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Andrzej Lewandowski, Joanna Samoćko,
Krystyna Boratyńska, Adam Boratyński

Genetic differences between two Polish populations of *Pinus uliginosa*, compared to *P. sylvestris* and *P. mugo*

Abstract: Genetic differences between two populations of *P. uliginosa* from Batorów and Węgliniec were assessed on the basis of 15 allozyme loci. The level of genetic differentiation between them was also compared with genetic differences among the three closely related pine taxa: *P. uliginosa*, *P. sylvestris* and *P. mugo*. A high level of genetic variation was found in both populations of *P. uliginosa*. The average (Na) and effective (Ne) numbers of alleles per locus amounted respectively to 2.47 and 1.50 in Węgliniec and to 2.67 and 1.52 in Batorów and the percentage of polymorphic loci was 80% and 87%, respectively. Close relationship between the three studied species were confirmed. The genetic differences between the two populations of *P. uliginosa* were substantial, as the Nei's genetic distance between the two populations (D = 0.040) was larger than between populations of *P. sylvestris* and between populations of *P. mugo*. The relatively high level of genetic differentiation between *P. uliginosa* populations may result from their isolation, small size and possibly different origin of these populations.

Additional key words: Peat-bog pine, allozymes, electrophoresis, genetic distance, *Pinaceae*

Address: A. Lewandowski, J. Samoćko, K. Boratyńska, A. Boratyński. Polish Academy of Sciences, Institute of Dendrology, 62-035 Kórnik, Poland, e-mail: alew@man.poznan.pl

Introduction

Peat-bog pine (*Pinus uliginosa* Neumann) was described for the first time in 1837, from Batorów in the Sudety Mts. It is one of four native pines in Poland. Despite of intensive research, its taxonomic rank has not been fully explained (Staszkiewicz and Tyszkiewicz 1972; Krzakowa et al. 1984; Christensen 1987; Siedlewska and Prus-Głowacki 1994; Lauranson-Broyer et al. 1997; Prus-Głowacki et al. 1998; Lewandowski et al. 2000). *P. uliginosa* reaches in Poland the northern limit of its natural range, and usually forms small isolated populations on peatland in the southern part of the country (Boratyński 1994). As a threatened species it has been included in the Polish Plant Red Data Book (Zarzycki and Kaźmierczakowa 1993).

Some of the best-known localities of Peat-bog pine are the nature reserves at Batorów and Węgliniec. Both populations are small and threatened with extinction because of lack of natural regeneration and the gradual decline of older specimens. There are still about 400 individuals at Batorów and nearly 100 at Węgliniec. Some populations of this species (e.g. at Węgliniec) are in the immediate neighbourhood of *P. sylvestris* and thus theoretically exposed to a constant inflow of its genes through pollen, especially that phenological observations revealed only slight differences in periods of macro- and microstrobils development of the two species (Boratyński et al., data not published).

The aim of this study was to assess the genetic differences between two populations of *P. uliginosa* from

Batorów and Węgliniec on the basis of allozyme markers. The level of their genetic differentiation was then compared with genetic differences between the three closely related pine taxa: *P. uliginosa*, *P. sylvestris* and *P. mugo*.

Materials and methods

Plant material

The material for the present study included seeds collected in one population of *P. uliginosa* from Węgliniec, two populations of *Pinus sylvestris* L. (from Krotoszyn and Węgliniec in south-western Poland) and two populations of *P. mugo* Turra (one from Kopa in the Sudety Mts. and the other from Chornohora in the Ukrainian Carpathians). Because of the lack of cones in the second population of *P. uliginosa* from Batorów dormant winter buds were collected. Detailed data on geographical location of populations and numbers of examined individuals are presented in Table 1.

