GENETIC VARIATION OF F₁ HYBRIDS
FROM CONTROLLED CROSSES BETWEEN
PINUS MONTANA VAR. *ROSTRATA* AND *PINUS SYLVESTRIS*
IN ANATOMICAL NEEDLE TRAITS

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ABSTRACT

One hundred and seventeen individuals representing ten hybrid families from controlled crosses between *Pinus montana* var. *rostrata* and *Pinus sylvestris* were examined with respect to eight traits of anatomy of their two-year old needles. The biometric data obtained by measurements of anatomical traits were used to conduct multivariate statistical analyses with discriminant analysis and Mahalanobis distances as principal methods. Some of the hybrid families were similar to parental species representing the typ *Montana* or *Sylvestris*, and some less numerous intermediate forms. The results of the anatomical studies correspond to a great extent to those obtained for isoenzymes and antigene proteins (Prus-Głowacki and Stephan, 1998).

KEY WORDS: *Pinus montana*, *Pinus sylvestris*, controlled F₁ hybrids, progenies, anatomy of needles, multivariate analysis.

INTRODUCTION

Pinus montana Mill. and *Pinus sylvestris* L. occur sympatrically and hybridize naturally. The fertile hybrids are often growing in hybrid swarms. The two species and their interspecific natural hybrids were investigated in numerous studies (e.g. Bobowicz 1984, 1990a, 1990b, 1993, Bobowicz and Krzakowa 1986, 1988; Bobowicz and Korczyk 1990, 1994, Bobowicz *et al.* 1995). Results have shown that genetic variation of hybrids and their parent species can be interpreted more easily and clearly when, for instance, morphological, anatomical and biochemical traits of needles, cones or seed are treated separately. Furthermore, studies on hybrids from controlled crosses would facilitate those investigations. The possibility was given by ten F₁ hybrid families from controlled crosses between *P. montana* Mill. var. *rostrata* (Ant.) Hoopes and *P. sylvestris* L. carried out in the Institute for Forest Genetics and Forest Tree Breeding, Grosshandorf, in 1977. The first results of serological and isoenzymatic studies (Prus-Głowacki and Stephan 1998), as well as of morphological needle traits (Bobowicz *et al.*, submitted) were already published. There are so far no published results about anatomical needle traits of hybrids from controlled crosses. The following studies involved descriptions of the variability pat-

tern between hybrid families and their parental species with respect to traits of needle anatomy. The attempt was made to find out whether the hybrid variation pattern in traits of needle anatomy is similar to the variation described earlier for the serological, isoenzymatic and morphological traits. The results were also expected to indicate principal traits of needle anatomy to determine characters suitable for studies on the variation within and between hybrid families.

MATERIAL AND METHODS

The plant material used for this study included seven clones of *Pinus montana* var. *rostrata* representing mother trees, two clones of *Pinus sylvestris* representing father trees, and 117 individuals representing ten hybrid families originating from controlled crosses. Both mothers and fathers were treated synthetically in the study and mean values of anatomical traits for each group of parents represented species specific patterns. Analysis of each hybrid family together with the respective parents will be presented in a subsequent paper.

The hybrid families originated from controlled pollination of *Pinus montana* var. *rostrata* with pollen of *Pinus sylvestris*. The paternal trees (*Pinus sylvestris*) no. S12 (Schl 77/1)

originated from the forest district Schleswig (Idstedt), and no. S11 (Hasl E203) from the forest district Rantzau (Hasloh). Both locations are situated in northern Germany. Trees of *Pinus montana* var. *rostrata*, marked with R (R1, R7, R10) originated from Ramsau, district of Wimbach (Bavaria), while those marked with K (K9, K11, K13, K14) originated from Koenigseggwald in the Franconian Alps (Schwaben), southern Germany.

The controlled crosses were carried out in spring 1977. Further details about the parent clones and hybrid families were given by Prus-Głowacki and Stephan (1998). Seeds were collected in autumn 1978 and sown in spring 1981 in the forest nursery of the Institute for Forest Genetics and Forest Tree Breeding, Grosshansdorf, near Hamburg (northern Germany). The seedlings were planted in a field trial with four plants per plot and in two replications in spring 1984.

