ISOENZYMATIC VARIABILITY IN PROGENY
OF *PINUS MUGO* TURRA × *PINUS SYLVESTRIS* L. HYBRIDS
FROM BÓR NA CZERWONEM, IN EXPERIMENTAL CULTURE

MARIA ANNA BOBOWICZ¹, WŁADYSŁAW DANIELEWICZ², BEATA PIECZYŃSKA¹
ALEKSANDRA WOJNICKA-PÓŁTORAK¹, WIESŁAW PRUS-GŁOWACKI¹

¹Department of Genetics

Institute of Experimental Biology, Adam Mickiewicz University
Międzychodzka 5, 60-371 Poznań

²Chair of Forest Botany, August Cieszkowski Academy of Agriculture
Wojska Polskiego 71d, 60-625 Poznań

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ABSTRACT

The article describes the genetic structure of 12-year old seedlings. They were grown in an experimental culture representing the progeny of natural hybrids of *Pinus mugo* Turra and *Pinus sylvestris* L. from the peatbog Bór na Czerwonem. The studies were carried out on seedlings, employing electrophoresis of enzymatic proteins from winter buds. Parallel isoenzymatic analyses were also conducted for trees of *P. mugo* Turra from Tatra Mountains and for *P. sylvestris* L. trees from Białowieża in order to determine similarities of the hybrids to the 'pure' species, thought to represent parental forms, and for natural hybrids from Bór na Czerwonem. The obtained electrophoretic patterns made the determination of genetic structure of the population possible, namely of the observed heterozygosity, expected heterozygosity, Wright's fixation index and coefficients of similarity according to Nei and Hedrick. Genetic distances between the populations were also estimated. The variability of 11 enzymatic loci demonstrated the highest level of variability in the population of hybrids. Coefficients of genetic differentiation (G_{ST}) and of gene flow, (N_m) suggested a close relationship of the hybrids to *P. mugo* which may indicate an introgressive type of the hybrid swarm, from which the studied trees originated. The analysis of genetic similarity coefficients, according to Nei and Hedrick, demonstrated also an extensive similarity of the two hybrid groups to *P. mugo*. However, upon analysis of individual enzymatic loci, some of them highly resembled *P. mugo* while others were similar to *P. sylvestris*. The closest genetic similarity was demonstrated for the group of hybrids from the peatbog and the respective of hybrid group from culture. The variable gene expression as well as asymmetric inheritance of isoenzymes provided additional evidence of the introgressive character of the studied trees from the hybrid population.

KEY WORDS: *Pinus mugo* Turra, *Pinus sylvestris* L., hybrids, genetic structure, isoenzymes, introgression.

INTRODUCTION

Natural hybridization between *P. mugo* Turra and *P. sylvestris* (L.) in regions of their sympatric occurrence has been known for a long time and analysed by numerous investigators (Pravdin 1964; Christensen 1987a, b, c; Kanak 1994). In the interspecies hybrids of low fertility, backcross is much more frequent and more effective with growing nearby parental species than within themselves. The backcrosses result in a gene flow between hybrids and the parental species and in the enrichment of a gene pool of one species with genes of another species. Anderson and Hubricht (1938) termed this process the introgression. The interspecies crosses, introgressive crosses, and hybrids of F₂ subsequent generations and all the other possible combinations of crosses result in the formation of a hybrid swarm, which exhibits a variability of forms. Survival and existence of the hybrid swarm depends, however, upon the inhabitation of appropriate ecological niches. The