Electrophoretic analysis

Individual trees were genotyped the basis of 8 to10 megagametophytes or, in the case of *P. uliginosa* from Batorów, extracts from 2 buds. The following 9 enzyme systems encoded by 15 loci were studied (Enzyme Commission numbers and locus abbreviations in parentheses): fluorescent esterase (EC 3.1.1.2; Fle-1), glutamate dehydrogenase (EC 1.4.1.2; Gdh), glutamate oxalo-acetate transaminase (EC 2.6.1.1; Got-1, Got-2, Got-3), isocitrate dehydrogenase (EC 1.1.1.42; Idh), leucine aminopeptidase (EC 3.4.11.1; Lap-1, Lap-2), malate dehydrogenase (EC 1.1.1.37; Mdh-1, Mdh-3), 6-phosphogluconate dehydrogenase (EC 1.1.1.44; 6Pgd-1, 6Pgd-2), phosphoglucomutase (EC 2.7.5.1; Pgm-1, Pgm-2), and superoxide dismutase (EC 1.15.1.1; Sod-1). The separation of isoenzymes on starch gels and the genetic interpretation of the results were performed according to Rudin and Ekberg (1978), Szmidt and Yazdani (1984) and Goncharenko et al. (1994). Alleles at each locus were numbered according to the electrophoretic migration of allozymes. The most anodally migrating band was named 1, the next 2, and so on.

Statistical methods

Genetic variation was described by the average (Na) and effective (Ne) numbers of alleles per locus, the percentage of polymorphic loci P (95% criterion) and expected heterozygosity (He) (Nei 1975). Wright`s fixation index (F) was estimated to measure the proportional extend of inbreeding using the formula: F = 1-Ho/He. Genetic differences between populations were measured by the genetic distance index D of Nei (1975). In order visualize genetic relationships between populations, matrices of D value were used to cluster populations by the UPGMA method (Sneath and Sokal 1973).

Results

Table 2 presents the frequencies of alleles in the studied loci for all populations. Out of 15 loci analysed in six populations, 13 were polymorphic in at least one population, and two loci were completely monomorphic (Idh and Sod-1). The most common alleles were the same in all six populations analysed at 10 polymorphic loci (Fle-1, Got-1, Got-2, Got-3, Lap-1, Lap-2, Mdh-1, 6-Pgd-1, Pgm-1 and Pgm-2), independent of the investigated taxa. The largest differences in allele frequency between the studied species were found in Mdh-3 and 6Pgd-2. The most frequent allele at locus Mdh-3 for *P. uliginosa* was allele 4. Its average frequency was 0.589 in that species, 0.264 in *P. sylvestris*, and 0.791 in *P. mugo*. Similarly, the frequency of allele 3 at 6Pgd-2 was low in *P. sylvestris*, moderate in *P. uliginosa* and high in *P. mugo*.

A summary of genetic variability measures at 15 loci for the analysed populations are given in Table 3. Generaly, a high and similar level of allozyme variation was observed in both Peat-bog pine populations. The average (Na) and effective (Ne) numbers of alleles per locus in the population from Batorów were 2.67 and 1.52, respectively, and 86.7% of loci were polymorphic. In Węgliniec, the respective values were: Na = 2.47, Ne = 1.50 and P = 80%. A slightly lower level of genetic variation was observed in *P. sylvestris*, where mean values were: Na = 2.44, Ne = 1.51 and P = 76.7%. The lowest values were recorded in *P. mugo*: Na = 2.43, Ne = 1.35, P = 73.4%. Ex-

Table 1. Origin and size of samples used for allozyme analyses

Taxa	Pop. No.	Total no. of trees	Stand	Localization
Pinus uliginosa	1	56	Węgliniec (WEG)	51°41'N, 17°15'E
	2	47	Batorów (BAT)	50°28'N, 16°23'E
Pinus sylvestris	3	30	Węgliniec (WEG)	51°41'N, 17°15'E
	4	50	Krotoszyn (KRO)	51°17'N, 15°14'E
Pinus mugo	5	41	Kopa (KOP)	50°45'N, 15°44'E
	6	39	Czarnohora (CZA)	48°10'N, 24°40'E