In the present study we analysed eight anatomical traits of the F₁ hybrids and compared them with those of the specific patterns of the parental species *P. sylvestris* and *P. montana*. The needles were collected from individual trees in the plantation in 1993, placed in labelled envelopes and conserved in 70% ethanol. This was followed by making semipermanent preparations. From each tree ten short shoots were taken randomly. From one needle of each short shoot a cross-section was made at half of the needle length which was embedded in polyvinyl alcohol. These preparations served for the following studies on needle anatomy. Each preparation was analysed with respect to the following traits (Fig. 1): 1. Number of resin canals. 2. Thickness of the epidermis on the flat side of the needle (in μm). 3. Mean width of three epidermal cells (μm). 4. Mean width of a transverse cross-section of the needle (mm). 5. Height of a transverse cross-section of the needle (mm). 6. Value of trait 5 divided by value of trait 4 (needle cross-section height divided by its width). 7. Distance between the edges of vascular bundles (μm). 8. Marcet's coefficient (Marcet 1967): trait 4 $\times$ trait 7, divided by trait 5 or height of needle cross-section (μm) $\times$ distance between vascular bundles (μm) divided by width of needle cross-section (μm).

Fig. 1. Scheme of the transverse cross-section of a pine needle. 1 – vascular bundle, 2 – resin canal, 3 – epidermis, 4 – stomata, 5 – needle width, 6 – needle height (after Szweykowska and Szweykowski 1982).

RESULTS

Variation of anatomical needle traits

The eight investigated anatomical needle traits show generally a high variation within and between the ten hybrid families and their parent species *P. montana* var. *rostrata*

(*Pm*) and *P. sylvestris* (*Ps*) (Table 1). The most variable trait was assessed for the number of resin canals (trait 1), which varied for the hybrid families with mean values between 2.5 (no. 63) and 5.4 (no. 60), single values between 1 (in seven of ten families) to 12 (nos. 60 and 62) and an overall average of 4.0. The observed variation was also expressed by relatively high variation coefficients between 24% (no. 56) and 52% (no. 64) and an average value of 35%. The number of resin canals showed also distinct differences between the parent species *Pm* and *Ps*, and was for *Pm* about 3 (1 to 5 resin canals) and for *Ps* about 7 (3 to 13 resin canals), but with high variation coefficients (Table 1).

A large variation existed also for the thickness of the epidermis on the flat side of the needle (trait 2) which showed mean values between 25.6 μm (nos. 51 and 57) and 32.3 μm (no. 60) with an overall average of 27.4 μm . For the parent species *Pm* 28.2 μm were measured, for *Ps* 22.4 μm . The variation coefficients had relatively stable values between 7.4% (no. 55) and 15.8% (no. 60). Also for the parent species the values ranged between these values.

For Marcet's coefficient (trait 8), the synthetic trait which integrates data on needle cross-section width (trait 4) and height (trait 5) and on distance between vascular bundles (trait 7), the variation coefficient varied between 21.6% (no. 55) and 36.9% for the ten hybrid families, and was about 52% for *Pm* and 26% for *Ps*. The least variable trait of the hybrid families was the width (trait 4) and the height (trait 5) of needle transverse cross-section, for which values of the variation coefficient approached about 10%. An even lower mean value of the coefficient, about 7%, was found for the ratio of the two traits 4 and 5 (trait 6). Also in the parent species the lowest variation was manifested by needle width and height (traits 4 and 5) and by their ratio (trait 6) and, in *Ps*, additionally by the width of three epidermal cells (trait 3).

Correlation between traits

Correlation coefficients were calculated for the investigated eight anatomical needle traits. For the ten hybrid families the correlation coefficients differed highly significantly ($\alpha = 0.01$). The highest fraction (50%) of highly significant correlation coefficients was found in the crosses nos. 61, 63 and 64, while their lowest proportion was disclosed in the cross no. 56. A significant difference was detected also between the parental species. *Pm* showed more than 46% highly significant correlation coefficients, while *Ps* demonstrated only correlations of about 11%. In all the examined hybrid families and in *Pm* the most strongly correlated traits were the width (trait 4) and the height (trait 5) of a transverse cross-section of the needle. In *Ps* the highest value of a positive correlation coefficient was observed for the number of resin canals (trait 1) and for the width of needle transverse cross-section (trait 4).

