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Genetic variation of F_1 hybrids from controlled crosses between *Pinus montana* var. *rostrata* and *Pinus sylvestris* in morphological needle traits

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Abstract. One hundred and seventeen hybrids, representing ten families from controlled crosses between *Pinus montana* var. *rostrata* and *Pinus sylvestris,* were examined in respect to six morphological traits of two-year-old needles. The biometric data obtained from the measurements provided a basis for multivariate statistical analyses, including discriminant analysis and Mahalanobis distances as principal methods. The analysed families formed two complexes, which were significantly different. The main traits responsible for the distinction were numbers of stomatal rows on both sides of the needles.

Key words: *Pinus montana*, *Pinus mugo*, *Pinus sylvestris*, F_1 hybrids, morphology of needles, multivariate analysis.

Introduction

Natural hybridization between species and introgression are important mechanisms of evolutionary processes, which can result in speciation and – as a last consequence – in the formation of new taxa. Hybrids increase the genetic variation and may have the ability to inhabit ecological niches which are not suitable for the parental species. Additionally, hybrids often possess traits useful for breeding purposes, e.g. good growth and resistance to biotic and abiotic stress factors (DE PHAMPHILIS, WYATT 1990, NASON et al. 1992, ARNOLD 1997, PRUS-GŁOWACKI, STEPHAN 1998).

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The two pine species *Pinus montana* Mill. (taxon of the *P. mugo* Turra complex) and *Pinus sylvestris* L. occur sympatrically in mountains of Central Europe and have, therefore, the potential for interspecific hybridization, presumably since the Pleistocene period (PRAVDIN 1964, CHRISTENSEN 1987a, b, c, KANAK 1994). Only some of the developed F_1 hybrids are fertile, while most of them are fully sterile. The fertile hybrids may form small amounts of viable pollen and seed due to disturbances during meiosis and by autogamy. Backcrossing of F_1 hybrids with both parental species, which grow nearby and produce large amounts of viable pollen, is more frequent phenomenon than reciprocal crosses between poorly fertile F_1 hybrids (STEBBINS 1963, STACE 1993). Backcrossing can cause gene flow from parental species to hybrids and can finally cause introgression (ANDERSON, HUBRICHT 1938). Thus, the fertile hybrids are capable of forming hybrid swarms, consisting of all possible backcross combinations, in F_2 hybrids and in subsequent generations. The presence of hybrid swarms may point to the existence of ecological niches that are suitable for highly variable hybrid families, but not for the parental species. This process has been defined as 'hybridization of a biotope' by ANDERSON (1949). Natural hybridization of *Pinus montana* with *Pinus sylvestris* has been confirmed by numerous studies of hybrid swarms, focused on anatomical and morphological traits of needles, cones and seed (MARCET 1967, STASZKIEWICZ, TYSZKIEWICZ 1969 a, b, 1972, SZWEYKOWSKI 1969, SZWEYKOWSKI, BOBOWICZ 1977, TYSZKIEWICZ 1979, KRZAKOWA et al. 1984, BOBOWICZ 1988, 1990 a, b, STASZKIEWICZ 1993 a, b, c, BĄCZKIEWICZ 1995, CHRISTENSEN, DAR 1996, LAURANSON-BROYER et al. 1997), by studies on serological similarity (PRUS-GŁOWACKI et al. 1978, 1981, PRUS-GŁOWACKI, SZWEYKOWSKI 1979, 1980), on enzymatic variation (PRUS-GŁOWACKI, SZWEYKOWSKI 1983, KRZAKOWA et al. 1984, SIEDLEWSKA 1994, SIEDLEWSKA, PRUS-GŁOWACKI 1994, 1995), on phenolic compounds (SZWEYKOWSKI, URBANIAK 1982, KRZACZEK, URBANIAK 1985), and on chloroplast DNA (FILPPULA et al. 1992). However, the results obtained were in many cases not unequivocal – in relation to the existence and rate of hybridization and pattern of inheritance of particular traits. Thus, corresponding studies were desirable on artificial hybrids of known parents.

DENGLER (1932, 1939, 1941), JOHNSON (1939) and SCHMIDT (1951) described for the first time artificial hybrids between *P. montana* and *P. sylvestris*, which were viable and fertile. However, the primary aim of the investigated crossings was to test if the resistance of *P. montana* against the needle-cast fungus (*Lophodermium seditiosum*) and the growth performance of *P. sylvestris* could be expressed in the progenies. Results regarding needle morphology of F_1 hybrids from controlled crosses have not been published yet. In this paper ten F_1 hybrid families were compared with each other and with their parents in respect of variation in needle morphology and the usefulness of the respective traits for their differentiation.

Material and methods

Material used in the study included seven maternal clones of *Pinus montana* var. *rostrata*, two paternal clones of *Pinus sylvestris* and ten hybrid families from controlled crosses (Table 1). We used for the maternal individuals the name *P. montana* after Professor W. Langner who collected the plants. We are aware of the difficulty in deciding of the taxonomically correct position of the plant in the complicated *P. mugo* complex (PRUS-GŁOWACKI, STEPHAN 1998). For each group of the parental trees mean values of morphological needle traits were estimated, as the patterns of the studied traits were species-specific. Detailed analysis of each hybrid family together with its parents will be described elsewhere.