Table 2. Frequencies of alleles in the investigated populations

Locus	Population					
	P. uliginosa		*P. sylvestris*		*P. mugo*	
	1. WEG	2. BAT	3. WEG	4. KRO	5. KOP	6. CZA
Fle-1						
1	0.009	0.022	0	0.020	0.049	0.026
2	0.607	0.797	0.684	0.710	0.792	0.577
3	0.036	0.096	0.133	0.070	0.159	0.295
4	0.348	0.085	0.183	0.200	0	0.102
Gdh						
1	0.036	0.053	0.167	0	0.122	0.269
2	0.196	0.426	0.233	0.360	0.171	0.385
3	0.768	0.266	0.600	0.640	0.707	0.346
4	0	0.255	0	0	0	0
Got-1						
1	0.027	0.011	0.133	0.010	0.024	0
2	0.714	0.842	0.717	0.990	0.890	1
3	0.259	0.138	0.150		0.086	0
Got-2						
1	0	0.011	0	0	0	0
2	0.009	0	0.050	0.020	0	0.077
3	0.429	0.340	0.450	0.320	0.171	0.103
4	0.562	0.649	0.483	0.650	0.817	0.820
5	0	0	0	0.010	0	0
6	0	0	0.017	0	0.012	0
Got-3						
1	0	0	0	0	0	0
2	0.188	0.117	0.333	0.370	0.098	0.141
3	0	0.011	0	0	0	0.038
4	0.812	0.872	0.667	0.630	0.902	0.821
Idh						
1	1	1	1	1	1	1
Lap-1						
1	0	0	0	0.010	0	0
2	1	0.957	1	0.960	0.927	0.782
3	0	0.043	0	0.030	0.073	0.218
Lap-2						
1	0	0	0	0.040	0.024	0.025
2	0.964	0.894	0.863	0.890	0.927	0.949
3	0.036	0.106	0.137	0.050	0.037	0.013
4	0	0	0	0.020	0.012	0.013
Mdh-1						
1	0.098	0.085	0.067	0.060	0.012	0
2	0.893	0.894	0.933	0.940	0.964	1
3	0.009	0.021	0	0	0.024	0
Mdh-3						
1	0	0	0	0.010	0	0
2	0.429	0.394	0.733	0.730	0.354	0.026
3	0	0	0	0	0	0.038
4	0.571	0.606	0.267	0.260	0.646	0.936

Locus	Population					
	P. uliginosa		*P. sylvestris*		*P. mugo*	
	1. WEG	2. BAT	3. WEG	4. KRO	5. KOP	6. CZA
6Pgd-1						
1	0	0.011	0	0	0.012	0.039
2	0	0.011	0	0.010	0	0.179
3	0.812	0.872	0.517	0.530	0.976	0.769
4	0.170	0.106	0.450	0.450	0.012	0.013
5	0.018	0	0.033	0.010	0	0
6Pgd-2						
1	0.330	0.319	0.666	0.700	0.012	0
2	0.018	0	0	0	0	0.013
3	0.652	0.681	0.317	0.290	0.988	0.987
4	0	0	0.017	0.010	0	0
Pgm-1						
1	0	0.053	0.017	0.030	0	0
2	0.982	0.947	0.983	0.940	1	1
3	0.009	0	0	0.020	0	0
4	0.009	0	0	0.010	0	0
Pgm-2						
1	0.009	0.330	0	0	0.049	0.192
2	0.536	0.628	1	1	0.841	0.680
3	0.455	0.042	0	0	0.110	0.128
Sod-1						
1	1	1	1	1	1	1

pected heterozygosity (He) also had high, very similar values in both *P. uliginosa* populations: 0.278 in Węgliniec and 0.279 in Batorów. Among the remaining populations, only the one of *P. sylvestris* from Węgliniec had a slightly higher heterozygosity (He = 0.282). Both populations of *P. mugo* had distinctly lower He values: 0.175 and 0.220. Two populations had an excess of homozygotes, according to the Hardy-Weinberg equilibrium. In the population of *P. uliginosa* from Batorów the excess amounted to 4%, and in the population of *P. mugo* from Chornohora it amounted to 7% (Tab. 3).