Comparison of anatomical traits in hybrid families and parental species

In order to determine if significant differences existed between mean values of the eight anatomical needle traits for ten hybrid families and of those for the parental species, the Student's *t* test was performed. The results are shown in Table 2. As demonstrated by the test, all hybrid families were found to differ significantly ($\alpha = 0.05$) from either of the parents, although each hybrid family differed from their parents in a different set of traits. None of the crosses differed from both parents with respect to all traits. Only hybrid family no. 51 differed significantly with respect to all traits from *Pm*. Each of the hybrid families and *Ps* differed at a high level of

TABLE 1. Eight anatomical 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	Anatomical needle traits								
		1	2	3	4	5	6	7	8
51 n=16	min ... $\bar{x}$... max s.d. coeff. of var.%	1.0 .. 3.6 .. 8.0 1.59 33.99	20.2 .. 25.6 .. 30.4 2.33 9.10	12.6 .. 14.8 .. 17.7 1.27 8.57	1.1 .. 1.4 .. 1.6 0.09 6.69	0.5 .. 0.7 .. 0.8 0.05 7.78	0.3 .. 0.5 .. 0.6 0.04 7.91	0.1 .. 0.1 .. 0.2 0.04 26.09	0.1 .. 0.3 .. 0.5 0.08 30.99
55 n=8	min ... $\bar{x}$... max s.d. coeff. of var.%	1.0 .. 2.8 .. 5.0 0.94 33.62	20.2 .. 26.0 .. 30.4 1.92 7.39	10.1 .. 14.4 .. 17.7 1.44 10.02	1.0 .. 1.3 .. 1.6 0.13 10.37	0.4 .. 0.7 .. 0.9 0.08 11.90	0.5 .. 0.6 .. 0.6 0.03 5.21	0.1 .. 0.1 .. 0.2 0.03 19.79	0.1 .. 0.3 .. 0.4 0.05 21.58
56 n=6	min ... $\bar{x}$... max s.d. coeff. of var.%	3.0 .. 5.2 .. 8.0 1.25 23.96	20.2 .. 27.3 .. 32.9 2.87 10.54	12.7 .. 15.1 .. 20.2 1.51 9.97	1.1 .. 1.4 .. 1.7 0.11 7.75	0.6 .. 0.8 .. 0.9 0.07 8.65	0.5 .. 0.5 .. 0.6 0.03 6.45	0.1 .. 0.2 .. 0.3 0.03 20.13	0.2 .. 0.3 .. 0.6 0.07 23.65
57 n=10	min ... $\bar{x}$... max s.d. coeff. of var.%	1.0 .. 3.6 .. 6.0 1.09 30.33	20.2 .. 25.6 .. 30.4 1.97 7.69	12.7 .. 15.5 .. 17.7 1.42 9.18	1.0 .. 1.4 .. 1.8 0.17 11.99	0.6 .. 0.8 .. 1.0 0.09 11.95	0.5 .. 0.5 .. 0.7 0.04 7.45	0.1 .. 0.2 .. 0.3 0.05 32.04	0.1 .. 0.3 .. 0.6 0.10 33.51
58 n=10	min ... $\bar{x}$... max s.d. coeff. of var.%	3.0 .. 4.7 .. 10.0 1.23 25.90	20.2 .. 26.6 .. 30.4 2.34 8.81	12.7 .. 15.5 .. 17.7 1.13 7.32	1.3 .. 1.5 .. 1.7 0.10 6.61	0.4 .. 0.8 .. 0.9 0.07 8.99	0.5 .. 0.5 .. 0.6 0.03 5.58	0.1 .. 0.2 .. 0.3 0.06 28.00	0.2 .. 0.4 .. 0.7 0.12 29.98
60 n=11	min ... $\bar{x}$... max s.d. coeff. of var.%	1.0 .. 5.4 .. 12.0 1.76 32.67	22.8 .. 32.3 .. 48.1 5.10 15.82	10.1 .. 14.4 .. 22.8 2.26 15.68	1.2 .. 1.4 .. 1.7 0.11 7.97	0.6 .. 0.8 .. 1.0 0.09 10.88	0.4 .. 0.6 .. 0.7 0.05 8.27	0.1 .. 0.2 .. 0.3 0.04 21.87	0.2 .. 0.4 .. 0.6 0.09 25.41