Table 1. Clones of *Pinus montana* var. *rostrata* and *P. sylvestris*, types of crosses and numbers of investigated hybrid progenies

P. montana var. *rostrata* maternal	*P. sylvestris* paternal	Hybrid family no.	Number of investigated progenies
Ramsau			
R1	Schl 77/1 (S12)	51	16
R7	Schl 77/1 (S12)	55	8
R10	Schl 77/1 (S12)	56	6
Königseggwald			
K9	Hasl E203 (S11)	57	10
K9	Schl 77/1 (S12)	58	10
K11	Schl 77/1 (S12)	60	11
K13	Hasl E203 (S11)	61	8
K13	Schl 77/1 (S12)	62	8
K14	Hasl E203 (S11)	63	20
K14	Schl 77/1 (S12)	64	20
Total 7	2	10	117

The hybrid families originated from controlled crosses of *P. montana* var. *rostrata* with the pollen of *P. sylvestris*. The paternal tree of *P. sylvestris* no. S12 (Schl 77/1) originated from the forest district Schleswig (Idstedt) and the paternal tree no. S11 (Hasl E203) from the forest district Rantzau (Hasloh). Both locations are situated in northern Germany. Trees of *P. montana* var. *rostrata* marked with R (R1, R7, R10) originated from Ramsau, district Wimbach (Bavaria), while

those marked with K (K9, K11, K13, K14) originated from Königseggwald in Franconian Alps (Schwaben), locations in southern Germany. The parental individuals were propagated by grafting and the clones were planted in the pine collection at Grosshansdorf (northern Germany).

The controlled crosses were carried out in the spring of 1977 (Table 1). Seed was collected in the autumn of 1978 and sown in the spring of 1981 in the forest nursery of the Institute for Forest Genetics and Forest Tree Breeding, Grosshansdorf. The seedling families were planted in a field trial with four plants per plot and in two replications in the spring of 1984. The existence of the F_1 hybrids offered the opportunity for investigations into patterns of variation in needle morphology.

In the present study six morphological needle traits of the F_1 hybrids were analysed and compared with those of the parental clones of *P. sylvestris* and *P. montana* var. *rostrata*. Two-year-old needles were collected from the individual trees in 1993 and preserved in 70% ethanol. This was followed by preparing semipermanent preparations. From each tree ten short shoots were taken randomly. From each short shoot one needle was used for the analysis of morphological traits. From the second needle a cross-section was made at half the length of the needle and embedded in polyvinyl alcohol. These preparations were used for anatomic analysis (BOBOWICZ et al. 2000).

The following morphological needle traits were analysed: (1) needle length (mm); (2) number of stomatal rows on the convex side of the needle; (3) number of stomatal rows on the flat side of the needle; (4) number of stomatal rows on the convex side of the needle divided by the number of stomatal rows on the flat side of the needle; (5) number of stomata per 2 mm length of the convex side at half the length of the needle; (6) number of stomata per 2 mm length of the flat side at half the length of the needle.

From the biometric data of the measured needle traits the following indices were calculated at the Centre of Informatics, Adam Mickiewicz University, Poznań: major statistical characteristics of the investigated traits, coefficients of correlation between the traits (WILLIAMS 1995, ŁOMNICKI 1999, FERGUSON, TAKANE 1999), Student's *t* distribution and test *t* (FISHER 1925, TRIOLA 1998), discriminant analysis (CALIŃSKI et al. 1975, KRZYŚKO 1979, 1982, 1990, SOKAL, ROHLF 1997), Mahalanobis distances between particular families of hybrids and parental species together with Hotelling's T^2 statistics and a minimum spanning tree, constructed on the basis of the shortest Mahalanobis distances (HOTELLING 1957, KRZYŚKO 1979, 1982, 1990). In order to detect the effect of each trait from the applied set of traits, for each pair of traits diagrams were prepared in all possible combinations of traits (FERGUSON, TAKANE 1999).

Results

Variation in needle traits

The six investigated needle traits show a high variation within and between the hybrid families and their parent species *P. montana* var. *rostrata* (*Pm*) and *P. sylvestris* (*Ps*) (Table 2). Particularly variable was needle length (trait 1), with mean values between 48.0 mm (no. 62) and 59.1 mm (no. 61), individual values between 18.0 mm (no. 57) and 96.0 mm (no. 64), and an overall average of 54.7 mm. The high variation in needle length was also expressed by the relatively high variation coefficients, between about 10% (no. 58) and 30% (no. 57), with an average value of 17%. Needle length of the parent species was generally smaller than that of the hybrids (mean values: 45.3 mm in *Pm* and 47.0 mm in *Ps*).

A generally high variation was also shown by the quotient of stomatal rows (trait 4), where the mean values of the variation coefficient amounted to more than 20% in five hybrid families. But also the other five families had high variation coefficients (over 17%). For *Pm* the variation coefficient of the trait was around 20.5%, while for *Ps* it amounted to 18.5%. Variation in simple traits, the number of stomatal rows on the convex side (trait 2) or on the flat side (trait 3) of the needle, which were used for the calculation of stomatal row quotient (trait 4), ranged from 18.5% (about 15% for each parental species) for trait 2 to 19.5% (20.4% for *Pm*, 12.4% for *Ps*) for trait 3.

The least variable was the number of stomata per 2 mm length of the flat side at half the length of the needle (trait 6), for which the average value of the variation coefficient was about 7%. The variation in trait 6 was also low in both parental species, with 7.8% for *Pm* and 4.8% for *Ps*. Also variation in the number of stomata per 2 mm length of the convex side at half the length of the needle (trait 5) was a relatively low, with mean coefficient values of 7.4%. Also for trait 5 *Pm* had a higher variation coefficient (8.5%) than *Ps* (about 5%).

