BIOMETRICAL STUDY OF SOME INDIVIDUALS CHOSEN FROM *PINUS MUGO* TURRA POPULATIONS IN THE PEAT BOG "BÓR NA CZERWONEM"

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ABSTRACT

Sixteen individuals were sampled for study of variation in 17 anatomical and morphological characters. Only individuals of low polycormic growth (trait typical for *Pinus mugo* Turra) connected with incurved one-year-cone stipes (a similar situation exists in *Pinus sylvestris* L.) were chosen, thus the sample studied cannot be treated as a random one. It has been shown by multivariate statistical analysis that these 16 individuals are quite different from each other, Mahalanobis' generalized distances between them being nearly 50% significantly different from 0. The sample studied in this respect is distinctly different from pure stands of both putative parental species (i.e. *Pinus mugo* and *P. sylvestris*). Every plant studied shows a different combination of traits typical (or nearly typical) for both the above-mentioned species and traits that are truly intermediate between them. The results support the frequently expressed opinion that the mountain pine population from the peat bog "Bór na Czerwone" is, in fact, a hybrid swarm formed by hybridization between *Pinus mugo* and *Pinus sylvestris*.

KEY WORDS: *Pinus sylvestris* L., *Pinus mugo* Turra, *Pinus x rhaetica* Brügger, variability, hybridization, multivariate analysis.

INTRODUCTION

Isolated stands of *Pinus mugo* are located in a few places in the northern foothills of the Tatra Mts. and the Sudetes. Such stands are usually treated as being of postglacial age.

The peat bog "Bór na Czerwone" in the valley Kotlina Nowotarska (S Poland) at elevation 620m is inhabited by a population of *Pinus mugo* surrounded by a Scots Pine (*Pinus sylvestris*) forest. It has been described by various authors, e.g. Szwejkowski 1969, Staszkiewicz and Tyszkiewicz 1961, 1972, Bobowicz et al. 1990.

This population comprises besides typical forms of both *Pinus sylvestris* and *P. mugo*, also many intermediate individuals, which were identified by Staszkiewicz (1994) as *Pinus x rhaetica* Brügger.

It is feasible that these intermediate individuals were formed by intercrossing of both pines inhabiting the bog (i.e. *Pinus sylvestris* and *P. mugo*). Some of these putative hybrids could have intercrossed with each other and with one or both parental species resulting in a hybrid swarm (Bobowicz 1990). The main argument for such an interpretation is the intermediate status of many morphological and anatomical characters of needles, cones, bark and growth type. However, no direct proof of the hybrid nature of at least some intermediate individuals has been reported so far.

The population from the peat bog "Bór na Czerwone" is, as it seems, sufficiently known as far as the anatomical and morphological characters are concerned; however, its genetic

structure is practically unknown. According to unpublished results of an electrophoretic study of enzyme proteins by K. Kuśik, the genetic structure of the population in question differs from the genetic structure of a pure *Pinus mugo* stand from the Tatry Mts. (west slope of the peak called "Żółta Turnia") having a higher share of heterozygotes and a higher value of polymorphic index.

Dr. Ireneusz J. Odrzykoski (Dept. of Genetics, Adam Mickiewicz University in Poznań) undertook a study of chloroplast DNA to obtain more detailed information about the putative hybrid nature of the *Pinus mugo* plants inhabiting the bog "Bór na Czerwone". He sampled (in 1989) 16 individuals which were chosen according to only two criteria:

(i) Polycormic growth, as in typical *Pinus mugo* plants, and
(ii) the stipes of one-year-old cones were incurved toward the base, as is observed in typical *Pinus sylvestris* trees; however, the setting angle was usually intermediate between states typical for *Pinus sylvestris* and *Pinus mugo*.

Results of the chloroplast DNA study will be published separately. In the present paper results of an anatomical and morphological analysis of these 16 individuals is given.

MATERIAL AND METHODS

As has been said in the Introduction, samples of 16 arbitrarily chosen individuals (polycormic growth connected with incurved stipes of the one-year-old cones) collected by dr. I.J. Odrzykoski, were studied.

