GENETIC STRUCTURE AND TAXONOMIC POSITION OF *PINUS ULIGINOSA* NEUMANN POPULATION FROM WIELKIE TORFOWISKO BATOROWSKIE IN STOŁOWE MTS. (*LOCUS CLASSICUS*)

ANNA SIEDLEWSKA¹ and WIESŁAW PRUS-GŁOWACKI²

¹Institute of Plant Genetics, Polish Academy of Sciences,
60-479 Poznań, ul. Strzeszyńska 37, Poland

²Department of Genetics, Adam Mickiewicz University,
60-594 Poznań, ul. Dąbrowskiego 165

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ABSTRACT

Genetic structure of *Pinus uliginosa* Neumann from Wielkie Torfowisko Batorowskie peat-bog (*locus classicus*) was studied by means of isoenzyme variability and compared with genetic structure of *P. mugo* Turra from Tatra Mts and *P. sylvestris* populations from Kłodzka Valley and Czersk. The level of genetic variability in the population of *P. uliginosa* (heterozygosity, genotypic polymorphism) was similar to that in populations of *P. mugo*, with an excess of homozygosity in relation to Hardy-Weinberg equilibrium. An average number of alleles per locus in population of *P. uliginosa* is the same as in the studied *P. sylvestris* populations, but the average number of genotypes is slightly lower. However, populations of *P. mugo* from montane stands in Tatra Mts. are characterized by a higher number of alleles per locus than that in *P. uliginosa* population. In populations of *P. mugo* from peat-bogs, a lower number of alleles and genotypes is noted than in *P. uliginosa* population. The coefficients of genetic similarity (Nei and Hedrick) show the distinctly separate character of *P. uliginosa* as compared to *P. sylvestris* and loose relationship of this taxon to *P. mugo*. Also the measure of genetic differentiation of populations (G_{ST}) confirms the specificity of this pine taxon.

KEY WORDS: *Pinus uliginosa*, genetic structure, taxonomy, isoenzymes.

INTRODUCTION

P. uliginosa Neumann, described for the first time from Wielkie Torfowisko Batorowskie peat-bog in 1838 (Neumann 1838), occurs in a few stands in Poland: at Węgliniec (Lower Silesia), at Wielkie Torfowisko Batorowskie (*locus classicus*), in peats at Zieleniec and on the Błędne Skały in the Stołowe Mts. (Sudeten Mts.), in pine forest of the Nowy Targ region and at Skamieniałe Miasto reservation near Ciężkowice (Boratyńska and Boratyński 1978, Staszkiwicz 1993). Some of them are shown in Figure 1.

The taxonomic position of *P. uliginosa* is still not unequivocally defined, but the majority of authors assume its hybrid origin. Some of them (Szweykowski 1969, Boratyński 1978, Prus-Głowacki and Szweykowski 1979) consider this pine to represent an introgressant of *P. mugo* $\times$ *P. sylvestris* into *P. mugo*. Similarly, Staszkiwicz and Tyszkiewicz (1972) have stated that "*P. uncinata* ssp. *rotundata* known in Poland as *P. uliginosa* is a hybrid between *P. mugo* and *P. sylvestris*". *Pinus uncinata* occurs in mountainous and submountainous parts of Western and Central Europe.

Krzakowa et al. (1984) suggest that Polish populations of *P. uliginosa* could be isolated, marginal populations of *P. uncinata* (which, however, are also "suspected" of a hybrid origin). Christensen (1987) describing complex *P. mugo* Turra divides it into two subspecies: the eastern ssp. *mugo* and western ssp. *uncinata*. *P. uliginosa* and *P. uncinata* ssp. *ro-*

tundata were classified by him as synonyms of *P. mugo* ssp. *mugo* $\times$ *P. mugo* ssp. *uncinata* (*P. x rotundata* Link).

Recently, Staszkiwicz (1993) classified *P. uliginosa* Neumann as one of *nothomorphs* of *P. x rhaetica* Brügger (*P. mugo* $\times$ *P. sylvestris* – see Christensen 1987).

