ISOENZYMATIC DIFFERENTIATION IN PUTATIVE HYBRID SWARM POPULATION (*PINUS MUGO* TURRA X *P. SYLVESTRIS* L.) FROM "TORFOWISKO ZIELENIEC" PEAT-BOG

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ABSTRACT

Isoenzyme variability of eight groups of individuals from different parts of putative hybrid swarm population (*P. mugo* Turra x *P. sylvestris* L.) from "Torfowisko Zieleniec" peat-bog complex was studied at ten enzymatic loci.

Differences in allelic frequencies distribution among particular samples of the Zieleniec population were statistically significant at 9 loci, as shown by G^2 -statistics. Chi-square test indicated that in each of studied subpopulations frequencies of alleles at some loci (2-5) differed significantly from frequencies expected for a homogeneous population. Heterozygosity values and also a genotypic polymorphism in studied population confirmed the existence of large genetic variation. Wright's fixation indices (F) showed some excess of homozygotes in majority studied groups of individuals, notably higher in some subpopulations. Gene diversity coefficient for 8 subpopulations was high ($G_{ST} = 9.4\%$). However, when MDH C locus was excluded from the calculation, its value decreased to 2.8%.

Presented data demonstrated notable differentiation of subpopulations within studied pine population, comparable with the differentiation among different populations of coniferous species.

KEY WORDS: *Pinus mugo*, isoenzymes, subpopulations, genetic structure, gene diversity, hybridization.

INTRODUCTION

The studied population of putative hybrid swarm *P. mugo* x *P. sylvestris* is situated within a big peat-bog complex called Torfowisko Zieleniec in the Bystrzyckie Mts. at an altitude of 700m.

The population consists of low polycormic bushes resembling typical *P. mugo* ssp. *mugo*, different forms of higher bushes (up to 4-5 m high), oligocormic trees (3-5 trunks growing from a common base) and also monocormic trees of highly variable appearance. A differentiation in distributions of these forms in peat-bog complex area can be easily observed.

According to certain hypothesis, the population from Zieleniec was formed after glacial period by hybridization of a relic *Pinus mugo* population with the surrounding *P. sylvestris* forest (Szweykowski – unpublished data). The previous morpho-anatomical and biochemical studies (Szweykowski and Bobowicz 1977, Prus-Głowacki et al. 1978, 1981, Prus-Głowacki and Szweykowski 1979, 1980, 1983, Bobowicz 1988) were concentrated mainly on the part of peat-bog called "Kieszeń" and some of works included also a detailed characteristic of the putative parents of this hybrid group.

Studies on additional individuals from this population have seemed to be necessary to see if there exists a common trend in the variation at particular enzymatic loci. The analysis of genetic structure of different groups of individuals from the possibly entire area of "Torfowisko Zieleniec" was the purpose of this paper.

MATERIAL AND METHODS

Plant material.

Isoenzyme variability in buds of 800 individuals was investigated. From each of eight studied parts of "Torfowisko Zieleniec" peat-bog 100 plants were collected (Fig. 1).

A, B, C, F and H stands consisted mainly of bushy-like individuals which resemble typical *P. mugo* ssp. *mugo*. The higher treelike mono- and polycormic forms prevailed at D, E and G stands.

Biochemical methods

The following enzymatic loci were studied: fluorescent esterase (Fest, E.C.3.1.1.1), glutamate oxalo-acetate transaminase (GOT, E.C.2.6.1.1 – 2 loci), diaphorase (DIA, E.C.1.6.4.3), glutamate dehydrogenase (GDH, E.C.1.4.1.3), alcohol dehydrogenase (ADH, E.C.1.1.1.1), shikimate dehydrogenase (ShDH, E.C.1.1.1.25 – 2 loci) and malate dehydrogenase (MDH, E.C.1.1.1.37 – 2 loci). The separation of isoenzymes in starch gels and genetic interpretation of the results were performed as described by Gulberg et al. (1985), Rudin and Ekberg (1978), Szmidt and Yazdani (1984), Yazdani et al. (1985).

