Anatomical differences between Pinus mugo Turra populations from the Tatra Mts.. expressed in needle traits and in needle and cone traits together

MARIA ANNA BOBOWICZ. MARIA KRZAKOWA

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

(Received: May 14, 1985, Revision accepted: December 3, 1985)

ABSTRACT

Two-year-old needles were collected from 429 individuals of dwarf mountain pine (Pinus mugo Turra) from 10 populations of this species growing in the Tatra Mts. (5 populations from calcareous and 5 from calcium-free undersoil). Thirteen anatomical traits of the needles were studied. For obtaining a full picture of the variability of the dwarf mountain pine, the differences were investigated in ten populations (385 plants) as regards 27 traits of needles and cones. The data obtained by measurements served as basis for performing multivariate analysis of variance and testing the statistical hypotheses, for discriminant analysis, calculation of the Mahalanobis distances and construction of a minimum spanning tree of the shortest Mahalanobis distances and agglomerative clustering by the method of nearest neighbourhood. The results of these analyses indicated that the dwarf mountain pine populations differ statistically significantly as regards the whole complex of needle traits and form three groups not correlated with the type of undersoil on which they grow. Most closely similar are the populations from Kominy Tylkowe, Morskie Oko and Wyżni Toporowy Staw. The population from Kobylarz is the most diverse. Investigation of the differences in the complex of needle and cone traits proved that the traits of cones are decisive for the variability picture.

Key words: dwarf mountain pine, variability, needles, cones

INTRODUCTION

More papers have been devoted to investigation of the dwarf mountain pine (*Pinus mugo* Turra) (sensu Rothmaler 1963, Gaussen et al. 1964), concerning both needle and cone traits (Szweykowski et al. 1976a, b,

Szweykowski and Bobowicz 1977, Krzyśko et al. 1980, Bobowicz et al. 1983b, c) than to needles alone (Szweykowski 1969) or cones only (Bobowicz and Krzakowa 1986).

In the present study variability was now investigated between the same *Pinus mugo* Turra populations from the Tatra Mts. on the basis of anatomical traits of needles, in which previously the cone traits were examined (Bobowicz and Krzakowa 1986). Trials were undertaken to establish whether there are significant differences between these populations in needle traits and whether they are correlated with the undersoil type on which the populations grow. In order to obtain a full picture of the morphological-anatomical variability, differences were also studied between these populations for the joint set of needle and cone traits.

The main cause why separate analyses were performed for the sets of cones and needles and the joint group of needles + cones was the fact, repeatedly confirmed in our investigations on the genus *Pinus* (e.g. Bobowicz et al. 1983a, b, c, Bobowicz 1984), that the pictures of variability both between populations and within them obtained for these three groups differ from one another.

Thus, the aim of our study was to obtain pictures of the variability of ten *P. mugo* Turra populations separately in each of these sets. Only this would give a full picture of the variability of the dwarf mountain pine.

From the methodical point of view most valuable are results based on a maximal number of traits which have not been evaluated a priori. By using the joint set of needle and cone traits we wanted to find which of them exerts a greater influence on the picture of dwarf mountain pine variability. With the available set of 27 traits it was possible to choose those with highest discriminative power, and, thus, most important in describing the differences between the studied populations.

MATERIAL AND METHODS

The material consisted of needle samples from the following sites in the Tatra Mts. (the number of examined plants is given in parentheses):

- 1. Kopa Magury (49),
- 2. Giewont (40),
- 3. Sarnia Skała (31),
- 4. Kominy Tylkowe (35),
- 5. Kobylarz (35),
- 6. Hala Gąsienicowa (98),
- 7. Hala Goryczkowa (30),

- 8. Morskie Oko (37),
- 9. Wyżni Toporowy Staw (38),
- 10. Ornak (36).

Populations from sites 1-5 grow on calcareous, those from sites 6-10 on granite substrate. The distribution of the stands is shown in Fig. 1a in the paper of Bobowicz and Krzakowa (1986). A total of 429 plants were examined, ten needles from each.

The following traits of the needles were elaborated:

- 1) number of rows of stomata on the convex side of the needle,
- 2) number of rows of stomata on the flat needle side,
- 3) quotient of stomatal lines (trait 1:trait 2),
- 4) mean number of stomata per a 2-mm length on the convex side of the needle,
- 5) mean number of stomata per a 2-mm length on the flat side of the needle.
- 6) number of resin canals.
- 7) epidermis thickness on flat side of needle,
- 8) mean width of three epidermis cells,
- 9) width of needle crossection.
- 10) height of needle crossection,
- 11) ratio of height to width of crossection (traits 10:9),
- 12) distance of vascular bundles
- 13) Marcet's index $\frac{\text{trait } 9 \times \text{trait } 12}{\text{trait } 10}$ (Marcet 1967).

The data obtained from measurements were trasmitted to the Computer Centre of the Agricultural University in Poznań where the following calculations were performed:

- 1. characteristics of the 13 needle traits separately for each of the ten populations and jointly for the "calcareous" populations (1+2+3+4+5) and the "calcium-free" populations (6+7+8+9+10),
- 2. multivariate analysis of variance together with testing of the statistical hypotheses,
- 3. discriminant analysis,
- 4. Mahalanobis distances with minimum spanning tree constructed on the basis of the shortest Mahalanobis distances.
- 5. agglomerative clustering by the method of nearest neighbourhood.