In an analysis of correlation coefficients of the six morphological traits, significant correlation coefficients could be found between the ten hybrid families. The highest proportion of almost 67% highly significant correlation coefficients was noted in progenies of cross no. 63 while the lowest proportion (20%) was recorded in cross no. 56. In all hybrids as well as in the parental species the strongest correlations were observed for the numbers of stomatal rows on both sides of the needle (traits 3 and 4), and for the numbers of stomata on both sides of the needle (traits 5 and 6).

Differences between hybrid families and parental species

To detect significant differences between mean values of the traits investigated in particular hybrid families and the parental species, Student's t distribution was calculated with the respective t test. Results of the testing provided data for Table 3.

Table 2. Six morphological needle traits of ten hybrid families and their parental species *Pinus montana* var. *rostrata* (*Pm*) and *P. sylvestris* (*Ps*): minimum (min), maximum (max) and mean ($\bar{x}$) values, standard deviation (s.d.) and variation coefficient (coeff. of var. %)

Family no. n = individuals	Statistical characteristic	Morphological needle traits (see: Material and methods)					
		1	2	3	4	5	5
1	2	3	4	5	6	7	8
51 n=16	min ... $\bar{x}$... max	38.0 ... **54.3** ... 80.0	6.0 ... **9.8** ... 14.0	4.0 ... **6.6** ... 10.0	0.7 ... **1.5** ... 3.0	13.0 ... **17.4** ... 20.6	14.3 ... **17.4** ... 20.3
	s.d.	7.63	1.60	1.24	0.35	1.50	1.12
	coeff. of var. %	14.06	16.30	18.81	22.58	8.59	6.43
55 n=8	min ... $\bar{x}$... max	40.0 ... **57.8** ... 80.0	7.0 ... **9.6** ... 13.0	4.0 ... **6.3** ... 9.0	1.0 ... **1.5** ... 2.5	15.0 ... **18.3** ... 22.0	16.3 ... **18.6** ... 22.3
	s.d.	9.73	1.71	1.20	0.24	1.37	1.04
	coeff. of var. %	16.83	17.88	18.95	17.76	7.50	5.59
56 n=6	min ... $\bar{x}$... max	38.0 ... **56.6** ... 71.0	7.0 ... **10.3** ... 15.0	4.0 ... **7.1** ... 10.0	1.0 ... **1.5** ... 2.5	16.3 ... **18.2** ... 20.6	16.3 ... **18.8** ... 20.6
	s.d.	8.84	1.54	1.21	0.32	1.19	1.10
	coeff. of var. %	15.62	14.99	16.98	21.45	6.55	5.84
57 n=10	min ... $\bar{x}$... max	18.0 ... **51.4** ... 78.0	7.0 ... **11.1** ... 15.0	5.0 ... **8.5** ... 15.0	0.9 ...**1.3** ... 1.9	15.6 ... **18.8** ... 21.3	16.0 ... **18.9** ... 21.6
	s.d.	15.80	1.87	1.89	0.24	1.35	1.33
	coeff. of var. %	30.72	16.87	22.21	17.80	7.19	7.03
58 n=10	min ... $\bar{x}$... max	39.0 ... **53.1** ... 65.0	6.0 ... **10.3** ... 13.0	5.0 ... **7.6** ... 11.0	0.9 ... **1.4** ... 2.0	14.3 ... **18.4** ... 21.3	15.0 ... **18.3** ... 21.6
	s.d.	5.26	1.59	1.55	0.26	1.52	1.43
	coeff. of var. %	9.91	15.46	20.25	18.71	8.28	7.82
60 n=11	min ... $\bar{x}$... max	46.0 ... **58.6** ... 72.0	4.0 ... **6.8** ... 10.0	5.0 ... **8.3** ... 12.0	0.4 ... **0.9** ... 1.2	14.3 ... **18.4** ... 22.6	15.6 ... **19.5** ... 22.6
	s.d.	6.38	1.23	1.24	0.17	1.66	1.73
	coeff. of var.%	10.89	18.07	14.99	19.89	9.02	8.87