Statistical methods.

Frequencies of alleles and genotypes, and on this base heterozygosity (H_O) and genotypic polymorphism indices (P_G acc. to Kahler et al. 1980) were calculated to determine differences in genetic structure among analyzed groups of individuals

Fig. 1. Localization of studied groups of individuals from putative hybrid swarm population from "Torfowisko Zieleniec" peat-bogs.

— subpopulations. Allelic frequencies in particular subpopulations were compared by means of G^2 -statistic (Gillula 1985, Siatkowski and Krajewski 1989). Significance of deviation of allele distribution in the groups of individuals against the distribution expected with assumption of homogeneity of Zieleniec population, was studied with χ^2 -test. Wright's fixation index (F) (Jain and Workman 1967) and also χ^2 -test were used to find whether analyzed subpopulations fulfil criteria of Hardy-Weinberg equilibrium.

Genetic differentiation of eight groups of putative hybrids was appraised using Nei's (1973) gene diversity statistics (GST).

RESULTS

Ten enzymatic loci with 36 alleles were detected (Table 1). In particular subpopulations I observed from 67% (C group) to

TABLE 1. Frequencies of alleles observed at 10 enzymatic loci in population from "Torfowisko Zieleniec".

Loci and alleles	Frequencies of alleles								
	Group A	Group B	Group C	Group D	Group E	Group F	Group G	Group H	In whole population
FEst									
1	.890	.905	.910	.945	.895	.856	.960	.930	.912
2	.095	.080	.090	.020	.065	.033	.020	.050	.057
3	.015	.015	.000	.035	.040	.111	.015	.020	.030
4	.000	.000	.000	.000	.000	.000	.005	.000	.001
GOT A									
1	.995	1.000	1.000	.970	.960	.983	.810	.990	.975
2	.000	.000	.000	.000	.000	.006	.000	.005	.001
3	.000	.000	.000	.010	.010	.006	.167	.000	.013
4	.005	.000	.000	.020	.025	.006	.024	.000	.009
5	.000	.000	.000	.000	.005	.000	.000	.005	.001
GOT B									
1	.925	.955	.890	.895	.864	.972	.688	.909	.888
2	.055	.030	.110	.075	.126	.028	.312	.091	.102
3	.020	.015	.000	.030	.010	.000	.000	.000	.010
DIA									
1	.980	.990	.995	.939	.952	.967	.907	.995	.972
2	.015	.010	.005	.020	.000	.006	.009	.005	.009
3	.005	.000	.000	.020	.000	.011	.046	.000	.008
4	.000	.000	.000	.020	.048	.017	.037	.000	.011
GDH									
1	.510	.465	.345	.460	.435	.528	.405	.550	.461
2	.465	.505	.645	.535	.565	.461	.515	.435	.514
3	.025	.030	.010	.005	.000	.011	.000	.000	.013
4	.000	.000	.000	.000	.000	.000	.080	.015	.012
ADH									
1	.140	.190	.098	.075	.062	.182	.092	.134	.122
2	.825	.730	.881	.851	.902	.807	.864	.825	.835
3	.030	.070	.021	.075	.036	.011	.044	.041	.041
4	.005	.010	.000	.000	.000	.000	.000	.000	.002
ShDH A									
1	.300	.315	.325	.505	.399	.244	.444	.330	.358
2	.700	.670	.660	.484	.548	.722	.546	.606	.616
3	.000	.000	.005	.000	.000	.028	.000	.032	.008
4	.000	.015	.010	.021	.053	.006	.010	.032	.018
ShDH B									
1	.970	.990	.965	.985	.974	.950	.970	.984	.974
2	.000	.000	.005	.005	.011	.022	.000	.000	.005
3	.030	.010	.030	.010	.011	.028	.030	.011	.020
4	.000	.000	.000	.000	.005	.000	.000	.005	.001
MDH A									
1	.995	.965	.985	.965	.970	.994	.990	.960	.978
2	.005	.035	.015	.035	.030	.006	.010	.040	.022
MDH C									
1	.205	.154	.242	.725	.853	.852	.748	.809	.609
2	.795	.846	.758	.275	.147	.148	.252	.191	.391

83% (F group) of all noted alleles. At GDH, ADH or ShDH A loci frequency of the most common allele varied to a great extent in analyzed groups of individuals in the population.