biotope, which fulfills the requirements, is the high peatbog, Bór na Czerwonem, within the peatbog belt of Bory Nowotarskie in Kotlina Nowotarska. In 1925 a reservation was established on a small part of the peatbog. In 1956 it was expanded to its present size (Staszkiewicz 1965). According to the latter author (Staszkiewicz 1985, 1992), *P. mugo* appeared in Bór na Czerwonem around 400 years ago, due to anthropogenic transformations associated with peat exploitation, which resulted in peatbog drainage, creating appropriate conditions for inhabiting of *P. mugo* to the biotope, inhabited from the Bölling interstadial by *Pinus sylvestris* (Koperowa 1962). According to Staszkiewicz (1992), this means also the beginning of introgressive hybrids between *P. mugo* and *P. sylvestris* (*P. × rhaetica* Brügg.). The hybrid swarm populations were already subjected to morphologic-anatomical studies (Staszkiewicz and Tyszkiewicz 1969a, b; Szweykowski 1969; Szweykowski and Bobowicz 1977; Bobowicz 1988, 1990a, b; Staszkiewicz 1993a, b, c, d, 1994, 1996; Bączkie-

wicz 1995), to studies on enzymatic variability and on variability of polyphenol compounds (Szweykowski and Urbaniak 1982; Urbaniak and Krzaczek 1985). Studies were also performed on the progeny of the natural introgressants from Bór na Czerwonym, maintained in the experimental culture, and pertained to the morphological and anatomical variability of needles (Tyszkiewicz 1979). Using the analysis of isoenzymatic variability the authors aimed at describing genetic structure of 12-year old seedlings of *P. × rhaetica*, representing the progeny of putative hybrids from Bór na Czerwonym, growing in the experimental culture. On the basis of the calculated parameters genetic structure and genetic similarities were established of the studied hybrid population and compared to the reference populations of *P. mugo* and *P. sylvestris* and to the population from Bór na Czerwonym, from which the seedlings originated.

MATERIALS AND METHODS

The studies were performed on 12 year old seedlings (termed SAR) of natural *Pinus mugo* Turra × *Pinus sylvestris* L. (*P. × rhaetica*) hybrids, grown in an experimental plot. The seedlings represented the progeny of putative hybrids (termed SBC), inhabiting the high peatbog of Bór na Czerwonym,

located on the right, high bank of the river Biały Dunajec, ca. 620 m above sea level in the valley of Kotlina Orawsko-Nowotarska (Fig. 1). In autumn 1983, two year old cones were collected from a few dozens of trees, inhabiting the peatbog and representing either the low, dwarf forms, the monocormic trees or the intermediate forms. From the few hundreds of cones seeds were extracted and were sown in 1985, in the glasshouse of the Dendrological Garden-Academy of Agriculture in Poznań. In the culture, the seedlings were grown in homogeneous conditions. All the factors which might present hazards for the young seedlings were eliminated but, nevertheless, out of a few hundreds of the obtained seedlings only few dozens of them survived. In the following 5 years, 30 seedlings remained in the field culture and they were permanently labelled. The distribution of the seedlings in the experimental plot was fully random. In 1990, studies were undertaken on the morphology and anatomy of the two year old needles of the seedlings. During annual collections of the material in autumn, observations were also carried out on the colour of needles, type of stem and on the angle of setting for one year old and two year old cones, which began to form. Results of morpho-anatomical analysis of the material collected in 1990-1994 will be published separately. In the spring of 1998, winter buds were collected from the 26 still surviving 12 year old seedlings and the buds were subjected to enzymatic analysis. Variability of 11 following loci were investigated (Fest (EC 3. 1. 1. 2.); GOT A, B (EC 2. 6. 1. 1.), DIA (EC 1. 6. 4. 3.); ADH (EC 1. 1. 1. 1.); GDH (EC 1. 4. 1. 3.); 6 PGD (EC 1. 1. 1. 44.); MDH A, C (EC 1. 1. 1. 37.); ShDH A, B (EC 1. 1. 1. 25.)) according to the procedure described by Gulberg et al. (1982).