RESULTS

NEEDLE TRAITS

The characteristics of 13 traits are included in Table 1. It is seen therefrom that most traits have variability coefficients not exceeding 20

per cent. Most variable are traits 12 and 13 (distance of vascular bundles and Marcet's index). The general means for the sum of "calcareous" populations (1+2+3+4+5) and "calcium-free" ones (6+7+8+9+10) indicate that, trait 5 excepted (mean number of stomata per 2 mm length on flat side of needle), all the remaining ones show in the "calcareous" populations higher values that in those from calcium-free substrate. Testing of the general hypothesis in multivariate analysis of variance (Caliński 1970. Caliński et al. 1976. Ceranka et al. 1976) indicates that, as regards the 13 traits treated jointly, the examined populations differ significantly in the statistical sense ($F_{cal} = 7.457 > F_{0.05} = 1.229$). The result of study of the differences in detail between the populations is given for each of the 13 traits separately in Table 2A. As seen, the means for the populations differ significantly in 12 traits, with the exception of trait 3 (quotient of stomatal lines) for which the differences are not significant $(F_{0.05} > F_{calc})$. The significance of differences was also tested for the "calcareous" population (1+2+3+4+5) from calcareous habitat and "calcium-free" population (6+7+8+9+10). This brought evidence that these groups differ significantly only in two of the 13 traits (Table 2B): mean width of three epidermis cells (trait 8) and number of resin canals (trait 6).

Discriminant analysis gives a graphic picture of the variability of dwarf mountain pine needles (Caliński and Kaczmarek 1973, Sneath and Sokal 1973. Caliński et al. 1975. Krzyśko 1982) within the space of the three first discriminant variables (U₁, U₂, U₃) (which supply 75.41°, of information from the used set of 13 traits of needles, Fig. 1a). It is seen from the diagram that: 1) there is a high similarity between populations 4 (Kominy Tylkowe). 8 (Morskie Oko) and 9 (Wyżni Toporowy Staw); 2) there are no distinct differences between populations in reference to the kind of undersoil on which they grow: 3) population 5 (Kobylarz) is distinctive. On the basis of correlation coefficients between traits and discriminant variables (Table 3) it may be concluded that in this picture of variability decisive are the following traits: 8 (mean width of three epidermal cells), 12 (distance of vascular bundles). 10 (height of needle crossection). 13 (Marcet's index), 9 (width of needle crossection) and 6 (number of resin canals) for the first discriminant variable; 4 (mean number of stomata per 2-mm length of needle on convex side). 5 (mean number of stomata per 2-mm length on flat needle side), 6 (number of resin canals) and 7 (thickness of epidermis on flat needle side) for the second discriminant variable; 9 (width of needle crossection), 10 (height of needle crossection), 2 (number of stomatal rows on flat needle side), 1 (number of stomatal rows on convex needle side) for the third discriminant variable

The minimum spanning tree constructed according to the shortest Mahalanobis distances (Caliński and Kaczmarek 1973, Fig. 1b) indicates

Table 1

Arithmetic means (m), standard deviations (s.d.) and correlation coefficients (c.var.) for 13 needle traits in ten *Pinus mugo* Turra populations from the Tatra Mts. The last two rows give general means for the "calcareous" (1+2+3+4+5) and "calcium-free" populations (6+7+8+9+10)

	Α		1			2			3			4			5			6		44.4	7			8			9			10	19.0		11			12			13	
В		m	s.d.	c.var.	m	s.d.	c.var.	m	s.d.	c.van.	m	s.d.	c.van.	m	s.d.	c.var.	m	s.d.	c.var.	m	s.d.	c.var.	m	s.d.	c.var.	m	s.d.	c.vr.	m	s.d.	c.vr.	m	s.d.	c.var.	m	s.d.	c.var. ,	m	s.d.	c.var.
1		9.64	1.17	12.11	7.73	0.73	9.49	1.26	0.09	7.68	16.96	0.88	5.20	16.85	0.85	5.06	4.62	0.62	13.36	28.28	1.28	4.52	14.85	0.63	4.27	1.50	0.14	9.16	0.84	0.06	6.92	0.56	0.02	3.20	0.11	0.03	25.22	0.19	0.05	28.04
3		10.00 8.94	1.08 1.21	10.84 13.48	7.63 6.99	0.67	8.83	1.32	0.11	8.18 9.28	16.49 17.24	0.65	5.20	17.11	0.67	4.08 4.74	4.30 3.90	0.76	15.51	27.63 29.50	2.08	7.55	14.77 15.10	0.88	5.77	1.47	0.13	8.74 5.30	0.83 0.77	0.07	8.34 5.81	0.56	0.02	3.63	0.09	0.03	28.02	0.16 0.15	0.05 0.04	28.03
5		9.06 9.57	0.98 0.77	10.76 8.09	7.11 7.45	0.77 0.51	10.84	1.29	0.10 0.06	7.51 4.99	16.99 17.51	0.95	5.57	16.85 17.50	0.83 0.63	4.94 3.58	3.99	0.47	11.85 5.78	27.36 27.57	1.37 1.89	5.02 6.36	15.01 16.13	0.68	4.52 5.12	1.37 1.37	0.09 0.05	6.21	0.79	0.04	5.51 3.73	0.58	0.02	3.39 3.30	0.08	0.01	19.14 10.22	0.13	0.03	18.84 10.72
6		9.36 9.73	0.94	10.04 11.22	7.43	0.71	9.60	1.28	0.09	7.16	.17.08	0.93	5.43	16.94	0.98	5.79	4.00	0.80	19.89	26.86	2.32	8.32	14.17	0.63	4.44	1.41	0.08	5.41	0.80 0.82	0.04	5.51	0.57	0.02	3.68	0.09	0.02	18.14	0.16	0.03 0.04	18.39
8	11	8.67	1.09 0.91	10.53	6.76	0.77	9.66 9.95	1.29	0.12	8.43	16.85	0.86	5.12	17.61 16.74	1.22 0.83	6.92 4.98	3.78	0.42	11.57 16.07	27.81	1.61	9.29 5.79	14.84	0.54	3.66	1.37	0.08	6.18	0.82	0.03	4.15	0.57	0.02	3.51	0.09	0.02	19.90	0.16	0.03	20.89
10		9.20 9.45	1.01	10.99 7.61	7.41 7.32	0.82	11.05 8.43	1.26	0.07	5.74 7.15	16.64	0.51	3.09 2.34	16.47 17.59	0.56	2.83	3.79 3.68	0.40 0.37	10.60 10.12	27.25 28.80	1.76 2.41	6.47 8.35	15.24 15.08	0.96	5.87	1.35 1.45	0.09	6.83	0.75 0.82	0.05	6.46	0.56	0.02	3.78 1.48	0.08	0.01	13.11 16.26	0.14 0.15	0.02	14.88 26.59
1-5		9.48			7.42	à		1.29			17.00			16.90		*	4.15			28.05			15.11			1.42			0.81			0.57			0.09			0.16		× 1.
6-10)	9.24			7.34			1.27			14.14			17.00			3.84		-	27.62			14.67			1.40			0.79			0.57			0.08			0.15		