The most striking differences in frequencies of two alleles detected at locus MDH C were found between groups of individuals from the reservation of Topielisko (A,B and C –

forms resembling typical *P. mugo* ssp. *mugo*), in which MDH C2 allele was the most frequent, and subpopulations in the remaining parts of this peat-bog complex (D,E,G – tree-like forms, F and H – similar to forms in groups A,B,C), in which MDH C1 allele was most frequent.

Some genetic parameters of studied population from Zieleniec

Fig. 2. Some genetic parameters of studied population from "Torfowisko Zieleniec".

Mean heterozygosity values are presented in Fig. 2. The level of heterozygosity was similar in subpopulations of studied population from Zieleniec and amounted to, approximately, 0.19-0.20. A higher value of this coefficient ($H_o = 0.28$) was observed only in G subpopulation.

Genotypic polymorphism in the studied population is also shown in Fig. 2. It was relatively high and uniform (approx. 0.3) in all groups except G, in which P_g value was higher (0.4), similarly to heterozygosity (H_o) which was also higher in the group.

Significant differences in allelic frequencies distribution among particular samples of the Zieleniec population were detected using G^2 - statistics in nine among ten studied (except locus MDH A) enzymatic loci (Table 2). Chi-square test indicated that in each studied subpopulation, frequencies of

alleles at some loci (from 2 to 5, depending up on the particular group of individuals) differed significantly ($\alpha = 0.05$) from frequencies expected for homogeneous population (Table 3).

χ^2 values obtained by testing the hypothesis on a panmictic character of pine population from Zieleniec are shown in Table 4. A, B and G samples remained in Hardy-Weinberg equilibrium at all studied loci. In the rest of the samples, significant deviations from Hardy-Weinberg expectations were found at one in groups F and H to five loci (D subpopulation). Deviations from Hardy-Weinberg equilibrium were detected at six enzymatic loci by χ^2 test when all 800 individuals were analyzed together as one population.

Wright's fixation indices (F) in studied population from Zieleniec are presented in Fig. 2. In all studied groups of individuals - except G, where F approximates zero, some excess of homozygotes was observed, notably higher in D, E and H subpopulations.

Nei's method (1973) was used to estimate the amount of genetic variation at different levels of population structure (within and between subpopulations. The total electrophoretically determined genetic variation (H_T), variation within and between subpopulations (H_S and D_{ST} , respectively) and gene diversity coefficient (G_{ST}) calculated for 10 enzymatic loci in the Zieleniec population are shown in Table 5.

At five enzymatic loci (FEST, ADH, ShDH A, ShDH B and MDH A) the majority of variation was found within subpopulations; G_{ST} value varied from 0.6-2.7%. At two GOT loci, investigated groups of individuals from Torfowisko Zieleniec peat-bog were highly differentiated (G_{ST} equal 9.3% and 7.0%, respectively). At the rest of studied loci (DIA, GDH and MDH C) a considerable amount of total genetic variation appeared between subpopulations reaching extreme at MDH C locus - 35% (Table 5).

TABLE 2. Significance level of G^2 values for differences in distributions of allelic frequencies between eight groups of individuals from investigated pine population near Zieleniec.

Locus	G^2	p
FEST	72.18	0.0000
GOT A	99.71	0.0000
GOT B	122.04	0.0000
DIA	57.06	0.0009
GDH	108.88	0.0000
ADH	54.32	0.0020
ShDH A	92.16	0.0000
ShDH B	28.00	0.1402
MDH A	14.99	0.0361
MDH C	554.53	0.0000

TABLE 3. Chi-square values for deviations of observed distribution of allelic frequencies in eight groups of individuals from studied pine population near Zieloniec, from distribution expected in homogenous population (significance level: * $p < 0.05$; ** $p < 0.01$).