As mentioned earlier, the seedlings represented the natural progeny of *P. × rhaetica* hybrids from Bór na Czerwonym. Therefore, the enzymatic analysis included also, as a reference material, winter buds of *P. mugo* Turra from Hala Gąsienicowa in Tatra Mountains and winter buds of *P. sylvestris* L. from Białowieża Forest (Fig. 1 and Table 1). It seemed also interesting to examine isoenzymatic variability of the group of natural hybrids from the peatbog, Bór na Czerwonym. The plant material in each of the sites was collected in spring 1998. Genetic variability of a population was estimated by the following parameters: 1. Mean number of alleles and genotypes per locus (A/L, G/L). 2. Observed heterozygosity (H_o), according to Crow and Kimura (1970), Nei and Roychoudhury (1974). 3. Expected heterozygosity (H_e), according to Nei and Roychoudhury (1974). 4. Index of genetic polymorphism (Pg), according to Kahler et al (1980). 5. Wright's fixation index (F), according to Wright (1969), served as a measure of population's deviation from Hardy-Weinberg's equilibrium. 6. The Nm parameter (indirect estimates of gene flow) was calculated according to the formula by Sladkin and Barton (1989) based on rare alleles that occur in compared populations. Statistical significance of the deviations was tested using χ^2 test.

Relative measure of genetic variability between particular populations was determined by G_{ST} coefficient (Nei 1973).

TABLE 1. Location of tree samples and their numbers (n) used in the investigation.

SAMPLES	LOCATION	n
<i>P. sylvestris</i>	Białowieża Primeval Forest	30
<i>P. mugo</i>	Tatra Mountains	33
hybrids	Bór na Czerwonym	33

Fig. 1. Location of populations from which the studied material was sampled. 1. Population of *P. mugo* Turra × *P. sylvestris* hybrids from the experimental plot in the Dendrological Garden, Academy of Agriculture in Poznań, 2. population of *P. × rhaetica* hybrids from peatbog Bór na Czerwonym, near Nowy Targ (according to Staszewicz 1965), 3. population of *P. mugo* Turra from Tatra Mountains, 4. population of *P. sylvestris* L. from Białowieża.

TABLE 2. Some genetic parameters of studied populations: H_e – heterozygosity expected, H_o – heterozygosity observed, F – fixation index, P_g – genotypes polymorphism index, A/L – mean number of alleles per locus, G/L – mean number of genotypes per locus. SAR – hybrid seedlings from culture, SBC – hybrids from "Bór na Czerwone".

POPULATIONS	H_e	H_o	F	P_g	A/L	G/L
SEEDLINGS SAR (HYBRIDS)	0.335	0.346	0.025	0.485	2.63	3.72
Bór na Czerwone SBC (HYBRIDS)	0.342	0.322	0.059	0.477	2.63	3.54
<i>Pinus mugo</i> Turra	0.297	0.270	0.091	0.423	2.54	3.45
<i>Pinus sylvestris</i> L.	0.298	0.293	0.016	0.407	2.36	3.18

Genetic similarities (S_N and S_H) and genetic distances according to Nei (D_N) and Hedrick (D_H) were also calculated (Nei 1972, Hedrick 1974). The obtained values of genetic distances between individual populations provided the basis for constructing dendrograms by UPGMA technique (Sneath and Sokal 1973). The dendrograms facilitated the determination of reciprocal genetic relationships between populations.

RESULTS

Genetic structure and gene flow

Genetic parameters of trees from the culture, trees from the hybrid swarm and of *P. sylvestris* and *P. mugo* populations are presented in Table 2. The observed heterozygosity (H_o) in the seedling population and the respective value for Bór na Czerwone trees were relatively high and exceeded values of the index for *P. mugo* and *P. sylvestris* groups by ca. 29%. Also the index of genetic polymorphism (P_g) as well as mean numbers of alleles and genotypes were higher in the two groups of hybrids.