The population of *P. uliginosa* from Wielkie Torfowisko Batorowskie has been studied by means of different methods (Szweykowski 1969, Szweykowski and Bobowicz 1977, Prus-Głowacki et al. 1978, 1981, Prus-Głowacki and Szweykowski 1979, 1983, Krzakowa et al. 1984). Morphological as well as biochemical studies indicated some distinctness of *P. uliginosa* from the two closely related species: Scots pine (*Pinus sylvestris*) and dwarf mountain pine (*Pinus mugo*) and, at the same time, showed considerable similarity to *P. mugo*.

The purpose of this study was to describe the genetic structure of the population of *P. uliginosa* at its *locus classicus* and to determine the taxonomic position of this population in relation to *P. sylvestris* and *P. mugo*.

MATERIAL AND METHODS

Isoenzymatic variability of 40 individuals from population of *P. uliginosa* Neumann from Wielkie Torfowiska Batorowskie (*locus classicus*) was examined. Dormant winter buds, used for analyses, were collected early autumn 1990. Variation in the following enzymatic loci was studied: fluorescent esterase (FEst, E.C.3.1.1.1.), glutamate oxalo-acetate trans-

Fig. 1. Some localities of *P. uliginosa* in Poland (Boratyński and Boratyńska 1978, Staszkiewicz 1993).

1. Węgliniec (Lower Silesia);
2. Wielkie Torfowisko Batorowskie (Sudeten Mts.);
3. Zieleniec (Sudeten Mts.);
4. Ludźmierz (Nowy Targ Valley);
5. Bór na Czerwonym (Nowy Targ Valley);
6. Skamieniałe Miasto (near Ciężkowice)

aminase (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 Gullberg et al. (1982), Rudin and Ekberg (1978), Szmidt and Yazdani (1984), Yazdani et al. (1985).

Isoenzymatic data of the studied population of *P. uliginosa* (signed hereafter as ul) were compared with results of isoenzymatic analyses (at the same loci) of three *P. sylvestris* and five *P. mugo* populations (see Table 1).

The following parameters were calculated for the studied groups of individuals: frequencies of alleles and genotypes, number of alleles and genotypes per locus (A/L, G/L), heterozygosity (H_o , H_e – observed, expected, respectively), Wright's fixation indices (F) (Jain, Workman 1967), genotypic polymorphism indices (P_G) (Kahler et al. 1980), genetic similarity coefficients (I_N , I_H) and genetic distances (D_N , D_H) according to Nei and Hedrick (Nei 1972, Hedrick 1974). Genetic differentiation coefficient G_{ST} (Nei 1973) was used to estimate an interpopulational variation in the analyzed groups of Scots pine and dwarf mountain pine populations.

The loci were denoted as mono- and polymorphic ones using the criterion of frequency. When the frequency of the most common allele was higher than 0.99, the locus was considered to be monomorphic. All calculations were made with assistance of the "Gen" computer program (Bzowy, Nowak-Bzowy 1986 – unpublished work).

RESULTS

Frequencies of alleles and genotypes

Frequencies of alleles and genotypes at ten enzymatic loci in the investigated population of *P. uliginosa* and also, in five *P. mugo* and three *P. sylvestris* populations are shown in Tables 2 and 3.

TABLE 1. Studied populations of *P. uliginosa*, *P. sylvestris* and *P. mugo* and their localization.

Species		Localization	Number of individuals per sample
Population			
Pinus uliginosa	ul	Wielkie Torfowisko** Batorowskie, Stołowe Mts.	40
Pinus sylvestris	bk	Bystrzyca Kłodzka,* Kłodzka Valley	29
	cz	Czersk,* forest region Toruń	29
	sz	Szczeliniec, Stołowe Mts.	51
Pinus mugo	kb	Kopa pass, Tatra Mts.	92
	kc	Czerwienica, Tatra Mts.	91
	kd	Litworowy Staw, Tatra Mts.	96
	pw	Polana Waksmundzka,** Tatra Mts.	25
	pt	Psia Trawka,** Tatra Mts.	63

* – material originates from provenance trial in Kórnik

** – peat-bogs

TABLE 2. Frequencies of alleles in nine pine populations.