⁻ traits, B - populations from: 1 - Kopa Magury, 2 - Giewont, 3 - Sarnia Skała, 4 - Kominy Tylkowe, 5 - Kobylarz, 6 - Hala Gasienicowa, 7 - Hala Goryczkowa, 8 - Morskie Oko, 9 - Wyżni Toporowy Staw, 10 - Ornak.

Table 2

F statistics value when comparing ten *Pinus mugo* Turra populations from the Tatra Mts. in reference to 13 traits of needles (A) and F statistics value when comparing populations from calcareous and calcium-free undersoil (B)

T'4-	A	1	В				
Traits	F _{calc} .	F _{0.05}	F _{calc} .	F _{0.05}			
1	6.007*	2.757	3.755	8.477			
2	8.472*	2.757	0.499	8.477			
3	2.494	2.757	2.545	8.477			
4	9.859*	2.757	2.428	8.477			
5	9.338*	2.757	0.829	8.477			
6	11.618*	2.757	21.850*	8.477			
7	8.037*	2.757	3.577	8.477			
8	24.515*	2.757	40.247*	8.477			
9	12.629*	2.757	3.369	8.477			
10	16.177*	2.757	2.485	8.477			
11	6.387*	2.757	0.463	8.477			
12	12.732*	2.757	1.700	8.477			
13	11.934*	2.757	1.696	8.477			

^{*} Value significant at = 0.05 level.

Table 3

Coefficients of correlation between 13 traits of needles and the three first discriminant variables U₁, U₂, U₃ for ten *Pinus mugo* Turra populations from the Tatra Mts.

Traits	U ₁ 42.64%	U ₂ 19.98%	U ₃ 12.79%
1	-3.283	0.200	-7.866
2	-6.396	-0.759	-7.904
3	3.710	0.799	-0.844
4	2.729	-13.127	-1.202
5	3.667	-12.468	-2.123
6	-12.536	8.472	-3.965
7	0.512	-8.052	-0.051
8	29.635	0.060	-3.325
9	-13.304	-0.539	-9.605
10	-18.719	-2.757	-8.454
11	-7.974	-3.736	3.645
12	-18.804	1.279	-3.658
13	-17.227	1.538	-4.093

wide differences between populations. a great diversity of the population from Kobylarz (No. 5) and a great similarity of populations 4 and 8. On the basis of critical values (Caliński and Kaczmarek 1969) of the Mahalanobis distances (Table 4) it may be affirmed that, the latter two populations excepted, the remaining ones show significant differences in the examined traits of needles. The dendrite does not show distinct differences between populations from the calcareous and calcium-free habitats.

Agglomerative clustering by the method of nearest neighbourhood (Karoński and Caliński 1973, Fig. 1c) seems to suggest that, among the examined populations three groups may be distinguished. The first comprises populations on stands 3 (Sarnia Skała), 7 (Hala Goryczkowa), and 10 (Ornak). The second consists of the population 5 from Kobylarz, and the third of the remaining six populations. In the latter group two subgroups can be distinguished: one with the population from Kopa Magury (1) and Giewont (2) and the second including populations 9, 4, 8 and that from Hala Gasienicowa (6). The result of this grouping also indicates a lack of differences between the populations in respect to the type of undersoil on which they grow. like populations 4, 8, 9 and population 6 close to them as well as the distinctiveness of population 5 from Kobylarz.