Two-year-old needles were preserved in 70% ethanol; hand-made cross-sections were made semi-permanent in polyvinyl alcohol; they were examined in respect of the following traits:

1. Number of stomatal rows on the abaxial (convex) side of the needle;
2. The same on the adaxial (flat) side of the needle;
3. Stomatal row index (value of trait # 1 divided by the value of the trait # 2);
4. Mean number of stomata in a 2 mm long section of the abaxial side of the needle;
5. The same on the adaxial side of the needle;
6. Number of resin canals;
7. Thickness of the epidermis on the adaxial side of the needle, in μm ;
8. Mean width of epidermal cells, measured as the average width of the 3 neighbouring cells, in μm ;
9. Width of the needle, in μm ; measured on cross-sections;
10. Thickness of the needle, in μm ; measured on cross-sections;
11. Thickness to width ratio (traits 10:9);
12. Distance between vascular bundles, in μm ;
13. Marcet's coefficient (values of traits # 9 x 12 divided by the value of the trait # 10, Marcet 1967);
14. Type of the sclerenchyma sheath around the resin canals, evaluated in three states: A, B, and C. Estimated according to Szweykowski 1968;
15. Type of the sclerenchyma lying between the vascular bundles, evaluated in four states: A, B, C, and D. Estimated according to Szweykowski 1989;
16. Angle between cone axis and cone stipe (= setting angle, in grades);
17. Growth type of the plant in question (polycormic, oligocormic or monocormic);

Values of characters nos. 1-13 were then processed statistically. The results obtained were then compared with four populations representing typical forms of the putative parental species. These were:

- A – I. A *Pinus sylvestris* sample from the vicinity of the village of Promno near Poznań – unpublished data were kindly supplied by professor M.A. Bobowicz; $n = 30$.
- B – II. A *Pinus sylvestris* sample from two nearly pure colonies of that tree on the peat bog "Bór na Czerwonym", growing on peaty, wet soil; $n = 30$; sampled by I. Odrzykoski.
- C – III. A *Pinus mugo* sample from a population growing on a peat bog in Polana Waksmundzka glade, eastern part of the Tatra Mts. elevation of 1365m; sampled by A. Bączkiewicz, W. Prus-Głowacki and J. Szweykowski.
- D – IV. A *Pinus mugo* sample from a population situated on a ridge called "Skupniów Upłaz", Tatra Mts., elevation 1490 m; $n = 30$; sampled by A. Bączkiewicz, W. Prus-Głowacki and J. Szweykowski.

The statistical analysis comprised the following procedures.

1. Discrimination analysis (Caliński, Kaczmarek 1973, 1975). 10 measurements of every trait (nos. 1-13) and of every individual plant were treated as one group, thus $n = 136$ (4 samples of typical populations of the putative parental species, $n = 30$ in each case, plus 16 plants studied).
2. Principal component analysis – here the samples of typical populations and the 16 plants studied were treated as one group, again n equalling 136.
3. Mahalanobis' generalized distances with Hotelling's T^2 (Caliński, Kaczmarek 1973) were calculated in three various combinations.

- a) Between all 16 individuals from the hybrid swarm population, $n=16$;
 - b) Between individuals of the samples taken from the typical populations of the putative parental species (A,B,C and D), each group was treated separately, thus the number of objects was $n=30$ in each case.
 - c) Between 4 samples of the populations of the putative parental species treated as one group each and, in addition, 16 individuals studied, treated also as one group; in this case $n=5$.
4. Student's t-test for differences between the 16 studied individuals.
 5. Calculation of characteristics of the 13 needle traits in the investigated group, $n=16$.
 6. Agglomerative clustering by the closest neighbours (UPGMA) method basing on Euclidean distances; graphically expressed in the form of dendrograms.

To show the individual character of the each of the 16 plants studied, cyclograms based on the following traits were constructed: nos. 2, 6, 12, 13, 14, 15, 16, 17. The choice of these characters was based on results of statistical analysis of the populations of the putative "parental" species: these were characters allowing the best discrimination between them. The discriminative power (obtained from the discriminant analysis, Table 1), the amplitude of particular characters and their averages were used for this purpose (Table 2). The cyclogram was constructed according to the diagram in Fig. 1, and in the Tables 3 and 4.