61 n=8	min ... $\bar{x}$... max s.d. coeff. of var.%	1.0 .. 4.1 .. 8.0 1.40 33.95	20.2 .. 30.9 .. 43.0 4.50 14.55	12.7 .. 16.0 .. 17.7 1.44 9.03	1.1 .. 1.4 .. 1.7 0.15 10.49	0.7 .. 0.8 .. 1.0 0.06 7.39	0.5 .. 0.6 .. 0.7 0.05 9.01	0.1 .. 0.2 .. 0.3 0.05 27.36	0.2 .. 0.3 .. 0.5 0.09 32.17
62 n=8	min ... $\bar{x}$... max s.d. coeff. of var.%	3.0 .. 5.1 .. 12.0 1.89 37.33	22.8 .. 27.1 .. 32.9 2.21 8.16	12.7 .. 15.5 .. 20.2 1.47 9.50	1.1 .. 1.4 .. 1.7 0.10 7.21	0.7 .. 0.8 .. 0.9 0.05 6.65	0.5 .. 0.6 .. 0.6 0.04 6.37	0.1 .. 0.2 .. 0.3 0.04 25.39	0.2 .. 0.3 .. 0.6 0.09 28.82
63 n=20	min ... $\bar{x}$... max s.d. coeff. of var.%	1.0 .. 2.5 .. 7.0 1.23 49.98	17.7 .. 26.0 .. 30.4 2.75 10.63	10.1 .. 14.8 .. 17.7 1.50 10.16	0.9 .. 1.3 .. 1.8 0.18 14.02	0.5 .. 0.8 .. 1.1 0.10 12.34	0.5 .. 0.6 .. 0.8 0.05 8.61	0.0 .. 0.1 .. 0.3 0.05 33.91	0.1 .. 0.2 .. 0.5 0.08 36.91
64 n=20	min ... $\bar{x}$... max s.d. coeff. of var.%	1.0 .. 3.1 .. 8.0 1.59 51.85	17.7 .. 26.3 .. 32.9 2.31 8.78	10.1 .. 13.8 .. 17.7 1.45 10.51	1.0 .. 1.3 .. 2.2 0.18 13.47	0.6 .. 0.7 .. 1.1 0.09 12.07	0.5 .. 0.6 .. 0.7 0.04 7.49	0.1 .. 0.1 .. 0.3 0.04 32.25	0.1 .. 0.2 .. 0.5 0.08 35.47
Pm n=7	min ... $\bar{x}$... max s.d. coeff. of var.%	1.0 .. 2.8 .. 5.0 0.92 32.74	17.7 .. 28.2 .. 35.4 3.97 14.11	12.7 .. 15.2 .. 17.7 1.36 8.97	1.0 .. 1.3 .. 1.5 0.15 11.57	0.6 .. 0.8 .. 0.8 0.07 8.71	0.5 .. 0.6 .. 0.7 0.05 8.25	0.0 .. 0.1 .. 0.2 0.05 46.63	0.0 .. 0.2 .. 0.4 0.10 51.69
Ps n=2	min ... $\bar{x}$... max s.d. coeff. of var.%	3.0 .. 6.8 .. 13.0 3.02 44.80	17.7 .. 22.4 .. 25.3 2.45 11.16	12.7 .. 15.1 .. 17.7 1.29 8.59	1.3 .. 1.5 .. 1.7 0.07 4.78	0.7 .. 0.8 .. 0.1 0.05 6.44	0.5 .. 0.5 .. 0.6 0.03 6.28	0.0 .. 0.1 .. 0.2 0.03 23.62	0.1 .. 0.3 .. 0.4 0.08 25.51

significance ($\alpha = 0.01$) in the number of resin canals (trait 1) and in the thickness of epidermis on the flat side of needle (trait 2). From the parent *Pm* each hybrid family differed highly significantly in the distance between vascular bundles (trait 7) and in Marcet's coefficient (trait 8). The hybrid families nos. 56, 57, 58, 62 and 63 did not significantly differ from either of the parents with respect to the mean width of three epidermal cells (trait 3). In this trait the hybrid families nos. 61 and 64 differed significantly from either of the parents, while the hybrid families nos. 51, 55 and 60 differed only from *Pm*. The test proved that thickness of epidermis on the flat side of the needle (trait 2) and the number of resin canals (trait 1) differentiated best the hybrids from their parents.