Loci/ Alleles	<i>Pinus uliginosa</i>	<i>Pinus mugo</i> populations from Tatra Mts.			<i>Pinus mugo</i> populations from peat-bogs		<i>Pinus sylvestris</i> populations		
	ul	kb	kc	kd	pw	pt	bk	cz	sz
FEst									
1	.863	.807	.808	.853	.980	.913	.879	.672	.726
2	.125	.167	.121	.116	.020	.056	.069	.086	.020
3	.013	.027	.071	.011	.000	.032	.017	.224	.226
5	.000	.000	.000	.021	.000	.000	.035	.017	.029
GOT A									
1	.950	.952	.973	1.000	.980	1.000	.948	.983	1.000
3	.000	.000	.006	.000	.020	.000	.052	.017	.000
4	.050	.038	.022	.000	.000	.000	.000	.000	.000
GOT B									
1	.850	.769	.835	.774	.680	.881	.552	.569	.520
2	.150	.204	.121	.221	.320	.111	.431	.328	.373
3	.000	.027	.044	.000	.000	.008	.017	.103	.108
4	.000	.000	.000	.005	.000	.000	.000	.000	.000
DIA									
1	.975	.855	.879	.922	.960	1.000	.948	.914	.824
2	.000	.059	.033	.037	.040	.000	.052	.086	.118
3	.000	.048	.044	.021	.000	.000	.000	.000	.059
4	.025	.038	.044	.016	.000	.000	.000	.000	.000
5	.000	.000	.000	.005	.000	.000	.000	.000	.000
GDH									
1	.188	.522	.608	.447	.560	.619	.707	.672	.755
2	.550	.441	.352	.474	.420	.278	.293	.328	.245
3	.262	.016	.006	.053	.020	.103	.000	.000	.000
4	.000	.022	.034	.026	.000	.000	.000	.000	.000
ADH									
1	.014	.048	.050	.065	.020	.111	.630	.586	.750
2	.943	.876	.922	.919	.980	.881	.370	.414	.250
3	.043	.005	.011	.000	.000	.000	.000	.000	.000
4	.000	.065	.017	.016	.000	.000	.000	.000	.000
5	.000	.005	.000	.000	.000	.008	.000	.000	.000
ShDH A									
1	.231	.677	.687	.469	.460	.516	.828	.897	.824
2	.744	.226	.291	.464	.540	.484	.103	.086	.118
3	.000	.005	.006	.005	.000	.000	.052	.000	.059
5	.026	.086	.006	.063	.000	.000	.017	.017	.000
7	.000	.005	.011	.000	.000	.000	.000	.000	.000
ShDH B									
1	.910	.995	.929	.927	1.000	.968	.946	.966	.775
2	.090	.000	.022	.005	.000	.000	.054	.035	.226
3	.000	.005	.050	.068	.000	.032	.000	.000	.000
MDH A									
1	.963	.925	.989	1.000	1.000	.985	1.000	.897	.990
2	.038	.075	.011	.000	.000	.016	.000	.103	.010
MDH C									
1	.736	.258	.187	.255	.060	.121	.643	.655	.745
2	.264	.742	.813	.745	.940	.879	.357	.345	.255

Some of the studied loci (GOT A, DIA, ShDH B and MDH A) were monomorphic in populations kd, pt, pw (*P. mugo*) and sz, bk (*P. sylvestris*). The polymorphic loci at which distributions of allelic and genotypic frequencies differentiated three pine taxa were GDH and ShDH A.

For example, in the *P. uliginosa* population the frequency of GDH 3 allele was 0.263, while in *P. mugo* populations it amounted to 0.006-0.103. In the populations of *P. sylvestris* this allele was not found. GDH 33 genotype occurred with relatively high frequency (0.1), only in *P. uliginosa* popula-

TABLE 3. Frequencies of genotypes in nine studied pine populations.