Locus	Number of degrees of freedom	χ^2 values in groups:							
		A	B	C	D	E	F	G	H
FEst	3	6.873	3.567	10.022*	5.409	1.155	42.052**	9.899*	1.110
GOT A	4	3.438	4.928	4.928	3.232	9.274	4.933	157.153**	11.067*
GOT B	2	6.640*	11.676**	2.126	9.440**	1.275	12.960**	90.510**	2.318
DIA	3	3.238	3.889	4.264	6.194	10.783*	0.968	26.920**	4.264
GDH	3	6.591	6.973	15.054**	3.557	6.305	4.930	81.028**	8.615*
ADH	3	2.045	20.723**	4.737	9.052**	7.299	9.452*	1.905	0.642
ShDH A	3	9.370*	3.680	2.173	18.277**	16.735**	20.130**	8.003	15.909**
ShDH B	3	2.203	2.253	1.217	1.225	5.466	11.509*	2.243	5.276
MDH A	1	2.686	1.571	0.455	1.571	0.634	2.262	1.303	3.117
MDH C	1	2.438	9.699**	0.117	5.395*	3.172	3.542	2.744	0.211

TABLE 4. Deviation from Hardy-Weinberg equilibrium. χ^2 values in groups of individuals and in entire population from Tofrowisko Zieloniec (+ $p < 0.05$, * $p < 0.01$; n – number of degrees of freedom).

Locus	χ^2 values in groups:								In whole population
	A	B	C	D	E	F	G	H	
FEst	6.168 n=6	0.511 n=6	0.978 n=3	0.339 n=6	1.376 n=6	0.982 n=6	0.176 n=10	2.696 n=6	5.640 n=10
GOT A	0.003 n=3	0.000	0.000	24.012* n=6	63.210* n=10	0.026 n=10	2.325 n=6	0.011 n=6	150.159* n=15
GOT B	3.246 n=6	0.222 n=6	0.046 n=3	21.641* n=6	0.619 n=6	0.073 n=3	2.103 n=3	2.066 n=3	19.808* n=6
DIA	0.042 n=6	0.010 n=3	0.003 n=3	0.310 n=10	0.105 n=3	0.107 n=10	0.562 n=10	0.003 n=3	0.589 n=10
GDH	0.865 n=6	3.121 n=6	0.225 n=6	0.894 n=6	0.715 n=3	1.832 n=6	6.047 n=6	2.512 n=6	36.729* n=10
ADH	11.999 n=10	3.210 n=10	5.974 n=6	98.684* n=6	65.770* n=6	0.965 n=6	3.158 n=6	8.894 n=6	59.555* n=10
ShDH A	0.227 n=3	7.254 n=6	100.414* n=10	24.261* n=6	4.504 n=6	9.146 n=10	0.416 n=6	0.278 n=10	18.638+ n=10
ShDH B	0.096 n=3	0.010 n=3	0.132 n=6	0.023 n=6	0.069 n=10	0.249 n=6	0.97 n=3	0.024 n=6	0.718 n=10
MDH A	0.003 n=3	0.132 n=3	0.023 n=3	0.132 n=3	0.097 n=3	0.003 n=3	0.010 n=3	0.176 n=3	0.420 n=3
MDH C	0.015 n=3	4.773 n=3	8.041+ n=3	19.241* n=3	22.831* n=3	35.929* n=3	0.807 n=3	32.469* n=3	174.563* n=3

TABLE 5. Relative measure of genetic differentiation between subpopulations (G_{ST}) of studied pine population from Torfowisko Zieleniec. H_T – total heterozygosity expected for studied subpopulations, H_S – mean expected heterozygosity, $D_{ST} = H_T - H_S$.