1	2	3	4	5	6	7	8
61	min ... x̄... max	37.0 ... **59.1** ... 75.0	5.0 ... **7.3** ... 12.0	6.0 ... **8.7** ... 13.0	0.6 ... **0.9** ... 1.5	14.0 ... **17.4** ... 20.6	14.3 ... **18.6** ... 21.3
	s.d.	9.36	1.52	1.75	0.18	1.72	1.48
n=8	coeff. of var. %	15.82	20.87	20.10	21.30	9.90	7.97
62	min ... x̄... max	36.0 ... **48.0** ... 66.0	3.0 ... **6.2** ... 10.0	5.0 ... **7.7** ... 11.0	0.4 ... **0.8** ... 1.2	15.0 ... **17.7** ... 21.0	15.6 ... **18.6** ... 22.0
	s.d.	7.50	1.32	1.52	0.15	1.29	1.14
n=8	coeff. of var. %	15.64	21.23	19.65	18.23	7.28	6.15
63	min ... x̄... max	25.0 ... **53.2** ... 79.0	3.0 ... **6.8** ... 12.0	3.0 ... **7.4** ... 12.0	0.4 ... **0.9** ... 1.8	11.0 ... **17.7** ... 20.6	13.6 ... **18.4** ... 21.0
	s.d.	9.31	1.55	1.68	0.19	1.80	1.35
n=20	coeff. of var. %	17.50	22.93	22.69	20.59	10.22	7.35
64	min ... x̄... max	31.0 ... **55.0** ... 96.0	3.0 ... **5.9** ... 9.0	3.0 ... **6.7** ... 10.0	0.4 ... **0.9** ... 1.8	14.3 ... **17.0** ... 19.6	15.0 ... **17.4** ... 20.0
	s.d.	12.26	1.22	1.40	0.21	1.30	1.24
n=20	coeff. of var.%	22.29	20.63	20.81	22.75	7.64	7.15
Pm	min ... x̄... max	34.0 ... **45.3** ... 63.0	6.0 ... **8.3** ... 12.0	4.0 ... **5.5** ... 9.0	0.9 ... **1.6** ... 2.3	13.6 ... **17.0** ... 20.3	13.6 ... **17.3** ... 20.0
	s.d.	6.69	1.25	1.11	0.32	1.44	1.35
n=7	coeff. of var. %	14.77	14.98	20.38	20.47	8.51	7.81
Ps	min ... x̄... max	32.0 ... **47.0** ... 64.0	8.0 ... **10.7** ... 14.0	6.0 ... **7.6** ... 9.0	1.0 ... **1.4** ... 2.0	17.3 ... **18.8** ... 21.0	17.0 ... **19.2** ... 21.3
	s.d.	8.98	1.63	0.94	0.26	0.93	0.92
n=2	coeff. of var. %	19.11	15.19	12.37	18.48	4.95	4.79

Table 3. Significance of differences between hybrid families nos.51-64 and their parental species *P. montana* var. *rostrata* (*Pm*) and *P. sylvestris* (*Ps*) in six morphological needle traits, assessed by Student's *t* test.

Comparison parental species/ hybrid family no.	Six needle traits (see Material and methods)					
	1	2	3	4	5	6
Pm/51	**	**	**	ns	*	ns
Ps/51	**	*	**	ns	**	**
Pm/55	**	**	**	ns	**	**
Ps/55	**	**	**	ns	ns	**
Pm/56	**	**	**	ns	**	**
Ps/56	**	ns	ns	ns	*	ns
Pm/57	**	**	**	**	**	**
Ps/57	ns	ns	*	ns	ns	ns
Pm/58	**	**	**	**	**	**
Ps/58	**	ns	ns	ns	ns	**
Pm/60	**	**	**	**	**	**
Ps/60	**	**	*	**	ns	ns
Pm/61	**	**	**	**	ns	**
Ps/61	**	**	**	**	**	ns
Pm/62	*	**	**	**	**	**
Ps/62	ns	**	ns	**	**	*
Pm/63	**	**	**	**	**	**
Ps/63	**	**	ns	**	**	*
Pm/64	**	**	**	**	ns	ns
Ps/64	**	**	**	**	**	**

** significant at $\alpha = 0.01$, * significant at $\alpha = 0.05$, ns = not significant.

It can be noticed that five hybrid families (nos. 57, 58, 60, 62 and 63) differed at various levels of significance from *Pm* in all studied traits. In contrast, only one hybrid family (no. 64) differed highly significantly from *Ps* in all six morphological needle traits. Especially hybrid family no. 57, which differed only in respect of the number of stomatal rows on the flat side of the needle (trait 3), showed a great similarity with the parental species *Ps*. Also nos. 56 and 58 could be distinguished from *Ps* by only two traits: needle length (trait 1) and number of stomata per 2 mm either on the convex (trait 5) or on the flat side (trait 6) of the needle.

The trait which differentiated the highest number of hybrid families and parental species was needle length (trait 1). Regarding this trait only nos. 57 and 62 could not be distinguished from *Ps*. The quotient of stomatal rows (trait 4) differentiated significantly the lowest numbers of hybrid families and parental species.

Table 4. Table of Mahalanobis distances (D) together with values of Hotelling's T^2 statistics for 10 examined hybrid families (nos. 51-64) and for their parental species *P. montana* var. *rostrata* (*Pm*) and *P. sylvestris* (*Ps*).

$T^2_{0.01} = 99.226$, $T^2_{0.05} = 88.856$

Hybrids parental species	Mahalanobis distance											
55	D	1.03										
	T^2	56.802										
56	D	1.15	0.79									
	T^2	57.790	21.604									
57	D	1.86	1.85	1.20								
	T^2	212.844	152.000	53.895								
58	D	1.13	1.18	0.72	0.83							
	T^2	78.515	61.962	19.221	34.119							
60	D	3.71	3.28	3.31	3.50	3.27						
	T^2	898.320	497.556	424.426	642.149	560.420						
61	D	3.41	3.23	3.19	3.36	3.10	1.05					
	T^2	619.921	417.190	349.194	500.899	427.142	50.586					
62	D	3.54	3.34	3.39	3.50	3.20	1.28	1.48				
	T^2	667.580	446.896	393.249	545.357	455.458	76.376	87.756				
63	D	2.94	2.72	2.79	3.05	2.65	1.18	1.25	0.80			
	T^2	770.893	421.538	356.372	621.527	469.630	98.945	88.975	36.496			
64	D	3.16	3.10	3.26	3.66	3.12	1.96	1.82	1.48	1.04		
	T^2	886.081	547.414	489.652	894.479	649.445	272.333	189.028	124.517	109.136		
Pm	D	1.55	1.86	2.27	2.89	2.24	3.80	3.60	3.29	2.93	3.11	
	T^2	117.222	128.901	166.039	343.592	206.754	617.269	484.333	404.574	445.136	500.302	
Ps	D	1.87	1.71	1.20	0.85	1.04	3.64	3.65	3.51	3.11	3.71	2.56
	T^2	61.859	46.897	21.764	12.164	18.087	224.664	212.598	197.271	176.003	254.001	101.959
		51	55	56	57	58	60	61	62	63	64	Pm