Loci/ Alleles	Pinus uliginosa	<i>Pinus mugo</i> populations from Tatra Mts.			<i>P. mugo</i> population from peat-bogs		<i>P. sylvestris</i> populations		
	ul	kb	kc	kd	pw	pt	bk	cz	sz
FEst									
11	.725	.699	.648	.737	.960	.825	.793	.483	.471
12	.250	.161	.198	.168	.040	.111	.069	.069	.039
22	.000	.086	.011	.032	.000	.000	.035	.000	.000
13	.025	.054	.121	.021	.000	.063	.035	.310	.431
23	.000	.000	.022	.022	.000	.000	.000	.069	.000
33	.000	.000	.000	.000	.000	.000	.000	.035	.000
15	.000	.000	.000	.000	.000	.000	.069	.000	.039
25	.000	.000	.000	.000	.000	.000	.000	.035	.000
35	.000	.000	.000	.000	.000	.000	.000	.000	.020
GOT A									
11	.900	.936	.945	1.000	.960	1.000	.897	.966	1.000
13	.000	.000	.011	.000	.040	.000	.103	.035	.000
14	.100	.032	.044	.000	.000	.000	.000	.000	.000
44	.000	.022	.000	.000	.000	.000	.000	.000	.000
GOT B									
11	.750	.570	.692	.611	.400	.778	.379	.207	.275
12	.200	.344	.198	.326	.560	.206	.310	.552	.373
13	.000	.054	.088	.000	.000	.000	.035	.172	.118
22	.050	.032	.022	.053	.040	.000	.276	.035	.137
23	.000	.000	.000	.000	.000	.016	.000	.035	.098
24	.000	.000	.000	.011	.000	.000	.000	.000	.000
DIA									
11	.950	.720	.780	.854	.920	1.000	.897	.828	.647
12	.000	.118	.044	.052	.080	.000	.103	.172	.235
13	.000	.097	.066	.042	.000	.000	.000	.000	.118
14	.050	.054	.088	.031	.000	.000	.000	.000	.000
15	.000	.000	.000	.010	.000	.000	.000	.000	.000
22	.000	.000	.011	.010	.000	.000	.000	.000	.000
33	.000	.000	.011	.000	.000	.000	.000	.000	.000
44	.000	.011	.000	.000	.000	.000	.000	.000	.000
GDH									
11	.025	.269	.398	.221	.360	.429	.517	.517	.588
12	.175	.473	.409	.400	.400	.270	.379	.310	.333
13	.150	.032	.011	.053	.000	.111	.000	.000	.000
22	.375	.204	.148	.221	.200	.095	.103	.172	.078
23	.175	.000	.000	.053	.040	.095	.000	.000	.000
24	.000	.000	.000	.053	.000	.000	.000	.000	.000
44	.000	.022	.034	.000	.000	.000	.000	.000	.000
33	.100	.000	.000	.000	.000	.000	.000	.000	.000
ADH									
11	.000	.011	.000	.000	.000	.000	.259	.310	.563
12	.029	.054	.100	.129	.040	.222	.741	.552	.375
13	.000	.000	.000	.000	.000	.000	.000	.000	.000
14	.000	.011	.000	.000	.000	.000	.000	.000	.000
15	.000	.011	.000	.000	.000	.000	.000	.000	.000
22	.914	.785	.856	.850	.960	.762	.000	.138	.062
23	.029	.011	.022	.000	.000	.000	.000	.000	.000
24	.000	.118	.011	.011	.000	.000	.000	.000	.000
25	.000	.000	.000	.000	.000	.016	.000	.000	.000
44	.000	.000	.011	.011	.000	.000	.000	.000	.000
33	.029	.000	.000	.000	.000	.000	.000	.000	.000
ShDH A									
11	.077	.473	.484	.260	.240	.270	.690	.793	.647
12	.282	.290	.374	.354	.440	.492	.138	.172	.235
13	.000	.000	.011	.000	.000	.000	.103	.000	.118
15	.026	.108	.000	.063	.000	.000	.035	.035	.000
17	.000	.011	.022	.000	.000	.000	.000	.000	.000
22	.590	.043	.099	.250	.320	.320	.035	.000	.000
23	.000	.011	.000	.010	.000	.000	.000	.000	.000
25	.026	.065	.011	.063	.000	.000	.000	.000	.000
35	.000	.000	.000	.000	.000	.000	.000	.000	.000
ShDH B									
11	.821	.989	.868	.865	1.000	.937	.893	.931	.628
12	.180	.000	.033	.010	.000	.000	.107	.069	.294
13	.000	.011	.088	.115	.000	.063	.000	.000	.000
22	.000	.000	.000	.000	.000	.000	.000	.000	.078
23	.000	.000	.011	.000	.000	.000	.000	.000	.000
33	.000	.000	.000	.010	.000	.000	.000	.000	.000
MDH A									
11	.925	.860	.978	1.000	1.000	.968	1.000	.793	.980
12	.075	.129	.022	.000	.000	.032	.000	.207	.020
22	.000	.011	.000	.000	.000	.000	.000	.000	.000
MDH C									
11	.611	.075	.033	.075	.000	.000	.464	.448	.529
12	.250	.366	.308	.365	.120	.242	.357	.414	.431
22	.139	.559	.659	.563	.880	.758	.179	.138	.039

tion. Frequency of the allele GDH 1 in this population was about three to four times lower than in the studied populations of *P. mugo* and *P. sylvestris*. The frequency of allele GDH 2 was in *P. uliginosa* population higher than in the Scots pine and dwarf mountain pine populations (Table 2).