NEEDLE AND CONE TRAITS TREATED JOINTLY

For obtaining a full picture of the dwarf mountain pine, variability was tested on the joint group of 13 needle and 14 cone traits (description of the latter traits in papers by Bobowicz et al. 1983a, Bobowicz and Krzakowa 1986). A total of 385 plants from ten populations were examined (no. of plants from each population indicated in Fig. 2). The material was subjected to the analyses described in Methods of needle study. In the study of differences concerning this joint group of needle and cone traits in multivariate analysis of variance a complete confirmation was obtained of the results of analyses performed for both the sets of traits separately. that is significant differences were found as regards 26 traits, the third trait of needles excepted (quotient of stomatal lines). Testing of significance of differences for the "calcareous" (1+2+3+4+5) and "calcium-free" populations (6+7+8+9+10) revealed significant differences between habitats as regards all the 27 traits treated jointly ($F_{calc.} = 4.464 > F_{0.05} = 1.517$) and in separate testing for each trait significant differences were noted concerning traits 6 (no. of resin canals) and 8 (width of three epidermis cells) for needles and 4 (diametre of cone apex). 12 (ratio of cone length to its number of scales). 9 (thickness) and 8 (width of scale) and 5 (cone diametre at mid distance between cone apex and greatest width of cone), with the exception of trait I of cones - its length - which in the newly

Table 4

Mahalanobis distances between ten *Pinus mugo* Turra populations plotted on the basis of 13 needle traits ($D_{calc.}$) and critical values for the same distances ($D_{0.05}$)

		7							
2	1.579 1.0633								
3	2.243 1.1106	2.558 1.1773	-						
4	2.004 1.0889	1.714 1.1391	1.941 1.2135						
5	3.501 1.0889	3.052 1.1391	2.915 1.2135	2.659 1.1761					
6	1.504 0.8465	1.624 0.9232	2.125 1.0139	1.578 0.9553	3.405 0.9553				
7	2.178 1.1406	2.593 1.1883	2.433 1.2601	2.366 1.2242	3.560 1.2242	1.823 1.0266			
8	2.189 1.0716	1.867 1.1226	1.685 1.1785	1.132 1.1411	2.700 1.1411	1.608 0.9494	2.405 1.2088		
9	2.365 1.0635	2.026 1.1146	2.024 1.1908	1.705 1.1339	2.052 1.1339	2.071 0.9403	2.885 1.2017	1.671 1.1173	
10	2.225 1.0800	2.281 1.1303	2.119 1.2056	1.748 1.1483	2.579 1.1483	1.983 0.9589	1.489 1.1962	1.809 1.1518	2.449 1.1443
	1	2	3	4	5	6	7	8	9
1 67	0 D								

1.579 D_{calc.} **1.0633** D_{0.05}

Table 5

Coefficients of correlation between 27 traits of needle and cones and three first discriminant variables U₁, U₂. U₃ for ten *Pinus mugo* Turra populations from the Tatra Mts.

Trai	ts U ₁ 43.23%	U ₂ 20.54%	U ₃ 10.62%	Traits	U ₁ 43.23%	U ₂ 20.54%	U ₃ 10.62%	Traits	U ₁ 43.23%	· U ₂ 20.54%	U ₃ 10.62%
1	1.686	2.362	→2.443	10	-2.940	4.087	-2.390	19	-3.329	4.560	0.850
2	0.303	3.081	-2.230	11	-4.539	-1.992	1.444	20	1.210	12.447	2.209
3	2.204	-0.555	-0.361	12	-5.101	3.985	-2.379	21	-7.738	10.229	1.837
4	2.421	0.205	4.471	13	-4.280	4.190	-2.443	22	-2.291	9.120	0.520
5	2.637	0.164	4.142	14	4.323	7.435	1.306	23	-6.139	0.472	-1.869
6	-5.317	3.738	-4.354	15	3.761	7.148	1.943	24	1.859	1.191	-0.573
7	1.340	-3.389	0.786	16	14.309	5.073	2.390	25	-4.064	1.705	3.558
8	12.537	-3.697	-0.181	17	-7.176	15.487	2.740	26	9.900	2.814	0.754
9	-0.519	4.663	-2.846	18	14.958	9.161	2.995	27	1.383	-0.267	-1.805

Table 6

Mahalanobis distances between ten *Pinus mugo* Turra populations plotted on the basis of 27 traits of needles and cones (D_{calc}) and critical values for these distances ($D_{0.05}$)

	3.064								
2	1.4715								
3	3.826	4.108							
	1.5517	1.6416		1					
4	3.336 1.4826	3.092 1.5756	2.254 1.6522						
5	4.999 1.6036	4.437 1.6906	5.451 1.7616	4.816 1.7009					
6	2.707 1.2423	3.968 1.3524	3.958 1.4401	3.375 1.3653	5.598 1.4958				
7	3.832 1.5517	3.641 1.6416	3.313 1.7146	2.972 1.6522	5.704 1.7616	3.735 1.4401			
8	3.464 1.4715	3.274 1.5652	2.332 1.6416	1.840 1.5756	4.677 1.6906	3.280 1.3524	3.077 1.6416		
9	3.853 1.4595	3.107 1.5545	4.225 1.6315	3.547 1.5658	2.938 1.6812	4.650 1.3401	4.774 1.6315	3.636 1.5545	
10	4.523 1.4951	4.438 1.5881	5.554 1.7886	4.947 1.5990	2.938 1.7112	5.328 1.3788	5.235 1.7886	4.922 1.5881	3.681 1.5776
	1	2	3	4	5	6	7	8	9

3.064 D_{calc.} 1.4715 D_{0.05}

formed set of 27 traits ceased to be a trait distinguishing the examined populations.

The result of **discriminant analysis** in the space of the three first discriminant variables U₁, U₂ and U₃ (supplying 74.39% of information from the used set of 27 traits) is shown in Fig. 2a. Considerable differences are seen between the populations and a complete lack of correlation of these differences with the type of substrate. On the basis of correlation coefficients between the traits and discriminant variables (Table 5) it may be stated that in the obtained picture of variability mainly decisive are the following cone traits: diametre of cone between top and greatest width (18), situation of greatest width in relation to top of cone (16) and mean width of three epidermis cells (8) for the first discriminant variable U₁, the cone apex diametre (17), length of scale (20), width of scale (21), diametre between top and greatest width of cone (18) for the second discriminant variable U₂ and the number of stomata on the convex side of the needle (4), the number of resin canals (6) and the number of stomata on the flat side of the needle (5) for the third discriminant variable U₃.