Trait 4 divided the examined hybrid families into three groups. The first group containing hybrid families nos. 51, 55 and 56 did not differ from the parental species *Ps* and *Pm*. The second group with hybrid families nos. 60, 61, 62, 63, and 64 differed highly significantly from both parental species. And the third group with hybrid families nos. 57 and 58 could only be distinguished from the parental species *Pm*.

Mahalanobis distances between hybrid families and parental species

For a precise intercross differentiation, Mahalanobis distances were calculated, the significance of which was tested using Hotelling's T^2 statistics. The results of analysis of Mahalanobis distances are presented in Table 4 and demonstrate the extensive intercross variation. Particularly hybrid family no. 64 differed highly significantly ($T^2_{calc.} > T^2_{0.01}$) from all the remaining families and parental species. All hybrid families differed highly significantly from *Pm*. Only nos. 60, 61, 62, 63 and 64 differed at a high significance level from *Ps* ($T^2_{calc} > T^2_{0.05}$), while hybrid families nos. 51, 55, 56, 57 and 58 did not differ significantly from *Ps*. The parental species *Ps* and *Pm* differed highly significantly from each other ($T^2_{calc} > T^2_{0.01}$).

The minimum spanning tree for ten crosses and for the parental species, constructed on the basis of the shortest Mahalanobis distances, is presented in Figure 1. Hybrid families nos. 51, 55, 56, 57 and 58 did not differ significantly from each other ($T^2_{calc} < T^2_{0.01}$) and occupied positions between the parental species. Only the distance between hybrid family no. 51 and *Pm* attests to a highly significant difference. This complex differed highly significantly from the other complex, containing families nos. 60, 61, 62, 63 and 64. The Mahalanobis distance between nos. 58 and 63 represented the greatest distance in the entire minimum spanning tree. Family no. 63 differed also at various levels of significance from nos. 64 and 60.

Discriminant analysis of morphological needle traits

The above-mentioned complexes of ten hybrid families were also easily distinguishable in the graph of the discriminant analysis in the space of the first three discriminant variables, U_1, U_2, U_3, (Figure 2). Determination coefficients between six traits of needle morphology in the hybrid families and their parental species *Pm* and *Ps* and the first three discriminating variables U_1, U_2, U_3, made it possible to distinguish the traits that exerted a major impact on the obtained hybrid variation patterns: the number of stomatal rows (trait 2) and the quotient of stomatal rows (trait 4) for the first discriminant variable U_1, and the number of stomatal rows (trait 3) and the number of stomata on the flat side of the needle (trait 6) for the second discriminant variable U_2.