Locus	H_T	H_S	D_{ST}	$G_{ST}(\%)$	Within groups (%)
FEst	0.1654	0.1626	0.0028	1.7	98.3
GOT A	0.0711	0.0645	0.0066	9.3	90.7
GOT B	0.2020	0.1878	0.0142	7.0	93.0
DIA	0.0670	0.0655	0.0113	16.8	83.2
GDH	0.5201	0.5114	0.0088	16.9	83.1
ADH	0.2852	0.2803	0.0049	1.7	98.3
ShDH A	0.4921	0.4790	0.0130	2.7	97.3
ShDH B	0.0520	0.0517	0.0003	0.6	99.4
MDH A	0.0431	0.0427	0.0004	0.9	99.1
MDH C	0.4892	0.3179	0.1713	35.0	65.0
Mean	0.2387	0.2164	0.0224	9.4	90.6
without MDH C	0.2169	0.2109	0.0060	2.8	97.2

The mean gene diversity coefficient calculated for 10 enzymatic loci amounted to 9.4%, but the value reduced to 2.8% when locus MDH C was excluded from calculations.

DISCUSSION

Pinus mugo and *P. sylvestris* are typical representatives of conifers, one of the most heterozygotic groups of plant species (Hamrick 1979, Hamrick et al. 1979, Prus-Głowacki 1991). Heterozygosity in eight groups of individuals from the putative hybrid swarm population at Zieleniec ($H_O = 0.19$; in G group = 0.28) approximates the minimal heterozygosity level observed in the European Scots pine populations ($H = 0.18-0.34$) (Prus-Głowacki 1991), and is slightly higher from mean heterozygosity noted in the North American populations of *Pseudotsuga menziesii*, *Picea sitchensis* and *Pinus contorta* ssp. *latifolia* (Yeh 1979) which amounted from 0.15-0.18 (standard error value of, approximately, 0.04). It is difficult to compare these results directly due to the different number of mono- and polymorphic analyzed loci and various number of studied populations (European Scots pine: 3-9 loci, 7-19 populations; American conifers: 21-25 loci and 9-11 populations).

Survey of genetic variation in European conifers, based on isoenzymes presented by Müller-Starck et al. (1992), showed extensive heterogeneity of results obtained by different investigators. For example, heterozygosity in *P. sylvestris* populations varied from 26.8%-38.0% (3-11 loci, 3-19 populations) and in other pine species (*P. cembra*, *P. halepensis*, *P. leucodermis*, *P. nigra*, *P. pumila* and *P. sibirica*) its value was lower and varied between 12.4% and 27.2% (4-23 loci, 3-28 populations). The reasons of these differences besides the ones mentioned above, could involved also specific features of tree species, connected with their geographic range and taxonomical division into different subspecies or races, heterogeneity of population types and environments (e.g. different population history, micro- and macrohabitat variation).

The sampling strategies – different life stages of studied trees and inclusion or exclusion of monomorphic gene loci in estimates of genetic variation by different authors, frequently without any data on the applied procedure also influenced differences between data obtained in individual studies.

Heterozygosity values and also genotypic polymorphism in studied population from Zieleniec (Fig. 2) have confirmed the existence of extensive genetic variation, consistent with the values described in a different species of conifers.

Genetic diversity in pine population from Torfowisko Zieleniec has been analyzed in eight groups of individuals (Fig. 1). Only in the case, when all subpopulations are members of a single large panmictic unit, all alleles will be evenly distributed over the entire range of population and H_T will be equal H_S . In nature, however, this kind of situations can be found very rarely. Natural populations tend to differentiate over time into subpopulations because of restricted gene flow, mating system, the processes of selection, genetic drift and also, but to a very small extent, due to mutations.

At some enzymatic loci G^2 and χ^2 tests have shown significant differences between allele and genotype frequencies observed in the studied groups of individuals on one hand and their frequencies expected in a genetically homogeneous population on the other (Tables 2 and 3). Significant deviations from Hardy-Weinberg equilibrium in population from Zieleniec have been observed in five subpopulations at some loci (only 15% of 80 analyzed cases), whereas differences in genotype frequencies tested for the entire sample (all 800 studied individuals together) have proven significant at six enzymatic loci (Table 4). These results can be explained by Wahlund's effect (if there are large differences among subpopulations there will be a deficiency of heterozygotes in the entire population even if Hardy-Weinberg proportions exist within each subpopulation) or by selection of specific genotypes. The best relevant example involves GDH locus, at which particular groups of individuals are in Hardy-Weinberg equilibrium, but when they are lumped together deviation

from the equilibrium due to an excess of GDH 44 homozygotes can be observed.