The coefficient of genetic differentiation (G_{ST}) for the studied groups of trees pointed to ca. 10% with the relatively small gene flow, as indicated by the low coefficient of migration per generation ($N_m = 2.47$) (Table 3). In pairwise comparisons of the hybrids (SAR vs. SBC), hybrid seedlings vs. parental species, and of the parental species between one another (Table 3), the closest relationship or the least pronounced genetic difference (G_{ST}) was demonstrated by hybrid seedlings (SAR) and *P. mugo* population, cultured seedlings (SAR) and hybrids from Bór na Czerwone (SBC). The most pronounced differences, as judged by G_{ST} coefficient, were observed for groups of *P. mugo* and *P. sylvestris*, for cultured seedlings (SAR) and *P. sylvestris*, for trees from Bór na Czerwone (SBC) and for *P. sylvestris*.

The N_m coefficient, taken as the level of possible hybridization, showed an inconsiderable extent of the latter phenomenon between the parental species, *P. sylvestris* and *P.*

mugo (1.9 migrant per generation), a much more extensive gene flow between the two groups of hybrids ($N_m = 6.62$) and hybrids and *P. mugo* ($N_m = 5.9$ and $N_m = 13.57$), and not so pronounced between the hybrids and *P. sylvestris* ($N_m = 3.75$ and $N_m = 2.07$).

Genetic similarity of populations according to NEI

For particular enzymes, the studied hybrids from the culture demonstrated various extent of similarities, whether compared to the parental population of natural hybrids from Bór na Czerwone or to the reference populations. Thus, the most extensive similarity of parents to the hybrids from Bór na Czerwone was detected in malate dehydrogenase A locus (MDH A), fluorescent esterase (Fest) and 6-phosphogluconate dehydrogenase (6PGD) (Fig. 2). In the case of Fest, the hybrid group from Bór na Czerwone, from the culture or from the peatbog, proved to be linked with the two reference populations of parents. In the case of MDH A and 6PGD, the hybrids formed a similar group to which the reference populations were linked: first *P. mugo* Turra and, then, *P. sylvestris* (Fig. 2). With respect to glutamate dehydrogenase (GDH) and alcohol dehydrogenase (ADH), the hybrids from experimental culture exhibited the highest similarity to *P. mugo* Turra (Fig. 3). In GDH, the group was linked with *P. sylvestris* reference population and, finally, the so – formed complex was joined by the hybrids from Bór na Czerwone. In the ADH, hybrids from the experimental plot and *P. mugo* formed a single group, linked with another group, formed of *P. sylvestris* and peatbog hybrids (Fig. 3).

With respect to glutamate-oxalacetic transaminase B locus (GOT B) and diaphorase (DIAF), the hybrids from experimental plot resembled most *P. sylvestris* (Fig. 4). The peatbog hybrids together with *P. mugo* formed another group. In the case of DIAF, hybrids from culture join to *P. sylvestris*

TABLE 3. Genetic differentiation of studied populations: H_T – total heterozygosity, H_e – expected heterozygosity, D_{ST} – genetic diversity within populations, G_{ST} – genetic diversity among populations, N_m – number of migrants for generation, SAR – hybrid seedlings from culture, SBC – hybrids from "Bór na Czerwone".

POPULATIONS	H_T	H_e	D_{ST}	G_{ST}	N_m
All populations	0.354	0.322	0.0325	0.0916	2.479
SAR and SBC	0.361	0.348	0.0131	0.0362	6.662
SBC and <i>P. mugo</i>	0.392	0.376	0.0159	0.0406	5.903
SBC and <i>P. sylvestris</i>	0.343	0.321	0.0214	0.0624	3.755
SAR and <i>P. mugo</i>	0.328	0.322	0.0059	0.0181	13.574
SAR and <i>P. sylvestris</i>	0.364	0.324	0.0391	0.1075	2.075
<i>P. sylvestris</i> and <i>P. mugo</i>	0.366	0.298	0.0384	0.1141	1.940

Fig. 2. Dendrogram presenting genetic similarity between *P. x rhaetica* hybrids in experimental culture (1), *P. x rhaetica* hybrids from Bór na Czerwone (2), *P. mugo* Turra from Tatra Mountains (3) and *P. sylvestris* L. from Białowieża (4), constructed on genetic distances for MDH A, 6PGD and FEst, according to Nei.