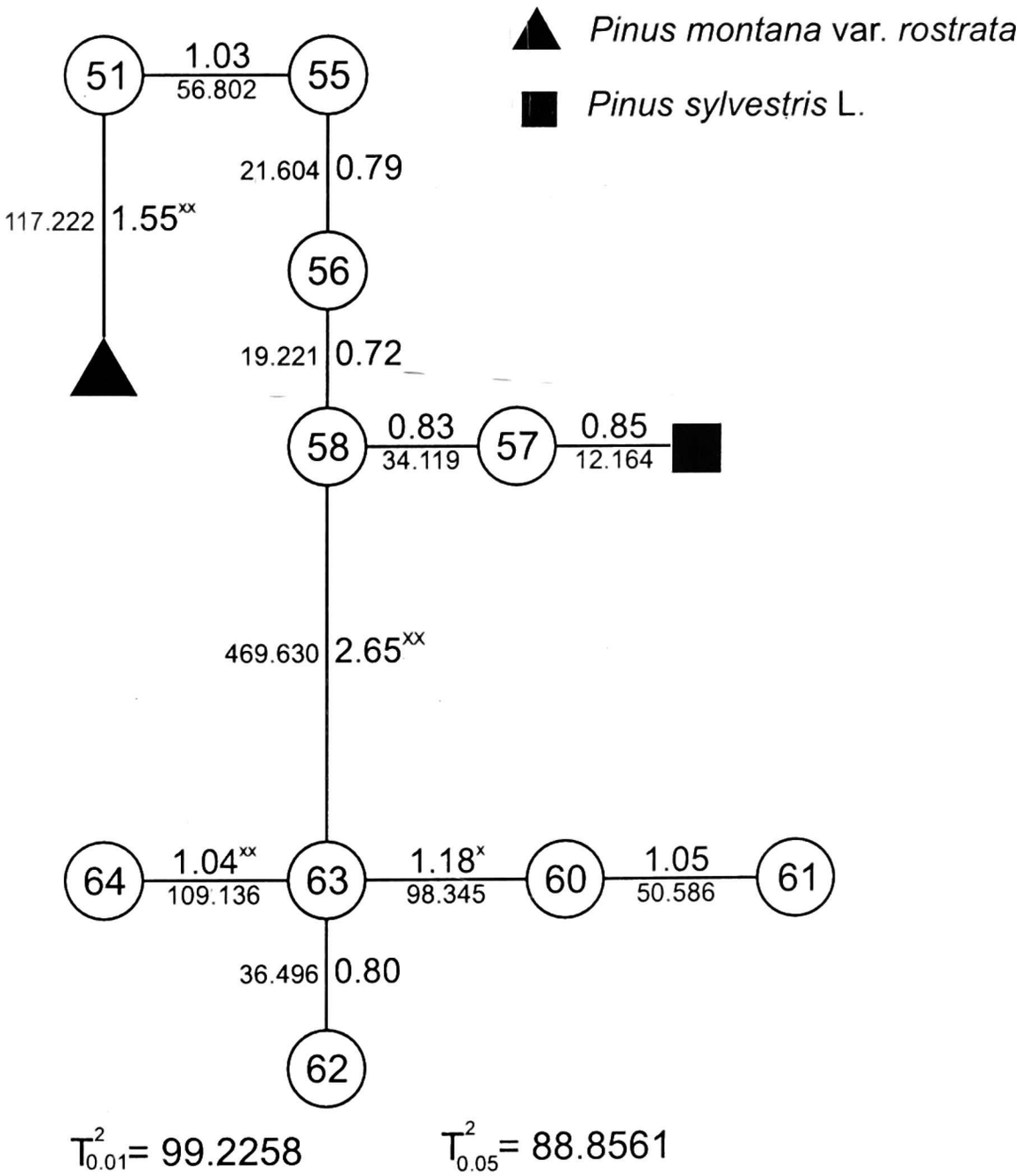

Figure 1. Minimum spanning tree for ten hybrid families of controlled crosses (nos. 51-64) and for their parental species *Pinus montana* var. *rostrata* and *Pinus sylvestris*, constructed on the basis of shortest Mahalanobis distances (large numbers) with values of Hotelling's T^2 testing statistics (small numbers). $T^2_{0.01}$ and $T^2_{0.05}$ = critical values of Hotelling's T^2 statistics. xx = highly significant Mahalanobis distances ($T^2_{calc.} > T^2_{0.01}$), × = significant Mahalanobis distances ($T^2_{calc.} > T^2_{0.05}$).

Pairwise comparison of needle traits

The separation of the hybrid families into two groups was also evident in two-trait scatter diagrams. Especially the comparison with respect to the numbers of stomatal rows on each side of the needle (traits 2 and 3) with the remaining traits supported the distinction of two hybrid family groups (Figure 3). The remaining trait pairs analysed in the two-trait scatter diagram failed to provide such a clear-cut pattern of two groups of the examined hybrid families. Thus, in scatter