At conifer populations a majority of their genetic diversity remains within populations, and a small part of it is between populations (El-Kassaby 1991).

Gene diversity coefficient values (G_{ST}) in different conifer species most often have amounted to several per cent and have varied according to the number and kind of enzymatic loci as well as the number and range of the analyzed populations. G_{ST} values in *Pinus sylvestris* ranged widely from 1.5% in three populations from Sweden (Muona and Szmidt 1984), 2% in 9 Swedish populations (Guldberg et al. 1985), 2.3% in 18 populations from the Lubień provenance trial (originating from 10 European countries) (Prus-Głowacki et al. 1993), 3% in 4 Swedish populations (Rudin et al. 1974) to 16% in 19 populations from Poland, Turkey, Germany, Russia and Hungary (Mejnartowicz 1979).

Gene diversity coefficient in 8 groups of individuals distributed over, approximately, 2 km² area of "Torfowisko Zieleniec" peat-bog has been relatively high (G_{ST} = 9.4%) but if MDH C locus is excluded from the calculations, its value would decrease to 2.8%. A similar case has been described in *Pinus muricata*, where G_{ST} value changed from 2.9% to 14.1% when G_{ST} value of one locus had been added. This phenomenon was probably related to one-way gene flow along north-south direction in the studied population (Millar 1983).

At MDH C locus differences in allelic frequencies have been observed between subpopulations from the "Topielisko" reservation, where frequencies of two alleles of MDH C was characteristic for *Pinus mugo* from Tatra Mts. (Filppula et al. 1992, Siedlewska and Prus-Głowacki 1994a,b). In the rest of studied subpopulations from "Torfowisko Zieleniec", the frequencies of MDH C alleles were characteristic for *P. sylvestris*. The reason of this phenomenon could have involved changes in the habitat in the part of the population. The "Topielisko" reservation started to be protected earlier than the remaining part of the peat-bog complex near Zieleniec, where a peat was still excavated and the population was destroyed.

The situation described above („pine” frequencies of MDH C alleles in longer exploited part of peat-bog) may represent the result of introgression of genes of *P. sylvestris* to *P. mugo* or genetic drift evolved by fluctuations in population size – bottle-neck effect. However, considering the lack of the species-specific isoenzymatic markers, the similarity in allelic frequency at one enzymatic locus to the frequency characteristic for *P. sylvestris* in part of the Zieleniec population cannot be considered to provide strong evidence for hybridization hypothesis.

The presented above data prove the differentiation of subpopulations within pine population from "Torfowisko Zieleniec", comparable with a differentiation among different populations of coniferous species. It is most likely that restricted gene flow, genetic drift and directional selection connected with adaptation to microhabitats has been responsible for the detected differences in the genetic structure of studied groups of individuals. However, involvement of a process of introgression cannot be also excluded.