The minimum spanning tree constructed on the basis of the **shortest Mahalanobis distances** is shown in Fig. 2b. On the basis of critical values for the calculated Mahalanobis distances (listed in Table 6) it may be said that all the populations differ significantly as far as the complex of needle and cone traits is concerned.

Agglomerative custering by the method of the nearest neighbourhood (Fig. 2c) disclosed two distinct groups among the ten examined populations. The first comprises population 9 (Wyżni Toporowy Staw). 4 (Kominy Tylkowe), 6 (Hala Gąsienicowa) and 3 (Sarnia Skała). The second group may be divided into three subgroups and comprises the remaining populations, that is 10, 5, 7, 2, 8 and 1. These groups correspond strictly to the groups obtained from cone traits exclusively.

DISCUSSION

The investigations performed indicated that among the ten elaborated populations of Pinus mugo Turra from the Tatra Mts. two do not differ from one another significantly in the statistical sense, whereas the remaining populations differ significantly as regards all the studied anatomical traits of needles, trait 3 (quotient of stomatal lines) excepted. The following populations do not differ significantly: that from Kominy Tylkowe and that from Morskie Oko. Their close similarity is seen in the diagram of dispersion of the examined populations (Fig. 1a) and in the minimum spanning tree (Fig. 1b) and the dendrogram (Fig. 1c). In the minimum spanning tree the Mahalanobis distance between the population from Kominy Tylkowe and that from Morskie Oko is 1.1316. Since it is smaller than the critical value amounting to 1.1411, one may consider that these populations do not differ from one another significantly. The population from Wyżni Toporowy Staw is very similar to both the above mentioned populations (Fig. 1a, c). On the basis of the Mahalanobis distance linking this population in the minimum spanning tree (Fig. 1b) with that from Morskie Oko and amounting to 1.6710 (critical value $D_{0.05} = 1.1173$), we can, however, consider that this population differs significantly from the other two. Most diverse from the remaining populations is that from Kobylarz. This diverseness is visible in the dispersion diagram (Fig. 1a), the minimum spanning tree (Fig. 1b) and most pronounced in the dendrogram. The Mahalanobis distance linking Kobylarz with Wyżni Toporowy Staw is the greatest distance in the minimum spanning tree (Fig. 1b) and amounts to 2.0515 (critical value $D_{0.05} = 1.1339$). In the dendrograms this population seems close to a large group of populations (Wyżni Toporowy Staw, Kominy Tylkowe. Morskie Oko, Hala Gasienicowa, Giewont. Kopa Magury), but actually it

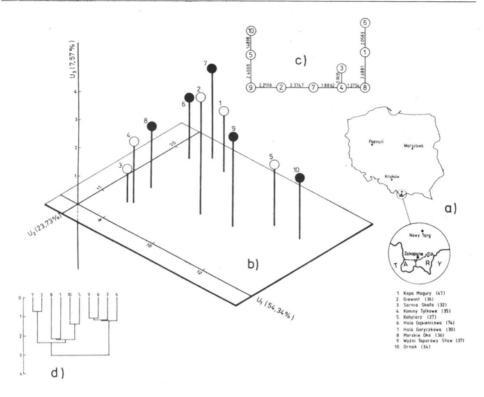


Fig. 1. a Result of discriminant analysis within the space of the three first discriminant variables (jointly 75.41% of information from the used set of 13 needle traits). Populations from the calcareous habitat are marked by clear cricles (nos. 1-5) and those from calcium-free habitat (nos. 6-10) by dark cricles. b — Minimum spanning tree constructed on the basis of the shortest Mahalanobis distances. c — Result of agglomerative clustering by the method of the nearest neighbourhood

forms as if a distinct "group". Rather diverse from the remaining populations are also the populations from Ornak, Hala Goryczkowa and Sarnia Skała (Fig. 1a). In the dendrogram (Fig. 1c) they form one distinct group. In the minimum spanning tree (Fig. 1b) the populations from Ornak and Hala Goryczkowa are linked with the population from Kominy Tylkowe by a distance second in size amounting to 1.1782 (critical value $D_{0.05} = 1.1483$). The population from Sarnia Skala is linked to the population from Morskie Oko by a distance equal to 1.6854 (critical value $D_{0.05} = 1.1785$) which is the third largest distance in the minimum spanning tree.

The detected variability of ten *Pinus mugo* Turra populations from the Tatra Mts. based on anatomical traits of needles shows that there are wide differences between the populations which may be classified to two groups: the first formed by the populations from Wyżni Toporowy Staw, Kominy

Tylkowe, Morskie Oko. Hala Gąsienicowa. Giewont, Kopa Magury and — as if a distinct group — Kobylarz, and a second one comprising the populatons from Ornak, Hala Goryczkowa and Sarnia Skała. It has been demonstrated in the present study that the differences in the undersoil do not correspond to the anatomical variability of the dwarf mountain pine. The existence