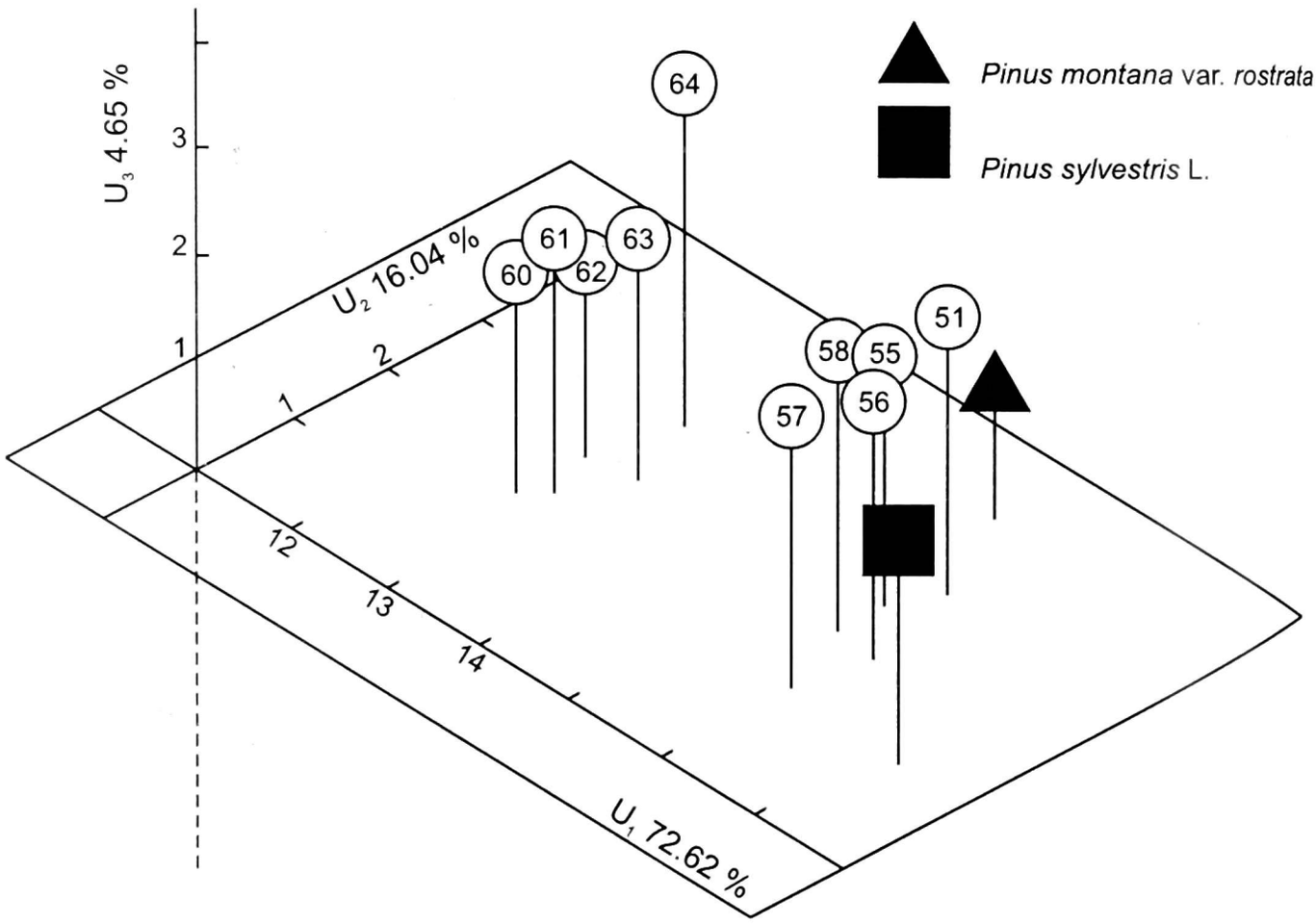

Figure 2. Results of analysis of disciminant variables for ten hybrid families of controlled crosses (nos. 51 - 64) and for their parental species *Pinus montana* var. *rostrata* and *Pinus sylvestris* in the space of the first three discriminant variables, U_1, U_2, U_3, including a total of 93.31% of information from the investigated set of six morphological needle traits.

diagrams analysing the number of stomatal rows on the flat side of the needle (trait 3) as related to the number of stomata on the convex (trait 5) or the flat (trait 6) side of the needle, most of the families took positions intermediate between those of the parental species. Some hybrid families transgressed even the variation range of *Ps* for each of the analysed traits (Figure 3). This pertained in particular to crosses nos. 57, 60, and 61. Comparing the trait pair of stomata numbers on each side of the needle (traits 5 and 6), all families showed positions intermediate between those of their parental species, with the exception of nos. 64 and 51, most similar to *Pm*, and no. 57, most similar to *Ps* (Figure 3).

If needle length (trait 1) was compared pairwise with the number of stomatal rows on the convex side of the needle (trait 2) or the stomatal row quotient (trait 4), the distinction between the earlier mentioned two groups was evident. Again, one group contained hybrid families nos. 51, 55, 56, 57 and 58, while the other group comprised families nos. 60, 61, 62, 63, and 64 (Figure 3).

