аллелей, а LAP-В — 6 аллелей. Констатирована большая генетическая разница между турецкими и венгерской популяциями. Эти популяции, с южной части ареала распространения сосны обыкновенной, отличаются от остальных европейских популяций, соединяясь с ними через южнопольскую популяцию Неполомице. Изменчивость сосны обыкновенной в мериднанном направлении оказалась значительно большей, чем в параллельном.

Известные своей адаптационной способностью популяции мазурских сосен характеризовались большой внутрипопуляционной изменчивостью, что выражалось большой степенью гетерозиготности этих популяций.

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o serve brow w 2 los z których LLAP-A com a a milel, w 2.17-b sars milet Des edama morecy dystam goneteczny like constructioni tured mile poscha i regenta, Populacji to z poludnicemi i zasego znacadi zóroby się ol preserte sele populacji curopetskich, bącząc ję z 10 milozzez potedniewepcieleg po-

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