The similarity of the ten hybrid families to one of the parents and the behaviour of the hybrid families on an intermediate position could easily be noticed by two-trait scatter diagrams. As an example the positions of the hybrid families and their parent species are demonstrated in Figure 4 for the number of resin canals (trait 1) and for Marcet's coefficient (trait 8). As already shown in Table 2, these traits were statistically most efficient in differentiating the studied hybrid families from the parental species. In Figure 4 a very extensive taxonomic distance could be noted between the parents, the hybrid families nos. 55, 63 and 64 of a so-called *Montana* group, the hybrid families nos. 56, 58, 60, 62 of a *Sylvestris* group, and obviously intermediate hybrid families nos. 51, 57 and 61.

TABLE 2. Variability in the examined ten hybrid families of controlled crosses and in their parental species, *Pinus montana* var. *rostrata* (*Pm*) and *P. sylvestris* (*Ps*) in respect to the applied anatomical needle traits (trait no. 1 to 8) at the significance level of $\alpha= 0.01$ (xx) or $\alpha= 0.05$ (x) detected by Student's *t* test, ns = not significant.

A	1	2	3	4	5	6	7	8
B								
Pm/51	xx	xx	x	xx	xx	xx	xx	xx
Ps/51	xx	xx	ns	xx	xx	x	ns	ns
Pm/55	ns	xx	xx	ns	xx	xx	xx	xx
Ps/55	xx	xx	ns	xx	xx	xx	ns	ns
Pm/56	xx	ns	ns	xx	ns	xx	xx	xx
Ps/56	xx	xx	ns	xx	ns	ns	xx	x
Pm/57	xx	xx	ns	xx	ns	xx	xx	xx
Ps/57	xx	xx	ns	x	ns	ns	ns	ns
Pm/58	xx	xx	ns	xx	xx	xx	xx	xx
Ps/58	xx	xx	ns	ns	ns	ns	xx	xx
Pm/60	xx	xx	x	xx	xx	xx	xx	xx
Ps/60	xx	xx	ns	ns	ns	x	xx	xx
Pm/61	xx	xx	xx	xx	xx	ns	xx	xx
Ps/61	xx	xx	x	x	x	xx	x	ns
Pm/62	xx	x	ns	xx	xx	xx	xx	xx
Ps/62	xx	xx	ns	ns	ns	xx	xx	ns
Pm/63	x	xx	ns	ns	x	ns	xx	xx
Ps/63	xx	xx	ns	xx	ns	xx	ns	ns
Pm/64	ns	xx	xx	xx	ns	xx	xx	xx
Ps/64	xx	xx	xx	xx	x	xx	ns	x

A – comparison parents/crosses
B – 8 needle traits (see text)

Mahalanobis distances between hybrid families and parental species

For the differentiation between the hybrid families and the parent species with respect to all eight anatomical needle traits the Mahalanobis distances were calculated (Table 3). The testing with the Hotelling's T^2 statistics demonstrated that most of the hybrid families were highly significantly different from each other and from their parents ($\alpha= 0.01$). With the exception of the hybrid families nos. 55, 63 and 64, all the remaining crosses differed highly significantly from *Pm*. From *Ps*, in contrast, all crosses were less significantly different except the hybrid families nos. 65 and 62. The family no. 55 did not vitally differ from the hybrid families nos. 63 and 64, which differed significantly from each other. Also the families nos. 56 and 62 did not crucially differ from each other. The hybrid family no. 60 was the only one, which differed from all the remaining families and from either of the parents at a high significant level.