Table 4 shows the values of Nei's genetic distance (D) separating the studied populations. The mean value for all populations was 0.064. The smallest distance (D = 0.010) was found between the pair of populations of *P. sylvestris*, and the largest (D = 0.140) between *P. sylvestris* from Węgliniec and *P. mugo* from Chornohora. The pair of populations of *P. sylvestris* was the most similar to each other (D = 0.010), the pair of populations of *P. mugo* was slightly less similar (D = 0.028), and the pair of populations of *P. uliginosa* (D = 0.040) was the least similar. Considering interspecific differences, the smallest distance separated *P. uliginosa* from *P. mugo*, and the distance between *P. uliginosa* and *P. sylvestris* was slightly larger (D = 0.063). The largest distance (D = 0.113) was between *P. sylvestris* from *P. mugo*.

Discussion

The high level of genetic variation in the studied *Pinus* populations is consistent with earlier reports (Gulberg et al. 1985; Mejnartowicz and Bergmann 1985; Wang et al. 1991; Goncharenko et al. 1994; Neet-Sarqueda 1994; Prus-Głowacki and Stephan 1994; Siedlewska and Prus-Głowacki 1994; Prus-Głowacki et al. 1998; Lewandowski et al. 2000; Odrzykoski 2002). It is noteworthy that both populations of *P. uliginosa* preserved a high level of genetic variation in spite of their isolation and small size. The level of genetic variation in *P. uliginosa* is comparable with the level of genetic variation in *P. sylvestris*, which is considered to be the most variable coniferous trees in Europe (Müller-Starck 1992).

Results of this study confirm the close relations between the three investigated *Pinus* species, and indicate that *P. uliginosa* is closer to *P. mugo* than to *P. sylvestris*. Similar results were earlier obtained with the use of isoenzymatic markers by Siedlewska and Prus-Głowacki (1995), and Prus-Głowacki et al. (1998). However, those studies and our results do not allow to confirm or reject the hypothesis that *P. uliginosa* is an old hybrid taxon between *P. sylvestris* with *P. mugo*. Our earlier investigation in populations from the boundary of the natural range of *P. sylvestris* and *P. mugo* provided less ambiguous evidence, attest-

Table 3. Genetic variability at 15 loci in the investigated populations (stand deviations in parentheses)

Taxa/population	Na	Ne	P	He	F
P. uliginosa					
1. WEG	2.47 (0.92)	1.50 (0.42)	80.0	0.278 (0.208)	–0.02
2. BAT	2.67 (1.04)	1.52 (0.55)	86.7	0.279 (0.055)	0.04
Pinus sylvestris					
3. WEG	2.20 (0.94)	1.54 (0.50)	73.3	0.282 (0.233)	–0.01
4. KRO	2.67 (1.18)	1.44 (0.41)	80.0	0.248 (0.208)	–0.05
P. mugo					
5. KOP	2.40 (0.91)	1.26 (0.29)	80.0	0.175 (0.160)	0
6. CZA	2.47 (1.19)	1.43 (0.58)	66.7	0.220 (0.231)	0.07

Table 4. Matrix of Nei's genetic distance coefficients between the studied populations (above diagonal) and the taxa (below diagonal)

Taxa/pop.	*P. uliginosa*		*P. sylvestris*		*P. mugo*	
	1. WEG	2. BAT	3. WEG	4. KRO	5. KOP	6. CZA
P. uliginosa						
1. WEG	–	0.040	0.056	0.063	0.038	0.072
2. BAT		–	0.067	0.067	0.032	0.043
P. sylvestris	0.063					
3. WEG			–	0.010	0.090	0.140
4. KRO				–	0.088	0.132
P. mugo	0.046		0.113			
5. KOP					–	0.028
6. CZA						–

ing to the hybrid origin of *Pinus uliginosa* (Lewandowski et al 2000).