TABLE 1. Coefficients of determination (Krzyśko 1982) for the first and for the second discriminant axes (see Fig. 2).

Traits	U ₁ (55.77%)	U ₂ (17.57%)
1	7.71	2.07
2	19.04	0.52
3	1.96	0.01
4	6.20	6.17
5	7.27	9.69
6	38.36	10.53
7	9.32	5.08
8	0.52	2.10
9	9.02	53.12
10	0.14	1.68
11	20.83	1.89
12	20.30	13.90
13	36.22	13.76

RESULTS

The results of all statistical analyses performed show clearly that populations of *Pinus sylvestris* (A, B) and of *Pinus mugo* (C, D) are distinctly separate from each other; at the same time, the studied group of 16 individuals taken from the putative hybrid swarm population (peat bog "Bór na Czerwonym") is of unequivocally intermediate character, occupying – in the hyperspace constructed – a place between the two putative parental species, i.e. *Pinus sylvestris* and *P. mugo*. It can be clearly seen on graphical presentations of the discriminant analysis both when the OTU was a separate individual (Fig. 2), and when the analysis was performed on particular groups: A,

TABLE 2. Arithmetical means, maximum and minimum values for the 13 characters in five samples studied; A, B – samples of two *Pinus sylvestris* populations; C, D – samples of two *Pinus mugo* populations; M – group of 16 plants from the peat bog "Bór na Czerwonym".

	M	M	A	A	B	B	C	C	D	D
traits	mean	min. max	mean	min. max	mean	min. max	mean	min. max	mean	min. max
1	11.1	6.0 16.0	11.8	7.0 18.0	15.4	9.0 24.0	9.3	5.0 16.0	10.3	6.0 15.0
2	7.6	5.0 12.0	11.2	7.0 19.0	11.0	6.0 19.0	6.6	4.0 11.0	6.2	3.0 12.0
3	1.5	0.9 2.7	1.1	0.7 1.9	1.4	1.0 2.3	1.5	0.8 2.7	1.7	0.8 3.3
4	19.0	14.0 25.0	23.2	13.7 29.2	20.2	13.3 26.0	18.2	13.0 24.0	18.6	12.3 24.0
5	18.4	13.0 25.0	23.2	18.3 29.3	20.2	15.3 25.3	17.7	10.3 24.0	18.7	14.0 23.7
6	6.6	3.0 13.0	8.5	3.0 15.0	9.3	5.0 16.0	4.1	3.0 6.0	3.5	1.0 6.0
7	26.9	17.0 43.0	17.4	10.1 30.4	18.8	12.5 27.5	25.9	15.0 37.5	28.3	17.5 45.0
8	16.7	12.5 21.7	14.3	10.1 19.4	16.2	11.7 20.0	16.1	10.8 21.7	16.5	10.0 21.7
9	1492	1232 1826	1464	1032 1979	1576	1166 2068	1435	1232 1804	1300	1012 1584
10	819	682 990	703	495 903	708	506 990	858	660 984	726	462 880
11	0.550	0.430 0.680	0.476	0.380 0.660	0.451	0.360 0.550	0.578	0.440 0.670	0.559	0.400 0.680
12	203	80 375	221	99 380	261	100 450	106	38 183	74	20 155
13	368	141 889	464	195 966	585	225 1138	186	67 726	132	33 278

Fig. 1. Structure of a cyclogram; the particular radii are scaled according to character values; numbers of characters are situated on the periphery of the circle. These are: 2. number of stomatal rows on the flat side of a needle, 6. number of resin canals, 12. distance between vascular bundles, 13. Marcet's coefficient, 14. type of sclerenchyma sheath around the resin canals, 15. type of interbundle sclerenchyma, 16. setting angle of one-year-old cones, 17. growth type (A – monocormic, B – oligocormic, and C – polycormic).

TABLE 3. Arithmetical means of eight characters chosen for the construction of the cyclograms in the figure 7A; A, B – whole samples of two *Pinus sylvestris* populations; C, D – whole samples of two *Pinus mugo* populations; 1B-5B – values for five individual plants randomly chosen from the sample B (pure stand of *Pinus sylvestris*); 1C-5C – values for five individual plants randomly taken from the sample C (pure stand of *Pinus mugo*).