The minimum spanning tree for the ten hybrid families, constructed on the basis of the shortest Mahalanobis distances is shown in Figure 2. The only significant different link was disclosed between the families nos. 60 and 61 ($T^2_{calc}>T^2_{0.01}$). This emphasizes the distinct character of the hybrid family no. 60 as compared with the remaining hybrids. The hybrid families nos. 55, 63 and 64 took positions close to that of *Pm* and did not significantly differ from this parent species. The family no. 56 was linked to family no. 62 and to the parent species *Ps*. To this group also family no. 62 is joined together with cross no. 61, both hybrid families are not significantly

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

different from *Ps*. Cross no. 58 took a position intermediate between the parental species, however resembling more closely *Ps* rather than *Pm* (see Table 2). A similar behaviour was found for the family no. 57, which was linked to the group of hybrid families similar to *Ps* and to *Pm*. The family no. 51 was most similar to family no. 57.

Discriminance analysis of the anatomical needle traits

The two distinguished groups of hybrid families similar to *Pm* (*Montana* group) including families nos. 55, 63 and 64, and of hybrid families similar to *Ps* (*Sylvestris* group) with the families nos. 56, 58, 60, 61 and 62 as well as the families of an intermediate position were well distinguishable by a discriminance analysis as shown in Figure 3. Determination coefficients between eight anatomical needle traits and the first three discriminating variables U_1 , U_2 , U_3 defined the main characters and contained 87.4% of the information of the applied set of eight needle traits. The traits included the thickness of epidermis of the flat side of the needle (trait 2), the number of resin canals (trait 1), the distance between vascular bundles (trait 7), Marcet's coefficient (trait 8), the ratio of the needle height to its width (trait 6), and the mean width of three epidermal cells (trait 3).

Fig. 3. Results of a discriminant analysis for ten hybrid families of controlled crosses and for their parental species (for description see Figure 2) in the space of the first three discriminant variables U_1 , U_2 , U_3 , containing a total of 87.39% information of the applied set of eight anatomical needle traits.

DISCUSSION

The studies show that among the ten hybrid families from controlled crosses, between the two species *P. montana* var. *rostrata* and *P. sylvestris* three groups can be distinguished, one forming a *Montana* group, another one a *Sylvestris* group, and a third one contains hybrid families intermediate between the parent species (Figure 3). The *Montana* group includes the families nos. 55, 63 and 64. The calculated Mahalanobis distances (Table 3) between two examined multitrait objects allowed to detect that these families were significantly different from *P. sylvestris*, but not significantly different from *P. montana*. Families of the *Montana* group have been situated close to each other in the minimum spanning tree, based on the shortest Mahalanobis distances (Figure 2), which confirms their high similarity.

Among the hybrid families, no. 55 (*P. montana* var. *rostrata* [clone R7] × *P. sylvestris* [clone S12]) is of particular interest. In studies of Prus-Głowacki and Stephan (1998) this hybrid family has been proven similar to *P. sylvestris* regarding the serological properties, and to *P. montana* and *P. sylvestris* in respect of the isoenzymatic characters. All individuals carried also novel protein fractions and possessed an "unexpected genotype". Studies of anatomical traits indicate that the hybrids of the cross no. 55 resemble more closely *P. montana* (Table 3). On the other hand, regarding morphological needle traits, the hybrid family no. 55 occupies a position intermediate between the parents (Bobowicz *et al.* 2000). In interspecific hybrids the parallel manifestation of traits resembling, to various extent, the parents and of traits, which exhibit an intermediate character, has been well established in studies on natural hybrids of *P. montana* (*P. mugo* Turra) × *P. sylvestris* (Staszkiewicz and Tyszkiewicz 1969a, 1969b, 1972, Bobowicz 1990a).

The hybrid families nos. 51 and 57 with their intermediate position (Figure 2 and 3) differed significantly from either of the parents (from *P. montana* at the significance level of ($\alpha=0.01$) and from *P. sylvestris* at ($\alpha=0.05$) (Table 3).

The *Sylvestris* group has comprised the remaining hybrid families nos. 56, 58, 60, 61 and 62 (Figs 2 and 3). The hybrids from crosses nos. 56 and 62 have not significantly differed from *P. sylvestris* and, thus, have been identical with the parent in the statistical sense. The remaining families of the *Sylvestris* group did not, however, demonstrate such a full identity with the standard.