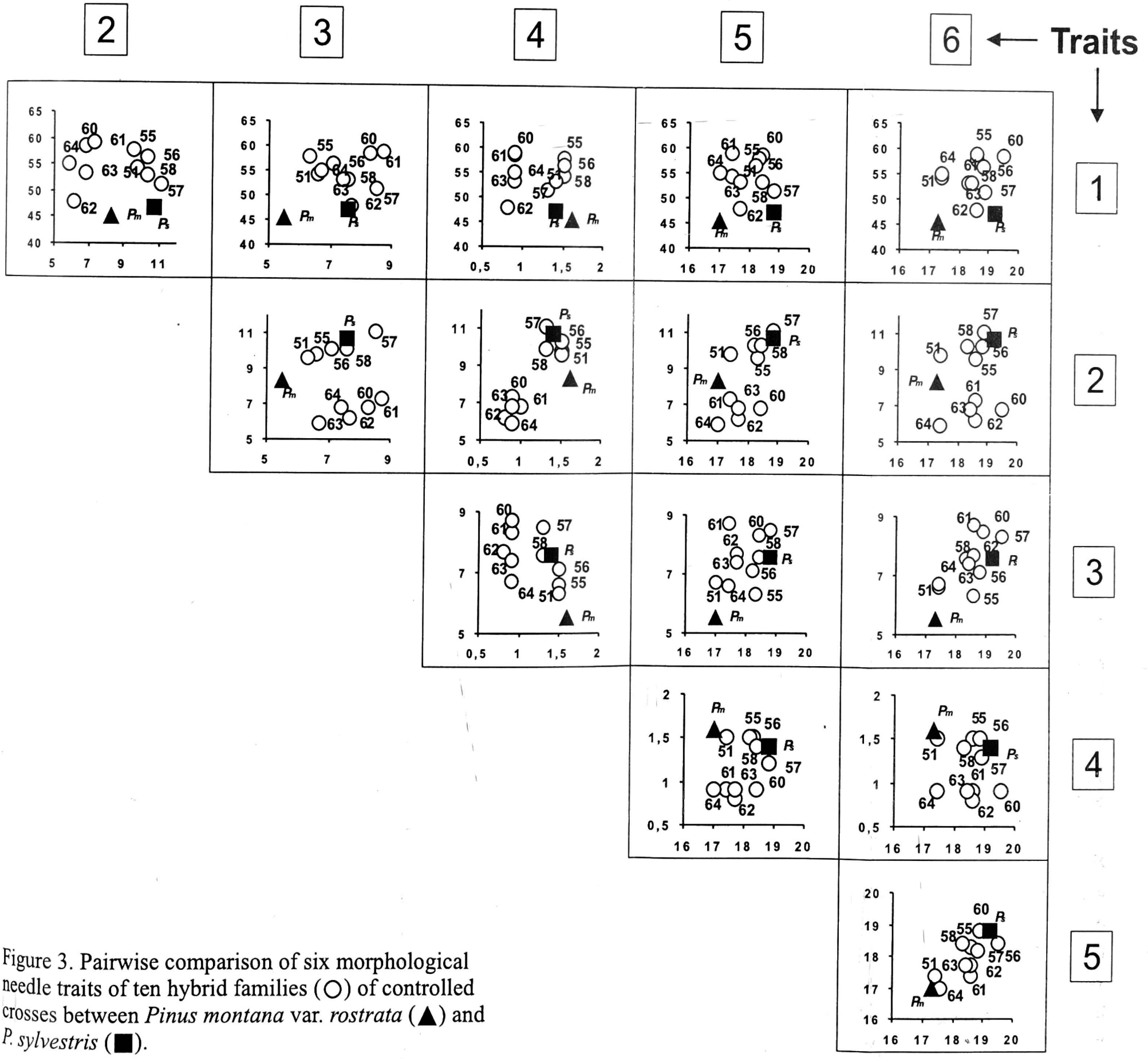

Figure 3. Pairwise comparison of six morphological needle traits of ten hybrid families (○) of controlled crosses between *Pinus montana* var. *rostrata* (▲) and *P. sylvestris* (■).

Discussion

The study shows that the ten hybrid families from controlled crosses between *Pinus montana* var. *rostrata* and *Pinus sylvestris* belong to two distinct, significantly different groups. One group of hybrid families with crosses nos. 51, 55, 56, 57, and 58 occupied intermediate positions between their parental species. For some traits they have transgressed the range shown by *P. sylvestris* and *P. montana* or have reached the parental values. The respective crosses are significantly distinct from *P. montana*, but less distinct from *P. sylvestris*. The conclusion is consistent with that of PRUS-GŁOWACKI and STEPHAN (1998), who analysed the same ten hybrid families in respect to the variation in antigenic proteins and isoenzymes. The authors found that most of the progenies decisively resembled *P. sylvestris* when tree S12 was the pollen donor. S12 was the pollen donor for crosses nos. 51, 55, 56, 58, except no. 57 with S11 as the father tree, but with the same mother tree as no. 58. Also hybrid family no. 57 resembled *P. sylvestris* regarding most traits of needle morphology.

The other group consisted of hybrid families nos. 60, 61, 62, 63, and 64. The complex was significantly distinct from the other group of hybrids and from its parental species. PRUS-GŁOWACKI and STEPHAN (1998) have linked the phenomenon of different characters of some crosses with the appearance of novel proteins in some hybrids, which were absent from the parents. The phenomenon has already been described in immunological studies of the alloploid grass hybrid *Lolium* × *Festuca* (PRUS-GŁOWACKI et al. 1971) and of *Alnus glutinosa* × *Alnus incana* (PRUS-GŁOWACKI, MEJNARTOWICZ 1992) as well as in isoenzymatic studies of hybrids from controlled pollination of *P. ponderosa* var. *ponderosa* × *P. ponderosa* var. *scopulorum* (LINHART et al. 1989). For hybrids of *Pseudotsuga menziesii* (NEALE et al. 1986, NEALE, WILLIAMS 1991), and for *P. contorta* and *P. banksiana* new variants of chloroplast DNA have been described, which were absent from the parents (WAGNER et al. 1987, GOVINDARAJU et al. 1989). Under natural conditions also interspecific hybrids of *P. montana* (*P. mugo* Turra) × *P. sylvestris* have manifested a similar phenomenon with the existence of 'nonparental' morphological and anatomical traits (STASZKIEWICZ, TYSZKIEWICZ 1969a, b, 1972, BOBOWICZ 1990a). However, spontaneous hybrids have been analysed in this aspect under varying environmental conditions and, therefore, they have not permitted to advance unequivocal hypotheses on the genetic basis of such variation. Detailed analysis of results obtained in this study allows to note that the division of hybrids into two complexes is reflected mainly by the numbers of stomata on each side of the needle. The traits exhibit a low variation and have obviously been highly conserved during evolution. Genes responsible for the development of appropriate stomata numbers and other morphological traits, most probably of polygenic type, may affect final needle characters both through their additive action, intragenic and intergenic

complementation and through the intragenic recombinations between individual alleles. Due to such processes, hybrid progenies include individuals resembling one or the other parental species as well as individuals with traits transgressing the parental traits which are distinct from either parental species.

Conclusions

The examined hybrid families formed two highly significantly different groups, determined by the quotient of numbers of stomata rows and particulary by the number of stomatal rows on the convex side of the needle. Hybrid families nos. 60, 61, 62, 63, and 64 formed one group and differed significantly from the other group of hybrid families and from either parental species. The hybrid families forming the second group (nos. 51, 55, 56, 57, and 58) exhibited characters intermediate between those shown by their parental species. In some traits, however, the hybrid families exhibited values transgressing ranges of parental species or closely resembled one of the parental species. They differed significantly from *P. montana* var. *rostrata*. Numbers of stomatal rows on the flat and the convex side of the needle represent the most variable traits. Numbers of stomata on the flat and the convex side of the needle are the least variable traits. Numbers of stomatal rows on either side of the needle seem to be under genetic control, expressed by their very high positive correlation coefficients. The same is true for numbers of stomata on either side of the needle. The present study once again proved that in population genetic studies a detailed analysis of individual traits is an indispensable supplementation of multivariate methods.

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