Fig. 3. Dendrogram presenting genetic similarity between hybrids of *P. × rhaetica* from experimental culture (1), hybrids of *P. × rhaetica* from Bór na Czerwone (2), *P. mugo* Turra from Tatra Mountains (3) and *P. sylvestris* L. from Białowieża (4), constructed on genetic distances for GDH and ADH, according to Nei.

Fig. 4. Dendrogram presenting genetic similarity between *P. × rhaetica* hybrids from experimental culture (1), *P. × rhaetica* hybrids from Bór na Czerwone (2), *P. mugo* Turra from Tatra Mountains (3) and *P. sylvestris* L. from Białowieża (4), constructed on genetic distances for GOT B and DIAF, according to Nei.

Fig. 5. Dendrogram presenting genetic similarity between *P. × rhaetica* hybrids from experimental culture (1), *P. × rhaetica* hybrids from Bór na Czerwone (2), *P. mugo* Turra from Tatra Mountains (3) and *P. sylvestris* L. from Białowieża (4), constructed on genetic distances for GOT A, ShDH A and MDH C, according to Nei.

population and then to hybrids from peat bog. The group is linked with *P. mugo* from Tatra Mts.

In patterns of GOT A, MDH C and ShDH A loci, hybrids from the culture were most similar to the complex of *P. mugo*

and hybrids from Bór na Czerwone (Fig. 5). The complex is linked with the *P. sylvestris* population.

Another pattern of the genetic similarity, shikimate dehydrogenase B locus (ShDH B): the hybrids from Bór na Czerwone were most similar to *P. sylvestris*. The group was linked with *P. mugo* and, then, the complex was joined to the hybrids from culture (Fig. 6).

Genetic similarity of populations according to Hedrick

In respect of the majority of the enzymatic genotypes (FEST, DIAF, 6PGD, MDH A, ShDH B) the studied hybrids from the experimental plot were most similar to hybrids from peat-bog Bór na Czerwone (Fig. 7). On the other hand, the similarity of reference populations to this complex varied for individual enzymes. For FEST, the references formed the group, which were linked to both hybrid groups. Except for DIAF, in the pattern of which the hybrid group was linked first with *P. sylvestris* and, then, with *P. mugo*, patterns for the remaining enzymes (MDH, 6PGD, ShDH B) exhibited more extensive similarity of *P. mugo* to the hybrid complex than to *P. sylvestris* (Fig. 7).

The studied hybrids from the culture were most similar to the *P. mugo* population in respect of ADH and GOT A. In the pattern of the two enzymes the second, separate group was formed the hybrids from the Bór na Czerwone and *P. sylvestris* (Fig. 8). With respect to MDH C and ShDH A, hybrids from experimental plot were most similar to the complex of *P. mugo* and peatbog hybrids. The three populations were joined to *P. sylvestris* (Fig. 9).

Still another pattern of reciprocal relations was demonstrated for GDH and GOT B (Fig. 10). In this respect, cultured hybrids resembled most extensively *P. sylvestris*. In the case of GDH the group was joined to *P. mugo* and then, to peatbog hybrids. For GOT B the peatbog hybrids and *P. mugo* formed a distinct group (Fig. 10) and to this group hybrids from the culture and population of *P. sylvestris* are joined.

Dendrograms constructed for mean genetic distances for all loci, calculated on the basis of either Nei's coefficient of similarity or coefficient of Hedrick, manifested the greatest resemblance between hybrid populations from the culture and the *P. mugo* population. The complex was joined to the hybrid population from Bór na Czerwone. *P. sylvestris* exhibited significant genetic distance from the complex (Fig. 11).

Fig. 6. Dendrogram presenting genetic similarity between *P. × rhaetica* hybrids from experimental culture (1), *P. × rhaetica* hybrids from Bór na Czerwone (2), *P. mugo* Turra from Tatra Mountains (3) and *P. sylvestris* L. from Białowieża (4), constructed on genetic distances for ShDH B, according to Nei.