At ShDH A locus, frequency of allele ShDH A1 in *P. uliginosa* population was two- to threefold lower in comparison to *P. mugo* populations and almost fourfold lower when *P. sylvestris* populations were taken into consideration. The frequency of the ShDH A2 in this population was, on average, about 200% higher than in dwarf mountain pine populations and as much as 700% higher in Scots pine populations. *P. uliginosa* was also different in showing the low frequency of ShDH A11 (0.077) and high frequency of ShDH A22 (0.59) homozygotes, as compared to *P. mugo* and *P. sylvestris* groups of individuals.

Polymorphic loci at which the allelic and genotypic frequencies differentiated *P. mugo* and *P. sylvestris* species involved FesT, GOT B and MDH C. *P. uliginosa* was similar in these frequencies to *P. mugo* at FesT and GOT B loci, and in locus MDH C to *P. sylvestris*.

The differences between the analyzed pine populations could be observed also in frequencies of rare alleles and genotypes, e.g., allele GOT A4 was restricted to *P. uliginosa* and two *P. mugo* populations and DIA 4 – to *P. uliginosa* and three *P. mugo* ones. ShDH B3 allele was observed only in four dwarf mountain pine populations (see Table 2).

Table 4 presents the number of alleles and genotypes per locus in the studied pine populations. The *P. uliginosa* population exhibited the same number of alleles per locus (2.4) as the Scots pine populations.

Within the studied dwarf mountain pine populations this number fluctuated with the habitat. *P. mugo* populations from peat-bogs had lower number of alleles per locus (1.9 and 2.2), than the rest of dwarf mountain pine populations, in which

TABLE 4. Mean number of alleles (A/L) and genotypes (G/L) per locus in the studied populations of *Pinus uliginosa* (ul), *P. mugo* (kb-pt) and *P. sylvestris* (bk-sz).

Population	A/L	G/L
ul	2.4	3.2
kb	3.2	4.3
kc	3.3	4.3
kd	3.0	4.0
pw	1.9	2.2
pt	2.2	2.5
bk	2.4	2.9
cz	2.4	3.1
sz	2.4	3.1

the highest number (≥ 3.0) of alleles per locus was noted among all the investigated populations.

Similarly to the number of alleles per locus, the number of genotypes was the highest in some dwarf mountain pine populations (≥ 4.0) and the lowest in populations of this species growing on peat-bogs (2.2 and 2.5). The *P. uliginosa* population was characterized by a similar to Scots pine number of genotypes per locus (3.2).

Genetic differentiation

The mean heterozygosity (for ten enzymatic loci) – H_o , Wright's fixation index (F) and genotypic polymorphism (P_g) in the studied *P. uliginosa*, *P. mugo* and *P. sylvestris* populations are shown in Fig. 2. The most heterozygotic were two populations of *P. sylvestris* (sz- $H_o = 0.326$ and cz- $H_o =$

Fig. 2. Some genetic parameters of the studied pine populations.

0.321), and among the *P. mugo* populations high heterozygosity was noted for population kb ($H_0 = 0.261$). The lowest values of this coefficient ($H_0 < 0.2$) were noted in two dwarf mountain pine populations from peat-bogs. The mean heterozygosity of *P. uliginosa* population ($H_0 = 0.202$), was within the range of values observed for *P. mugo* populations. The values of genotypic polymorphism indices (P_g) fluctuated in a similar way as did heterozygosity in the analyzed pine populations.

Except *P. uliginosa* population, where a significant excess of homozygotes was noted, all studied *P. mugo* and *P. sylvestris* populations were close to the Hardy-Weinberg equilibrium as indicated by the fixation indices (F) (Fig. 2).