Considerable genetic differences were observed between the two populations of *P. uliginosa*. Nei's genetic distance between them was larger than between populations of *P. sylvestris* or *P. mugo*. In contrast, the geographical distance between the populations of *P. uliginosa* (Batorów–Węgliniec) was similar to that between populations of *P. sylvestris* (Krotoszyn–Węgliniec), but much smaller than between populations of *P. mugo* (Sudety Mts. – Ukrainian Carpathians). Moreover, both studied populations of *P. uliginosa* were less similar to each other than to the population of *P. mugo* from the Sudety Mts.. Nevertheless, mean genetic distances between species were always larger than distances between populations of the same species. Relations between the studied populations are illustrated in a dendrogram (Fig. 1). The two populations of *P. uliginosa* differed also in many morphological and anatomical characters (Boratyńska et al., data not published).

The large differences between populations of *P. uliginosa* may result from their isolation and small size. Consequently, the differences may be caused by genetic drift or more likely founder effect. This seems to be confirmed by the frequency of alleles at locus Pgm-2. In both populations the most frequent is allele 2, which frequency amounts to 0.536 at Węgliniec and 0.628 at Batorów. In both populations two other alleles also occur (Table 2). Allele 1 is rare in Węgliniec (0.009) but quite common in Batorów (0.330), and allele 3, which is common in Węgliniec (0.455), and rare in Batorów (0.042). Prus-Głowacki et al. (1998) also claim that the specific genetic structure of the population of *P. uliginosa* at Batorów is due to its isolation. The differences between populations of *P. uliginosa* may also result from an inflow of genes of *P. sylvestris*. Phenological observations (Boratyński et al., data not published) indicate that the periods of flowering in the two species overlap, so there may be some exchange of genes between those species. If this is true, one might expect that the population of *P.*

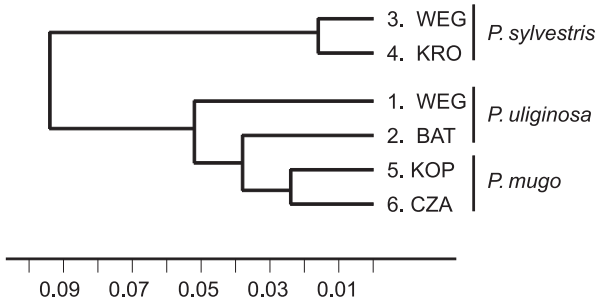

Fig. 1. UPGMA dendrogram based on Nei's genetic distances

uliginosa at Węgliniec should be more similar to the neighbouring population of *P. sylvestris* than to the isolated population of *P. uliginosa* at Batorów. In fact, the frequencies of three alleles (allele 4 at Mdh-3, allele 3 at 6Pgd-1, and allele 3 at 6Pgd-2) in Węgliniec slightly deviate towards those of *P. sylvestris* (Table 2). Similarly, a smaller genetic distance separates the two populations of *P. sylvestris* from the population of *P. uliginosa* from Węgliniec than from Batorów (Table 4). Nonetheless, the observed small deviation of allele frequencies of *P. uliginosa* towards *P. sylvestris* may be accidental, resulting from genetic drift or the founder effect. This is apparently confirmed by the distribution of alleles at locus Pgm-2.

It is interesting that there is an excess of heterozygotes in the population of *P. uliginosa* from Węgliniec, considering the Hardy-Weinberg equilibrium. By contrast, in such small and isolated populations one could expect to find an excess of homozygotes caused by inbreeding. Thus it can be concluded that there is some selection leading to elimination of homozygotes. In the population at Batorów, an excess of homozygotes is observed, but it reaches only 4%. A higher level of inbreeding was recorded in that population by Prus-Głowacki et al. (1998), who reported that the mean excess of homozygotes was as high as 15%. The differences in the estimated levels of inbreeding probably result from the fact that we analysed a different set of loci. Values of the inbreeding coefficient (F) usually had wide ranges of variation in different loci. For example, F values for the Batorów population ranged from –0.176 at locus Fle-1 to +0.671 at locus Mdh-1.

Because of the high level of genetic differentiation between the two studied populations of Peat-bog pine both populations should be strictly protected, to preserve genetic pool of this species.

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