Fig. 7. Dendrogram presenting genetic similarity between *P. × rhaetica* hybrids from experimental culture (1), *P. × rhaetica* hybrids from Bór na Czerwonym (2), *P. mugo* Turra from Tatra Mountains (3) and *P. sylvestris* L. from Białowieża (4), constructed on genetic distances for MDH A, 6PGD, ShDH B, DIAF and FEst, according to Hedrick.

DISCUSSION

All the studies performed till now have proved or suggested that *Pinus* sp. population inhabiting Bór na Czerwonym peat-bog exhibits hybrid character (Staszkiwicz 1965; Staszkiwicz and Tyszkiewicz 1969a, b; Szweykowski 1969; Szweykowski and Bobowicz 1977; Tyszkiewicz 1979; Szweykowski and Urbaniak 1982; Urbaniak and Krzaczek 1985; Bobowicz 1988, 1990a, b; Staszkiwicz 1992, 1993a, b, c, d, 1994, 1996; Bączkiewicz 1995).

Detailed studies on 400 individuals, drawn at random from the population, with respect to the extensive complex of traits of morphology and anatomy, have proven that all the individuals represent introgressive hybrids (Bobowicz 1990a). Among others, the phenomenon of introgressive individuals has been indicated by variable expression of genes inherited from pure parental species of *P. mugo* Turra and *P. sylvestris* L.

The variable expression of parental genes in the introgressive hybrids has also been confirmed by multiannual observations of seedlings developed from hybrids from Bór na Czerwonym in the experimental culture and this, as already

Fig. 8. Dendrogram presenting genetic similarity between *P. × rhaetica* hybrids from experimental culture (1), *P. × rhaetica* hybrids from Bór na Czerwonym (2), *P. mugo* Turra from Tatra Mountains (3) and *P. sylvestris* L. from Białowieża (4), constructed on genetic distances for ADH and GOT A, according to Hedrick.

Fig. 9. Dendrogram presenting genetic similarity between *P. × rhaetica* hybrids from experimental culture (1), *P. × rhaetica* hybrids from Bór na Czerwonym (2), *P. mugo* Turra from Tatra Mountains (3) and *P. sylvestris* L. from Białowieża (4), constructed on genetic distances for MDH C and ShDH A, according to Hedrick.

Fig. 10. Dendrogram presenting genetic similarity between *P. × rhaetica* hybrids from experimental culture (1), *P. × rhaetica* hybrids from Bór na Czerwonym (2), *P. mugo* Turra from Tatra Mountains (3) and *P. sylvestris* L. from Białowieża (4), constructed on genetic distances for GDH and GOT B, according to Hedrick.

Fig. 11. Dendrogram presenting genetic similarity between *P. × rhaetica* hybrids from experimental culture (1), *P. × rhaetica* hybrids from Bór na Czerwonym (2), *P. mugo* Turra from Tatra Mountains (3) and *P. sylvestris* L. from Białowieża (4), constructed on mean genetic distances for all examined enzymes, according to Nei and Hedrick.

mentioned in the "Introduction", will provide subject for a separate paper (in preparation).

Studies of enzymatic genes presented here have also confirmed the introgressive character of the population of hybrids from Bór na Czerwonym, whether examined cultured seedlings or the peatbog individuals. This is indicated by the variable pattern of similarities of hybrids to the parental species. Depending upon the enzymatic system, on some occasions, the examined sample has exhibited more extensive similarity to one parental (*P. mugo*) and, on other occasions, it resembled more closely the other parental species (*P. sylvestris*). In cases of GDH and ADH, the cultured hybrids (Fig. 3) have been most similar to *P. mugo*. On the other hand, in patterns of GOT B and DIAF they have been most similar to *P. sylvestris* (Fig. 4). Genetic similarity and, thus, close relationship of the populations from the culture plot and the peatbog has been indicated for MDH A, 6PGD and FEst (Fig. 2). In all the dendrograms, the individuals from Bór na Czerwonym and cultured seedlings have been most similar to each other.