Genetic differentiation (G_{ST}) between five dwarf mountain pine populations for 10 analyzed loci was 3.3% and between three Scots pine ones – 2.1% (Table 5). After including the population of *P. uliginosa* to the group of *P. mugo* populations ($G_{ST} = 3.3\%$) the average G_{ST} value for the entire group increased to 8.6%. This was caused mainly by high values of G_{ST} at GDH, ShDH A and MDH C loci.

Genetic distances and similarities

The dendrograms, constructed using Nei's genetic distances (based on allele frequencies) as well as based on genotype frequencies acc. to Hedrick's, showed that analyzed populations formed two distinct groups (Fig. 3 and 4). The first group comprised dwarf mountain pine populations and the second consisted of Scots pine ones. The population of *P. uliginosa* was loosely connected with the *P. mugo* group. Nei's genetic distance between *P. mugo* and *P. uliginosa* amounted to 0.0813 and the distance between *P. sylvestris* and *P. mugo* was 0.1484.

Fig. 3. Dendrogram constructed on the base of Nei's genetic distances (1972) showing similarities between the population of *P. uliginosa* (ul) and populations of *P. mugo* (kb, kc, kd, pw, pt) and *P. sylvestris* (bk, cz, sz).

TABLE 5. Genetic differentiation acc.to Nei (1973) between populations (G_{ST}) of *Pinus mugo* (K), *P. mugo* with *P. uliginosa* (Kul) and *P. sylvestris* (S).

Locus	G_{ST} (K) %	G_{ST} (Kul) %	G_{ST} (S) %
FEst	3.4	2.9	4.6
GOT A	1.5	1.8	2.1
GOT B	3.1	3.0	0.7
DIA	2.7	3.0	2.2
GDH	1.9	6.5	0.6
ADH	1.8	2.0	2.1
ShDH A	5.0	10.0	0.8
ShDH B	2.4	3.4	7.9
MDH A	3.9	3.1	6.0
MDH C	4.1	24.6	1.0
Mean	3.3	8.6	2.1

DISCUSSION AND CONCLUSIONS

Previous investigations of population of the *P. uliginosa* from Wielkie Torfowiska Batorowskie (*locus classicus*) with reference to *P. mugo* and *P. sylvestris* have shown that the most distinct species were *P. mugo* and *P. sylvestris*. The peat bog pine (*P. uliginosa*) is different from the two taxa, but shows greater similarity to *P. mugo* than to *P. sylvestris* (Szweykowski 1969, Szweykowski and Bobowicz 1977, Prus-Głowacki et al. 1978, 1981; Prus-Głowacki and Szweykowski 1979, 1983). The results presented in this paper are in agree-

Fig. 4. Dendrogram based on Hedrick's genetic distances showing similarities between population of *P. uliginosa* (ul) and the populations of *P. mugo* (kb, kc, kd, pw, pt) and *P. sylvestris* (bk, cz, sz).

ment with the conclusions of the authors mentioned above. Our studies have not confirmed or excluded the hybrid origin of *P. uliginosa*. At the two enzymatic loci, GDH and ShDH A, we found that there are clear differences in distributions of allelic and genotypic frequencies between the three investigated pine taxa (Tables 2 and 3). The rare allele, GOT A4, which appeared only in the studied *P. mugo* populations has been found also in the group of trees of *P. uliginosa* with a twofold frequency. This allele was not observed in *P. sylvestris* populations from Europe and can be considered a species specific marker for *P. mugo* (Prus-Głowacki et al. 1993, Prus-Głowacki and Stephan 1994, Prus-Głowacki and Bernard 1994). At the same time, the distribution of allelic and genotypic frequencies at MDH C locus in the *P. uliginosa* population has been similar to those noted in the studied populations of *P. sylvestris*.

This phenomenon can support the hypothesis of hybrid origin of *P. uliginosa*, or introgression of the genes of both taxa to this population.

The significant excess of homozygosity ($F = 0.144$) in the population of *P. uliginosa*, which has not been noted in the other studied pine populations, could have been created by selection pressure in the specific habitat of this pine or by inbreeding processes and restricted gene flow since the nearest population of this taxon is situated ca 15 km from the Wielkie Torfowisko Batorowskie.