		P O P.				I N D I V I D U A L S									
		A	B	C	D	1B	2B	3B	4B	5B	1C	2C	3C	4C	5C
T R A I T S	2	1.4	12.0	6.0	6.2	10.3	12.0	11.9	15.5	11.0	5.8	7.0	4.6	6.6	5.6
	6	8.5	9.3	4.1	3.5	7.1	9.2	9.0	12.0	9.9	3.8	4.0	4.0	4.0	3.8
	12	221	261	106	74	221	204	260	385	209	100	90	144	105	120
	13	464	585	186	132	466	449	597	921	444	153	145	289	172	215
	14	A	A	C	C	A	A	A	A	A	C	C	C	C	C
	15	A	A	C+D	C+D	A	A	A	A	A	C	C+D	D	C	D
	16	210	190	23	30	200	180	190	190	195	30	20	30	32	35
	17	A	A	C	C	A	A	A	A	A	C	C	C	C	C

TABLE 4. Values of the eight characters chosen for the construction of the cyclograms for the 16 individual plants from the peat bog "Bór na Czerwonym" (Fig. 7B); each value is an arithmetical average of measurements of the particular characters in 30 needles from every single plant.

		I N D I V I D U A L S															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
T R A I T S	2	7.3	6.6	5.9	7.5	9.0	7.2	6.2	7.1	8.8	9.2	7.4	8.4	8.4	7.8	6.4	8.2
	6	8.6	5.3	6.3	6.2	6.5	6.5	3.3	5.8	7.0	9.7	7.9	6.5	8.4	7.2	5.3	4.1
	12	228	252	141	178	173	199	106	212	295	319	194	248	209	176	140	169
	13	375	392	262	327	328	351	199	378	582	621	346	483	239	308	237	304
	14	A+B	A+B	A+B	B	B+C	A+B	A+B	B	A	A+B	A+B	B	A+B	B	B+C	A+B
	15	B+C	B+C	C	C	C+D	C	B+C	C	B	C+D	B	C	C	C	C+D	C
	16	100	90	165	115	100	100	100	110	45	100	90	100	105	105	100	100
	17	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C

B, C, D, and M for the group of 16 individuals from the peat bog "Bór na Czerwonym". The same results were obtained from the principal components analysis (Fig. 4), clustering by the UPGMA method (Fig. 5) and on the dendrite constructed on the basis of Euclidean distances (Fig. 6).

Table 1. shows values of the coefficients of determination for the particular characters in the first and in the second discriminant axes. It follows that the most important characters influencing the distribution of the individual plants on the plane formed by these axes (Fig. 1) are: number of stomatal rows on the convex side of the needle (#1); the same on the flat side of the needle (#2); mean number of stomata in a 2 mm long section of convex side of the needle (#4); the same

on the flat side of the needle (#5); the ratio of the thickness of the needle to its width (#11); distance of the vascular bundles (#12); and value of Marcet's coefficient (#13).

Mahalanobis' generalized distances show a high degree of heterogeneity in the group of 16 plants from the peat bog "Bór na Czerwonym" studied: nearly one half (45.8%) of all possible distances were significantly different from 0 (Table 5). This result should be emphasized, as such a heterogeneity is quite unusual in pure stands of *Pinus sylvestris* and *Pinus mugo*: no Mahalanobis' generalized distance between individuals of the A, B, C, and D samples was found to be significantly different from 0 (results not shown). The same situation was also observed in other pine populations (Bobo-

Fig. 2. Distribution of the studied individuals on the plane of the first and second discriminant axes; A,B - *Pinus sylvestris*; C, D - *Pinus mugo*, and M - 16 plants from the peat bog "Bór na Czerwonym"; information contents is given (in per cent) at the apices of the particular axes.

Fig. 3. Distribution of the samples studied (each treated as a whole) in the three-dimensional discrimination space; A, B - *Pinus sylvestris*, C, D - *Pinus mugo* and M - sample of 16 individuals from the peat bog "Bór na Czerwonym"; information contents is given (in per cent) at the apices of the particular discriminant axes.