In some enzymes an interesting phenomenon has been disclosed, namely of a distinct similarity of the hybrid populations in the same enzymatic system, but in its different loci to both reference species. The phenomenon has been observed

for MDH, GOT and ShDH systems. In locus A of MDH, the hybrids resembled most closely the individuals from the peatbog (Fig. 2) while in locus C they have joined the hybrids group from the peatbog and *P. mugo* (Fig. 5). For GOT locus B, the examined seedlings were genetically most similar to *P. sylvestris* (Fig. 4), while in locus A they were linked to the pair of populations from Bór na Czerwonym and *P. mugo* population (Fig. 5). The cultured seedlings have been linked with the same group, i.e. to the peatbog hybrids – *P. mugo* group in the locus ShDH A (Fig. 5) while in the locus B of the enzyme the seedlings were linked with both reference species and with the peatbog introgressants population (Fig. 6). The phenomenon points to an introgressive character of seedlings from the experimental plot and of the peatbog population.

The most extensive genetic similarity of the cultured seedlings to the peatbog introgressants (Fig. 2) was noted for FEst, genetic dissimilarity from either of the parents or, as found in the case of 6PGD (Fig. 2), distant similarity to one of the parents (*P. sylvestris*) may possibly indicate the existence of new, non-parental gene combinations in the hybrids. A similar phenomenon has also been disclosed in studies on controlled hybrids of *P. montana* × *P. sylvestris* (Prus-Głowacki and Stephan 1998).

Genetic parameters which characterise genetic structure of the studied groups of trees have documented the high genetic variability of either group of hybrids as compared to the populations of parental species. This has been expressed by higher levels of heterozygosity, of genotype polymorphism and of mean number of alleles and genotypes per locus. Such pattern is typical of hybrids, the genomes of which comprise genes of both parental forms (Linhard et al. 1989; de Phamphilis and Wyatt 1990; Prus-Głowacki and Stephan 1998; Krutowski and Bergmann 1993). In fact, in some types of the hybrids one of the parental genomes may remain unexpressed (Nanson et al. 1992; Prus-Głowacki and Mejnartowicz 1992), but our results suggest a more or less pronounced expression of both parental genomes in the hybrids.

In this respect, however, the situation seems somewhat more complicated since the obtained results, in particular those pertaining to genetic variability (Table 2), clearly suggest that the analysed hybrids are introgressants with a clear-cut prevalence of *P. mugo* genes and not *P. sylvestris*, as indicated by the Nm coefficient, which reflects a much more pronounced gene flow at present or in the past, or possibility of this phenomenon between *P. mugo* and the hybrids than that between *P. sylvestris* and the hybrids. The relatedness of the hybrids and *P. mugo*, as well as, the two groups of hybrids to each other is also evident when coefficients of interpopulation differentiation (G_{ST}) are considered, very low between *P. mugo* and both groups of hybrids, and between the two groups of hybrids.

Considering the significant age of the peatbog Bór na Czerwonym and the multiple generations of the hybrids and of the putative (*P. mugo* and *P. sylvestris*) parents, however, in much less extent which co-inhabit the peatbog one may assume that at present mainly the introgressant trees hybridise, which can be concluded from the intensity of gene flow (Nm) between the hybrids, as well as between the hybrids and *P. mugo*.

Summing up, it may be stated that: 1. Seedlings grown in the experimental plot represent hybrids of *P. mugo* Turra and of *P. sylvestris* L.; 2. The hybrid seedlings show the introgressant type; 3. The sample of population from peatbog Bór na Czerwonym, from which the seedlings originated, mani-

fects also the character of introgressant hybrids; 4. The peat-bog Bór na Czerwonem is inhabited by the hybrid swarm, (*P. mugo* × *P. sylvestris*) (*P.* × *rhaetica*.)