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LITERATURE CITED

- BOBOWICZ M.A., 1988. Differentiation of *Pinus sylvestris* L., *Pinus mugo* Turra, pines from Bór na Czerwonym and from Zieleniec in 12 traits of one- and two-years-old cones. Bull. Sci. Ami. Poznań D, 26: 99-108.
- BZOWY P., NOWAK-BZOWY R., 1986. Gen computer program. (unpublished).
- EL-KASSABY Y.A., 1991. Genetic variation within and among conifer populations: review and evaluation of methods. In: Fineschi S., Malvolti M.E., Cannata F. and Hattemer H.H. (eds.). Biochemical markers in the population genetics of forest trees, pp. 63-74, Acad. Pub., Hague.
- FILPPULA S., SZMIDT A.E., SAVOLAINEN O., 1992. Genetic comparison between *Pinus sylvestris* and *P. mugo* using isozymes and chloroplast DNA. Nord. J. Bot. 12: 381-386.
- GILLULA Z., 1985. On the analysis of heterogeneity among populations. J. R. Statist. Soc. 47: 76-83.
- GULLBERG U., YAZDANI R., RUDIN R., RYANNEN N., 1985. Allozyme variation in Scots pine (*Pinus sylvestris* L.) in Sweden. Silvae Genet. 34: 193-201.
- HAMRICK J.L., 1979. Genetic variation and longevity. In: Solbrig O.T., Jain S. and Raven P.H. (eds.), Plant Population Biology, pp. 84-113. Columbia University Press, N.Y.
- HAMRICK J.L., LINHART Y.B. and MITTON J.B., 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Ann. Rev. Ecol. Syst. 10: 173-200.
- JAIN S.K., WORKMAN P.L., 1967. Generalized F-statistic and the theory of inbreeding and selection. Nature 214: 674-678.
- KAHLER A.L., ALLARD R.W., KRZAKOWA M., WEHRHAHN C.F., NEVO E., 1980. Associations between isozyme phenotypes and environment in the slender wild oat (*Avena barbata*) in Israel. TAG 56: 31-47.
- MEJNARTOWICZ L., 1979. Genetic variation in some isozyme loci in Scots pine (*Pinus sylvestris* L.) populations. Arboretum Kórnickie 24: 91-104.
- MILLAR C.I., 1983. A steep cline in *Pinus muricata*. Evolution 37: 311-319.
- MUONA O., SZMIDT A.E., 1984. A multilocus study of natural populations of *Pinus sylvestris*. In: Szmidt A.E. (ed.) Genetic studies of Scots pine (*Pinus sylvestris*) domestication by means of isozyme analysis. The Swedish University of Agricultural Sciences, Umeå.
- MÜLLER-STARCK G., BARADAT Ph., BERGMANN F., 1992. Genetic variation within European tree species. New Forests 6: 23-47.
- NEI M., 1973. Analysis of gene diversity in natural populations. Proc. Natl. Acad. Sci. USA 70: 3321-3323.
- PRUS-GŁOWACKI W., SADOWSKI J., SZWEYKOWSKI J., WIATROSZAK I., 1981. Quantitative and qualitative analysis of needle antigens *Pinus sylvestris*, *Pinus mugo*, *Pinus uliginosa* and *Pinus nigra* and some individuals from a hybrid swarm population. Genet. Polon. 22, 4: 447-454.
- PRUS-GŁOWACKI W., SZWEYKOWSKI J., SADOWSKI J., 1978. Studies on serological similarity of *Pinus sylvestris* L., *P. mugo* Turra and individuals from a hybrid swarm population. Genet. Polon. 19, 3: 321-338.
- PRUS-GŁOWACKI W., SZWEYKOWSKI J., 1979. Studies on antigenic differences in needle proteins of *Pinus sylvestris* L., *P. mugo* Turra, *P. uliginosa* Neumann and *P. nigra* Arnold. Acta Soc. Bot. Polon. 68, 2: 217-238.
- PRUS-GŁOWACKI W., SZWEYKOWSKI J., 1980. Serological characteristics of some putative hybrid individuals from a *P. sylvestris* x *P. mugo* swarm population. Acta Soc. Bot. Polon. 49, 1/2: 127-142.
- PRUS-GŁOWACKI W., SZWEYKOWSKI J., 1983. Studies on isoenzyme variability in populations of *Pinus sylvestris* L., *Pinus mugo* Turra, *Pinus uliginosa* Neumann and individuals from a hybrid swarm population. Bull. Sci. Amis. Poznań D, 22: 107-122.
- PRUS-GŁOWACKI W., 1991. Biochemical polymorphism. In: Gierzych M., Mátyás C. (eds.), Genetics of Scots pine, pp. 73-86. Akadémiai Kiadó, Budapest.