Fig. 4. Distribution of individuals studied on the plane of the first and the second principal component axes; A, B – *Pinus sylvestris*, C, D – *Pinus mugo* and M – sample of 16 individual plants from the peat bog "Bór na Czerwonym"; information contents is given (in per cent) at the apices of the particular principal component axes.

Fig. 5. Graphical presentation (dendrogram) of results of the clustering performed according to the UPGMA procedure, based on Euclidean distances; A, B – samples of the populations of *Pinus sylvestris*, C, D – samples of the populations of *Pinus mugo*, and M – group of 16 individual plants from the peat bog "Bór na Czerwonym".

Fig. 6. Minimum spanning tree (dendrite) constructed on the basis of Mahalanobis' generalized distances; large figures (with asterisks) = values of Mahalanobis' generalized distance; and smaller figures = values of Hotelling's T^2 function. The critical values for T^2 are given on the right side of the figure; A, B – samples of the populations of *Pinus sylvestris*, C, D – samples of the populations of *Pinus mugo*, M – group of 16 individual plants from the peat bog "Bór na Czerwonym".

$$120 = 100\%$$

TABLE 6. Values of all Mahalanobis' generalized distances between 16 individual plants taken from the population situated on the peat bog "Bór na Czerwonym" (figures in the upper rows) and critical values of Hotelling's T^2 function (figures in the lower rows); one asterisk = value of Mahalanobis' generalized distance significantly different from 0 on the level 0.01; two asterisks = value of Mahalanobis' generalized distance significantly different from 0 on the level 0.05.

Traits	Factions (in per%) of highly signific. differences (level 0.01)	Factions (in per%) of the stat. signific. differences (level 0.05)	Factions (in per%) of the stat. signific. differences (values of columns 2 and 3 added)
1.	30.0%	10.8%	40.0%
2.	66.7%	7.5%	72.2%
3.	39.2%	5.0%	44.2%
4.	63.3%	8.3%	71.6%
5.	62.5%	10.0%	72.5%
6.	79.2%	5.0%	84.2%
7.	54.2%	12.5%	66.7%
8.	30.8%	17.5%	48.3%
9.	79.2%	5.8%	85.0%
10.	65.0%	11.7%	77.7%
11.	75.0%	4.2%	79.2%
12.	72.5%	6.7%	79.2%
13.	75.0%	5.0%	80.0%

states typical for the two species in question. This is found in a population growing on the peat bog called "Bór na Czerwonym" on the foothills of the Tatra Mts. Plants from this population are usually identified – because of the low polycormic growth habit and the deep dark green needles – as belonging to *Pinus mugo* (Bobowicz 1988). This is, however, an awkward solution to the problem, first of all because of the intermediate mentioned of the plants in question. Therefore, some authors – e.g. Staszkiwicz in 1994 – call the plants of this population *Pinus x rhaetica* Brügger.

The mentioned intermediacy is – as has been shown in "Results" – of two different kinds. Either the values of the particular characters are truly intermediate between those of the both species, or the individual plants show unique combinations of typical (or nearly typical) *Pinus mugo* and *Pinus sylvestris* traits (Figs. 7A and 7B). According to some authors (e.g. Bobowicz 1990, Staszkiwicz 1994) the interspecific hybridization can be made responsible for this intermediacy of the particular plants.

Further reasons for seeing a hybrid swarm in the population discussed come from comparison of Mahalanobis' generalized distances between individuals of the sample taken from the peat bog "Bór na Czerwonym", and those between individuals coming from pure stands of the both putative parental species (see "Results" for details). Nearly one half (48.5%) of the Mahalanobis' generalized distances are a significantly different form 0 only in the studied sample of the 16 individuals, whereas none such distances were found in the pure stands. Thus, every member of the studied sample shows a unique combination of characters not encountered in the pure stands. And the lack of the statistically significant distances between members of such pure stands seems to be a rule rather than an exception: at least papers by Bobowicz and Krzakowa (1988a, b), Bobowicz and Krzakowa (1990, 1994), Bobowicz (1993) show this very clearly. Thus, the heterogeneity observed in the studied sample from the peat bog "Bór na Czerwonym" seems to be a rare find. And as it has been discovered in a

place, where both putative parental species are growing in close proximity, their hybridization and the subsequent introgression seems to be possible.