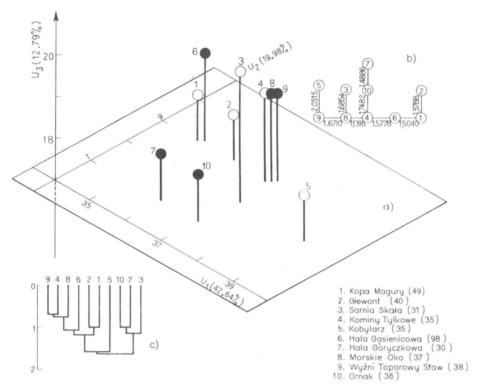


Fig. 2. a — Result of discriminant analysis within the space of the three first discriminant variables (jointly 74.39% of information from the used set of 27 traits of needles and cones). Populations from calcareous habitat marked with clear circles (nos. 1-5) and those from calcium-free habitat (nos. 6-10) with dark circles. b — Minimum spanning tree constructed on the basis of the shortest Mahalanobis distances. c — Results of agglomerative clustering by the method of nearest neighbourhood

of a relation between taxonomic differentiation in dependence on the substrate on which it grows has been suggested by Rothmaler (1963). The explantation experiments of Pilichody (1908) and Fankhauser (1926) contradict this. Neither did the investigations of Szweykowski (1969) unequivocally demonstrate such a relation. The author says: "There is no clear-cut difference between dwarf mountain pine growing on lime and growing in limeless substrate. However, a rather distinct tendency of the lime plants to group in the left part and of the granite plants in the right side of the *Pinus mugo* group can be observed in the dendrite. It is probable then, that

special conditions of living on lime or in limeless areas exert an influence on the anatomy of dwarf mountain pine. But it is evident from the dendrite that conditions of living on lime can be met sometimes in limeless areas and vice versa. The problem needs further study". (Szweykowski 1969, page 47).

In the present paper a group of populations from calcareous and one from limeless habitat have been tested and it was proved that these groups differ only in two traits of the 13 tested, namely mean width of three epidermal cell and number of resin canals. No correlation between the revealed character of variability and the kind of undersoil is visible in any pictures obtained by all the methods applied and in the dispersion diagram (Fig. 1a) and minimum spanning tree (Fig. 1b) and in the dendrogram (Fig. 1c). The best example of lack of such a correlation is the similarity of the populaton from Kominy Tylkowe (calcareous habitat) and Morskie Oko (calcium-free habitat). These are the only populations which do not differ significantly, each from a different undersoil. Another problem dealt with in the present paper is the geographical (spatial) differentiation of the examined populations. Bryant's test (1977) was applied to answer the question, whether the revealed anatomical variability of *Pinus mugo* Turra is in some way connected with the distribution of the populations.

The histogram plotted from the result of testing the needle traits indicates that the populations do not form groups as regards these traits, the histogram has one apex. Thus, it may be affirmed that as far as anatomical traits of needles are concerned the dwarf mountain pine from the Tatra Mts. forms 2 (3) groups which are correlated neither with the habitat or the geographical distribution, and the altitude above sea level. Results obtained from the study of the variability of the dwarf mountain pine according to the jointly considered traits of needles and cones should be discussed separately. It results from testing the significance of differences between the populations by means of F statistics that significant differences in the populations occur in reference to 12 traits of needles and all the fourteen traits of cones. Trait 3, that is the quotient of stomatal lines in the needles does not show significant differences. The discriminant power of cone traits is much higher that of needles, as seen both in the values of F statistics and in the number of needle and cone traits deciding mainly of the differentiation of the populations. To the best differentiating traits of populations belong those describing the shape of the cone (17, 18, 16). of the cone scales (20, 21), the mean width of three epidermal cells (8), the height (10) and width (9) of the needle and the number of resin canals (6).

In the picture of discriminant analysis in the space of three first variables U_1 . U_2 and U_3 (Fig. 2a) and in the picture of the minimum spanning tree constructed on the basis of the shortest Mahalanobis distances

(Fig. 2b) wide differences between the dwarf mountain pine populations are seen. Particularly diverse in Fig. 2a are the populations from Kopa Magury (1), Giewont (2) and Wyżni Toporowy Staw (9) as well as the population from Kominy Tylkowe (4). Their diverseness is due to the same traits which mainly form the third discriminant variable: the number of stomata on the convex and flat sides of the needle, the number of resin canals and the ratio of length of the cone to the number of scales.

Characteristic are in the minimum spanning tree the large Mahalanobis distances (especially as compared with those in the minimum spanning tree for the needle traits). These distances are all larger than the corresponding critical ones, thus, they are significant, this proving the significant differentiation of all the pairs of populations in the minimum spanning tree. The shortest distance of 1.8397 (critical value 1.5756) joins the populations from Kominy Tylkowe and Morskie Oko (differing nonsignificantly in needle traits). The populations from Sarnia Skała and Kominy Tylkowe (differing nonsignificantly in cone traits) are joined by a distance of 2.2545 (critical value $D_{0.05} = 1.6522$). The remaining Mahalanobis distances show high similar values, the largest of them is equal to 3.1071 and joins the population from Giewont with that from Wyżni Toporowy Staw.

There is no univocal grouping of populations from the calcareous and calcium-free habitat in the joint traits of needles and cones, like in the separately studied traits of cones and needles from both habitats and there are no significant differences, although in the minimum spanning tree, Fig. 2b a joint population group is visible from calcareous habitats: Sarnia Skała. Kominy Tylkowe, Giewont, Kopa Magury.

By clustering two distinct population groups were obtained. Their composition is identical with that obtained for clustering of cones (see Fig. 1d in paper by Bobowicz and Krzakowa 1986). No correlation is noticeable between the composition of the groups and the habitat from which they derive. In the two groups populations are present both from the calcareous and the calcium-free substrates.