If the studied *P. uliginosa* population is the marginal population of *P. uncinata*, as suggested by Krzakowa et al. (1984), the deviation from Hardy-Weinberg equilibrium could have been caused by founder effect or genetic drift, often observed in isolated, marginal populations (Yeh and Layton 1979, Babel and Selander 1974, Nei 1987).

The genetic distinct character of *P. uliginosa* in relation to *P. mugo* could be also evaluated by the coefficients of G_{ST} , presented in Table 5, showing the genetic differentiation of populations. After including the *P. uliginosa* population to calculations, the G_{ST} index increased from 3.3% to 8.6%. This has been caused mainly by loci of MDH C (4.0% $\Rightarrow$ 24.6%), ShDH A (5.0% $\Rightarrow$ 10.0%) and also GDH (1.9% $\Rightarrow$ 6.5%) (see Table 5).

Both the dendrogram constructed on the ground of frequencies of alleles (Nei 1972) and that based on frequencies of genotypes (Hedrick 1974) have shown that *P. uliginosa* is connected with the group formed by *P. mugo* populations (second group consists only of *P. sylvestris* populations) (Figs 3 and 4). However, Nei's genetic distance between them ($D_N = 0.0813$) is relatively high and approximates to the lower values of limit of genetic distances observed between species estimated by Nei (1976) as 0.1-0.2.

Nei's genetic distance coefficients may be used to estimate the approximate time of divergence of the taxa. According to the formula $t = 7.4 \times 10^5 \times D_N$ (where t is the time of the divergence, 7.4×10^5 is the proportion of aminoacid substitutions which can be detected by electrophoresis, D_N is the genetic distance acc.to Nei (1971), the time of divergence of *P. uliginosa* (*P. uncinata* ?) and *P. mugo* is roughly 60000 years B.P. and the time of divergence of and *P. mugo* and *P. sylvestris* is about 100000 years ago. If these calculations are based on correct assumptions, *P. uliginosa* (*P. uncinata* ?) has separated by hybridization process or by geographic isolation from the common ancestor during the pleistocene glaciation period, when the conditions for speciation connected with migrations of flora and isolations in glacial refuges, were especially favourable.

Nevertheless, further comparative studies including additional isoenzymatic or DNA markers are necessary to clarify the origin and taxonomic status of *P. uliginosa*.

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STRUKTURA GENETYCZNA ORAZ STANOWISKO TAKSONOMICZNE POPULACJI *PINUS ULIGINOSA* NEUMANN Z WIELKIEGO TORFOWISKA BATOROWSKIEGO W GÓRACH STOŁOWYCH (*LOCUS CLASSICUS*)

STRESZCZENIE

Przy pomocy metod izoenzymatycznych badano strukturę genetyczną populacji *Pinus uliginosa* Neumann z Wielkiego Torfowiska Batorowskiego (*locus classicus*) porównując ją z populacjami *P. mugo* Turra z Tatr i *P. sylvestris* z Kotliny Kłodzkiej i Czerska. Badania wykazały, że poziom zmienności tej populacji (heterozygotyczność, polimorfizm genotypowy) jest podobny do obserwowanego w populacjach *P. mugo* przy nadmiarze homozygot w stosunku do wartości oczekiwanych przy równowadze Hardy-Weinberga. Średnia liczba alleli na locus jest taka sama jak w populacjach *P. sylvestris*, a średnia liczba genotypów nieco wyższa niż u *P. sylvestris*, a niższa niż w populacjach *P. mugo* z Tatr ze stanowisk górskich. Populacje torfowiskowe *P. mugo* wykazują niższą liczbę alleli i genotypów na locus w stosunku do badanej populacji *P. uliginosa*. Podobieństwa genetyczne wg Nei'a i Hedrick'a wykazują zdecydowaną odrębność *P. uliginosa* od *P. sylvestris* i luźne powiązanie tego taksonu z *P. mugo*. Współczynnik zróżnicowania genetycznego (G_{ST}) także wskazuje na specyficzność tej populacji. Przeprowadzone badania nie potwierdziły, ani nie wykluczyły mieszańcowego pochodzenia *P. uliginosa*.

SŁOWA KLUCZOWE: *Pinus uliginosa*, struktura genetyczna, taksonomia, izoenzymy.