- RUDIN D., ERIKSSON G., EKBERG I., RASMUSON M., 1974. Studies of allele frequencies and inbreeding in Scots pine populations by the aid of the isozyme technique. *Silvae Genet.* 23: 10-13.
- RUDIN D., EKBERG T., 1978. Linkage studies in *Pinus sylvestris* using macrogametophyte allozymes. *Silvae Genetica* 27: 1-11.
- SIATKOWSKI I., KRAJEWSKI P., 1989. Grouping multinomial objects according to minimal within-groups variability. *Stat. Appl.* 1, 1: 33-43.
- SIEDLEWSKA A., PRUS-GŁOWACKI W., 1994a. Allozyme variability in putative hybrid swarm population (*Pinus mugo* Turra x *P. sylvestris* L.) from Topielisko peat-bog near Zieloniec. *Genet. Polon.* – in press.
- SIEDLEWSKA A., PRUS-GŁOWACKI W., 1994b. Genetic structure and taxonomic position of *Pinus uliginosa* Neumann population from Wielkie Torfowiska Batorowskie in Stołowe Mts. (locus *clascis*). *Acta Soc. Bot. Pol.* – submitted.
- SZMIDT A.E., YAZDANI R., 1984. Electrophoretic studies of genetic polymorphism of shikimate and 6-phosphogluconate dehydrogenases in Scots pine (*Pinus sylvestris*). *Arboretum Kórnickie* 29: 63-72.
- SZWEYKOWSKI J., 1969. The Variability of *Pinus mugo* Turra in Poland. *Bull. Soc. Am.* 10: 39-54.
- SZWEYKOWSKI J., BOBOWICZ M.A., 1977. The Variability of *Pinus mugo* Turra in Poland. IV. Needles and cones in some Polish populations. *Bull. Soc. Am.* 17: 3-14.
- YAZDANI R., MUONA O., SZMIDT A.E., 1985. Genetic structure of a seed tree stand and naturally regenerated plants of *Pinus sylvestris* L. *Forest Sci.* 31, 2: 430-436.
- YEH F.C., 1981. Analysis of gene diversity in some species of conifers. *Proc. Symp. Isozymes of North American Forest Trees and Forest Insects*, Berkeley, California.

ZRÓŻNICOWANIE IZOENZYMATYCZNE POPULACJI PRZYPUSZCZALNEGO
ROJU MIESZAŃCOWEGO (*PINUS MUGO* TURRA x *P. SYLVESTRIS* L.)
Z TORFOWISKA "ZIELENIEC"

STRESZCZENIE

Badano zmienność izoenzymatyczną w 8 grupach osobników (sto osobników każda) z populacji przypuszczalnego roju mieszańcowego *P. mugo* x *P. sylvestris* z Torfowisk Zielonieckich. W 9 loci enzymatycznych (na 10 analizowanych) stwierdzono istotne statystycznie różnice w rozkładach częstości alleli pomiędzy poszczególnymi próbami z tej populacji. Test χ^2 wykazał, że częstości alleli w niektórych loci w każdej z badanych subpopulacji różniły się istotnie od ich frekwencji oczekiwanych dla populacji homogenicznej. Wartości heterozygotyczności oraz współczynnika polimorfizmu genotypowego potwierdziły istnienie dużej zmienności genetycznej porównywalnej z opisaną dla różnych gatunków z rodzaju *Pinus*. Wartości fixation index Wright'a wykazały pewien nadmiar homozygot (w stosunku do wartości oczekiwanych dla stanu równowagi Hardy'ego-Weinberga) w prawie wszystkich analizowanych grupach osobników.

Współczynnik zróżnicowania genetycznego Nei'a (1975) badanych subpopulacji był stosunkowo wysoki ($G_{ST}=9.4\%$), lecz po wyłączeniu z obliczeń locus MDH C, jego wartość obniżyła się do 2.8%.

Przedstawione dane wykazały znaczne zróżnicowanie subpopulacji w obrębie badanej populacji, porównywalne ze stopniem zróżnicowania pomiędzy różnymi populacjami zbadanych gatunków drzew szpilkowych.

SŁOWA KLUCZOWE: *Pinus mugo*, izoenzymy, subpopulacja, struktura genetyczna, zróżnicowanie genetyczne, mieszańczość.