It seems, moreover, that the introgression was realised only in one direction: genes from *Pinus sylvestris* were introduced to the genome of *Pinus mugo*. This follows from the fact that the Scots pine forests surrounding the peat bog population seem to belong to the typical form of *Pinus sylvestris*, although possibly rare crossings of *Pinus sylvestris* x *Pinus mugo* would be rather difficult to discover because of the intense human activity in the Scots pine forests.

CONCLUSIONS

1. The group of 16 individuals taken from the peat bog "Bór na Czerwonym" (foothills of the Tatra Mts., close to the city of Nowy Targ, S. Poland) is characterized by a large variability in comparison with the pure populations of *Pinus sylvestris* and *Pinus mugo*. This statement follows, first of all, from the discussion on Mahalanobis' generalized distances, which are frequently (in 48.5% of cases) statistically significant, while such distances are very rare or lacking entirely in the pure populations.
2. Both cyclograms (Figs 7A and 7B) and results of the multivariate statistical analysis (principal components – Fig. 4; discriminant variables – Figs 2 and 3; dendrite and dendrogram – Fig. 5) show that each individual examined – in spite of being similar in many respects to the mountain pine (i.e. *Pinus mugo*) – occupies an intermediate place between *Pinus mugo* and *Pinus sylvestris* in the character space. This can be the result of, possibly, an interspecific hybridization. The postulated hybrid character of the studied individuals explains their unique combinations of characters.
3. Predominance of mountain pine traits connected with abundant flowering and cone production (at least in some years) may be considered as evidence that the studied in-

Fig. 7A. Cyclograms constructed for the samples of the studied populations based on averages of the particular characters; A, B – samples of the populations of *Pinus sylvestris*, C, D – samples of the populations of *Pinus mugo*; in addition, cyclograms for five randomly chosen individuals from the sample of the population B (i.e. *Pinus sylvestris*) and from sample of the population C (i.e. *Pinus mugo*) are given. For details see Figure # 1.

Fig. 7B. Cyclograms constructed for each of the 16 individual plants from the peat bog "Bór na Czerwonym". For details – see Figure # 1. Diagrams showing the setting angles typical for *Pinus sylvestris* (a), *Pinus mugo* (c); also the setting angle of every one of the 16 individual plants from the population on the peat bog "Bór na Czerwonym" (b).

dividuals are, in fact, introgressants with genes of *Pinus sylvestris* in the *Pinus mugo* genome.

4. The distinct influence of the genes of *Pinus sylvestris* can be observed in the following traits: 2, 6, 13, 14, 15, and 16.

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BIOMETRYCZNE STUDIA WYBRANYCH OSOBNIKÓW *PINUS X RHAETICA* BRÜGGER NA TORFOWISKU "BÓR NA CZERWONEM"

STRESZCZENIE

Celem pracy było zbadanie 16 osobników rosnących na torfowisku "Bór na Czerwonym", charakteryzujących się pokrojem polikormicznym – typowym dla *Pinus mugo* Turra, ale posiadających typowy dla *Pinus sylvestris* L. kąt osadzenia szyszek jednorocznych. Jednoczesne występowanie tych cech, wskazywało na mieszańcowy charakter badanych roślin. W wyniku analizy matematyczno-statystycznej, której poddano 13 cech morfologiczno-anatomicznych igieł, stwierdzono silne zróżnicowanie (45,8% odległości Mahalanobisa) było istotnych statystycznie) oraz pośredni (między *Pinus sylvestris* L. i *Pinus mugo* Turra) charakter budowy igieł. Stwierdzono również, że igły te mimo pośredniego charakteru swojej budowy są bardziej podobne do igieł kosodrzewiny niż do sosny. Wszystko to wskazuje na mieszańcowy charakter badanej grupy roślin. Jednak przewaga cech kosówkowych (wzrost, charakter igieł), jak i stosunkowo obfite kwitnienie, wskazują – był może – na to, że osobniki te są introgresantami kosodrzewiny z domieszką genów sosny zwyczajnej.

SŁOWA KLUCZOWE: sosna zwyczajna, kosodrzewina, mieszańce, zmienność, analizy wielozmienne.