This lack of correlation may be due to the fact that of the 27 traits of cones and needles only seven distinguish significantly populations from calcareous and calcium-free substrates. They are the same traits which distinguish significantly populations in reference to needle traits and those of cones treated separately, with the exception of the first cone trait (length of cone) which in the newly formed set of needle and cone traits has lost its discriminant power. These traits are: mean width of three epidermal cells, number of resin canals, diametre of cone apex, ratio of cone length to number of scales, scale thickness, width of scale and cone diametre at mid distance between cone apex and greatest cone width. The ordering of traits is according to the values of statistics F. Thus, it is seen that in testing the significance of differences concerning the

habitat in reference to the joint set of needle and cone traits, those of needles have a higher discriminant power, although the number of distinguishing traits is larger for cones. We have, therefore, a different situation than in the case of significance testing between populations.

Like in the case of needle and cone traits separately, geographical

differentiation was investigated for the joint set of these traits for the dwarf mountain pine population by applying Bryant's test. The histogram demonstrated the existence of two groups in these traits. After mapping standardised values of the first discriminant variable U_1 on the map of sites and plotting the isophene "O", a division into two groups was obtained: one comprising the populations from Giewont, Kobylarz, Wyżni Toporowy Staw and Ornak and the other including the remaining six populations from: Kopa Magury, Sarnia Skała, Kominy Tylkowe, Hala Gąsienicowa, Hala Goryczkowa and Morskie Oko. These groups are identical with those obtained for cones alone (see Fig. 1a in paper by Bobowicz and Krzakowa 1986). They are not correlated with the groups obtained for the anatomical variability of needles, they do not reflect the connection of the given population to the habitat and do not correspond to the altitude a.s.l. at which the populations grow.

- To sum up the results of the present investigations:

 1. The populations of dwarf mountain pine from the Tatra Mts., those from Kominy Tylkowe and Morskie Oko excepted, differ significantly in needle traits.
- 2. Statistically significant differences between the populations concern all the 13 needle traits jointly and each trait singly, with the exception of trait 3 describing the quotient of stomatal lines.
- Among the examined populations 2 (3) groups were distinguished.
 Among the studied populations those from Kominy Tylkowe, Morskie Oko and Wyżni Toporowy Staw are similar.
- 5. The most diverse population is that from Kobylarz.
- 6. The main traits deciding of the picture of variability are those describing: three epidermal cells width (8), distance of vascular bundles (12), height of needle crossection (10), Marcet's index (13), width of needle crossection (9), number of resin canals (6) and number of stomata on convex (4) and flat (5) sides of needle.
- 7. No relation was revealed between the antomical variability character of the needles and the type of habitat.
- 8. From the geographical aspect the Pinus mugo Turra populations are uniform—in needle traits—that is do not form groups.
- 9. The results of investigations of differences between populations as regards the complex of needle and cone traits confirm that the dwarf mountain pine populations are highly differentiated, decisive being in this respect the influence of the differentiation of cone traits.

- 10. No relation was detected between the undersoil type and population groups distinguished for the complex of needle and cone traits.
- 11. Variability pictures obtained for needle and cone traits studied separately and jointly are not similar.
- 12. As far as joint traits of needles and cones are concerned, geographical (spatial) variability is of identical character as that of cone traits: two groups of populations have been distinguished of composition identical as in the case of cones alone.
- 13. No relation was revealed between the character of the anatomical-morphological variability character for the joint needle and cone traits to the altitude a.s.l. at which the populations grow.

Acknowledgments

The authors wish to express their thanks to the Management of the National Park of the Tatra Mountains for permission of needles collection, to Professor Jerzy Szweykowski for reading the manuscript as well as to Mrs. Barbara Malchrowicz and Miss Izabela Freitag for technical assistance. This study was supported by the Polish Academy of Sciences, grant No. MR II/6.

REFERENCES

- Bobowicz M. A. 1984. Variability of needles in Polish populations of Scots pine (*Pinus sylvestris* L.). Bull. Soc. Amis. Sci. Lett. Poznań, Ser. D Sci. Biol. 24: 97-104.
- Bobowicz M. A., Błasińska A., Szweykowski J., 1983a. Variability of cones in Polish populations of Scotch pine (*Pinus sylvestris* L.). Bull. Soc. Amis. Sci. Lett. Poznań, Ser. D. Sci. Biol. 22: 51-61.
- Bobowicz M. A., Krzakowa M., 1986. Morphological differences between *Pinus mugo* Turra populations from Tatra Mts. revealed by cone traits. Acta Soc. Bot. Pol. 55: 000-000.
- Bobowicz M. A., Szweykowski J., Koźlicka M., 1983b. The variability of morphological traits in the population of *Pinus mugo* Turra on Borowina peat bog in the Izerskie Mts. (SW Poland). Bull. Soc. Amis. Sci. Lett. Poznań. Ser. D Sci. Biol. 22: 83-105.
- Bobowcz M. A., Szweykowski J., Mendelak M., 1983c. Morphological characteristics of an arificial sea-shore *Pinus mugo* Turra population. Bull. Soc. Amis. Sci. Lett. Poznań, Ser. D Sci. Biol. 22: 63-82.
- Bryant E. H. 1977. Morphometric adaptation of the housefly *Musca domestica* L. in the United States. Evolution 31: 580-596.
- Caliński T., 1970. Wielozmienna analiza wariancji i pokrewne metody wielowymiarowe. Materiały Kursu Szkoleniowego PTB i Wydziału V PAN, Warszawa.
- Caliński T., Czajka S., Kaczmarek Z., 1975. Principle component analysis and its application, (In Polish). Rocz. Akad. Roln., Poznań, Algor. Biometr. Statyst. 36: 159-185.
- Caliński T., Dyczkowski A., Kaczmarek Z., 1976. Testing the hypotheses in multivariate analysis of variance and covariance. (In Polish). Rocz. Akad. Roln., Poznań, Algor. Biometr. Statyst. 45: 77-113.