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IZOENZYMATYCZNA ZMIENNOŚĆ POTOMSTWA
NATURALNYCH MIESZAŃCÓW *PINUS MUGO* TURRA x *PINUS SYLVESTRIS* L.
Z BORU NA CZERWONEM W UPRAWIE DOŚWIADCZALNEJ

STRESZCZENIE

Opisano strukturę genetyczną 12 letnich siewek stanowiących potomstwo naturalnych mieszańców *Pinus mugo* Turra i *Pinus sylvestris* L. z torfowiska "Bór na Czerwonym", rosnących w uprawie doświadczalnej. Równolegle analizy izoenzymatyczne przeprowadzono także dla 33 osobników *P. mugo* Turra z Tatr i 30 drzew *P. sylvestris* L. z Białowieży w celu określenia podobieństwa mieszańców względem tych "czystych" gatunków, uznanych za formy rodzicielskie, oraz wobec 33 naturalnych introgresantów z "Boru na Czerwonym". Na podstawie otrzymanych rozdziałów elektroforetycznych obliczono frekwencje poszczególnych alleli i genotypów do obliczenia parametrów statystycznych określających strukturę genetyczną populacji: heterozygotyczności obserwowanej, heterozygotyczności spodziewanej, indeksu wsobności Wright'a oraz współczynników podobieństwa według Nei'a i Hedrick'a oraz wyznaczono odległości genetyczne między badanymi populacjami. Badania zmienności 11 loci enzymatycznych w grupie mieszańcowych osobników z "Boru na Czerwonym" i siewek uzyskanych z nasion z wyżej wymienionej populacji, a także populacji gatunków rodzicielskich *P. mugo* Turra z Tatr i *P. sylvestris* z Białowieży wykazały wyższy poziom zmienności genetycznej populacji mieszańców. Heterozygotyczność obserwowana (H_0) wynosiła tutaj odpowiednio 0,322, 0,346, 0,297 i 0,298. Liczba alleli i genotypów na locus u obu grup mieszańców wynosiła 2,63, a u gatunków rodzicielskich 2,54 (*P. mugo* Turra) i 2,36 (*P. sylvestris* L.). Dla genotypów liczby te wynoszą odpowiednio $G/L = 3,72, 3,54, 3,45$ i $3,18$. Współczynnik polimorfizmu genotypowego był również wyższy w grupie mieszańców $P_g = 0,485$ i $0,477$, a u gatunków rodzicielskich P_g (*P. mugo* i *P. sylvestris*) = $0,423$ i $0,407$. Współczynniki różnicowania genetycznego (G_{ST}) i migracji (N_m) (przepływu genów) sugerują wysokie powinowactwo mieszańców do *P. mugo*, co może być dowodem na introgresywny charakter roju mieszańcowego, z którego pochodzą badane osobniki. Współczynnik N_m dla populacji *P. mugo*/mieszańce z uprawy (siewki), *P. mugo*/mieszańce z "Boru na Czerwonym" wynosił $13,57$ i $5,903$. W zestawieniu z *P. sylvestris*/mieszańce wartości te są znacznie niższe $N_m = 2,09$ i $3,75$. Analiza współczynników podobieństwa genetycznego wg Nei'a i Hedrick'a wykazała także znaczne podobieństwo obu grup mieszańców do *P. mugo*. Jednak poszczególne loci enzymatyczne wykazywały częściej podobieństwo mieszańców do *P. mugo* niż do *P. sylvestris*. Grupy mieszańców z torfowiska i mieszańców z uprawy są sobie genetycznie najbliższe. Różnicowana ekspresja genów, jak również asymetryczne dziedziczenie dostarczają dodatkowego dowodu na introgresywny charakter badanych osobników z populacji mieszańcowej.

SŁOWA KLUCZOWE: *Pinus mugo* Turra, *Pinus sylvestris* L., hybrydy, struktura genetyczna, izozymy, introgresja.