- Caliński T., Kaczmarek Z., 1969. A note on the calculation and use of the generalized distance between multivariate samples. Zesz. Nauk. UAM. Geografia 8: 7-13.
- Caliński T., Kaczmarek Z., 1973. Metody kompleksowej analizy doświadczenia wielocechowego. Wydz. V PAN i PTB, Warszawa-Wrocław.
- Ceranka B., Chudzik H., Czajka S., Kaczmarek Z., 1976. Multivariate analysis of variance for one-way classification. (In Polish). Rocz. Akad. Roln., Poznań, Algor. Biometr. Statyst. 86: 3-21.
- Fankhauser F., 1926. Beitrage zur Kenntnis der Bergfohren, Festschr. z. 50. Jahr. Bestehen Eidgenonss. Insp. Forstwesen, Jagd Fischerei, pp. 65-126.
- Gaussen H., Heywood V. H., Chater A. O., 1964. Flora Europea. Cambridge Univ. Press. Cambridge.
- Karoński M., Caliński T., 1973. Grouping in multivariate populations on the basis of Euklidean distances. Rocz. Akad. Roln., Poznań, Algor. Biometr. Statyst. 17: 117-129.
- Krzyśko M. 1982. Discriminant analysis. (In Polish). Wyd. UAM w Poznaniu, Ser. Matematyka. No 16. Poznań.
- Krzyśko M., Szweykowski J., Bobowicz M.A., Krzakowa M., 1980. Canonical analysis in genetic research. X. Kollokwium Metodologiczne Agrobiologii PAN, Warszawa, pp. 279-300.
- Marcet E., 1967. Über den Nachweis spontaner Hybriden von *Pinus mugo* Turra und *Pinus sylvestris* L. auf. Ground von Nadelmerkmalen. Ber. Schweiz. Bot. Ges. 77: 314-361.
- Pilichody A., 1908. Über die Bergkiefer im Jura und ihre Verwendung bei Aufforstungen von Frostlochern, Schweiz. Z. Forstwes, 59: 175-181.
- Rothmaler W., 1963. Eskursionsflora von Deutschland. IV. Kritischer Erganzungsband, Gefasspflanzen. Volk und Wissen Volkseigenen Verlag, Berlin.
- Sneath P. H., Sokal R. R., 1973. Numerical taxonomy. The principles and practice of numerical classification. W. H. Freeman and Comp., San Francisco.
- Szweykowski J., 1969. The variability of *Pinus mugo* Turra in Poland. Bull. Soc. Amis. Sci. Lett. Poznań, Ser. D Sci. Biol. 10: 39-54.
- Szweykowski J., Bobowicz M. A., 1977. Variability of *Pinus mugo* Turra in Poland IV. Needles and cones in some Polish populations. Bull. Soc. Amis. Sci. Lett. Poznań, Ser. D Sci. Biol. 17: 3-14.
- Szweykowski J., Bobowicz M. A., Koźlicka M., 1976a. The variability of *Pinus mugo* Turra in Poland. III. A natural population from Borowina in Góry Izerskie Mts. (SW Poland). Bull. Soc. Amis, Sci. Lett. Poznań, Ser. D Sci. Biol. 16: 17-28.
- Szweykowski J., Mendelak M., Bobowicz M. A., 1976b. The variability of *Pinus mugo* Turra in Poland. II. An artificial sea-shore population. Bull. Soc. Amis. Sci. Lett. Poznań, Ser. D Sci. Biol. 16: 3-16.

Różnice w budowie anatomicznej igiel naturalnych populacji kosodrzewiny (Pinus mugo Turra) z równoczesnym uwzględnieniem cech szyszek

Streszczenie

Z 429 osobników kosodrzewiny. *Pinus mugo* Turra, pochodzących z 10 populacji tego gatunku z Tatr – po 5 populacji z podloża wapiennego i bezwapiennego – zebrano 2-letnie igly, które opracowano pod względem 13 cech anatomicznych. Aby uzyskać pełny

obraz zmienności kosodrzewiny wykonano także badanie zróżnicowania 10 populacji (385 roślin) pod względem 27 cech igieł i szyszek. Dane uzyskane z pomiarów były podstawą do wykonania wielozmiennej analizy wariancji wraz z testowaniem hipotez statystycznych, analizy zmiennych dyskryminacyjnych, obliczenia odległości Mahalanobisa wraz z dendrytem zbudowanym na najkrótszych odległosciach Mahalanobisa oraz przeprowadzenia grupowania aglomeratywnego metodą najbliższego sąsiedztwa. Na podstawie wyników z wymienionych analiz stwierdzono, że rozpatrywane populacje kosodrzewiny różnią się statystycznie istotnie pod względem całego kompleksu badanych cech igieł i tworzą 3 grupy nie skorelowane z typem podłoża, na którym rosną. Najbardziej podobne są populacje z Kominów Tylkowych, Morskiego Oka i Wyżniego Toporowego Stawu. Najbardziej odrębna jest populacja z Kobylarza. Badanie zróżnicowania populacji w kompleksie cech igieł i szyszek dowiodło, że decydujący wpływ na obraz zmienności mają cechy szyszek.