Also the hybrid family no. 60 (*P. montana* var. *rostrata* [clone K11] × *P. sylvestris* [clone S12]) deserves a particular attention. This hybrid family was the only one, which differed significantly ($\alpha=0.01$) from all the other remaining hybrid families and from both parental species (Table 3). It has been linked by the shortest Mahalanobis distance with the family no. 61 and the distance was highly significant ($T^2_{\text{calc}} > T^2_{0.01}$). In the minimum spanning tree (Figure 2), the link between families nos. 60 and 61 has represented the only significant different Mahalanobis distance. This fact indicates an exquisitely distinct character of hybrids in the cross, noted already in studies of needle morphology (Bobowicz *et al.* 2000). This has been confirmed by an expression in most of the hybrids of cross no. 60 of "novel proteins" fractions, as detected immunochemically by Prus-Głowacki and Stephan (1998). Four of 11 individual hybrids examined in this family have demonstrated in the antigens and isoenzymes new, nonparental proteins (Prus-Głowacki and Stephan 1998).

Prus-Głowacki and Stephan (1998) have reported that the progenies of F_1 hybrids have mostly demonstrated traits resembling *P. montana* when the clone K14 was used as the mother. In the present studies the conclusion has been confirmed by the families nos. 63 and 64. The families, which did not differ significantly from the *Sylvestris* pattern, i.e. the hybrid families nos. 56 and 62, have originated from the paternal clone S12. The fact is also consistent with the inheritance pattern dependent upon the applied pollen donor, as noted in the study of Prus-Głowacki and Stephan (1998). The family no. 62 has also been regarded to resemble *P. sylvestris* in studies on antigenic proteins and isoenzymes.

Considering the results of all applied multivariate analyses of this study, we may conclude that in anatomical needle traits F_1 hybrids of controlled crosses resemble, to a greater or smaller extent, (about 50%) *P. sylvestris*, 30% of the crosses are more similar to *P. montana*, and the remaining 20% include hybrid families of an intermediate position. The percentage values resemble closely those noted for the same hybrid families in respect to antigenic proteins (Prus-Głowacki and Stephan 1998). The authors estimate that 30% of the *P. montana*-resembling progenies have represented the matroclinal type of inheritance of antigenic proteins, 43% of the progenies resembled *P. sylvestris* and exhibited the patroclinal type of inheritance, while approximately 25% of the hybrids were of an intermediate type.

TABLE 3. Table of Mahalanobis distances together with values of Hotelling’s T^2 statistics for ten hybrid families and for their parental species (*Pinus montana* [no. 11], *Pinus sylvestris* [no. 12]) determined for eight anatomical needle traits. $T^2_{0.01}$ – critical value of Hotelling’s statistics at the level of significance of 0.01. $T^2_{0.05}$ – critical value of Hotelling’s statistics at the significance level of 0.05; xx – value significant at the level of $\alpha=0.01$, x – value significant at the level of $\alpha= 0.05$.

51												
55	3.78 101.189											
56	xx 1.71 128.125	x 1.85 117.272										
57	1.36 113.015	1.15 58.642	1.38 71.456									
58	xx 2.02 250.64	xx 2.09 194.843	1.26 59.438	1.26 79.408								
60	xx 3.41 759.485	xx 3.11 448.912	xx 2.09 169.268	xx 2.95 455.85	xx 2.36 292.867							
61	xx 3.01 484.509	xx 2.47 244.868	xx 1.94 129.09	xx 2.17 208.708	xx 2.18 211.019	xx 1.78 146.12						
62	xx 2.06 225.956	xx 1.9 144.067	0.68 15.947	1.28 72.518	1.27 72.222	xx 2.15 214.26	1.57 98.223					
63	xx 2.43 539.194	1.33 101.534	xx 2.45 277.08	xx 1.63 177.256	xx 2.45 401.258	xx 3.34 793.96	xx 2.27 293.82	xx 2.14 260.825				
64	xx 1.51 201.865	0.64 23.161	xx 1.88 163.142	xx 1.4 130.653	xx 2.21 324.363	xx 3.04 655.28	xx 2.46 345.716	xx 1.9 206.606	xx 1.34 179.221			
11	xx 2.72 251.408	1.47 80.788	xx 2.17 151.864	xx 1.82 136.547	xx 2.57 271.056	xx 3 384.147	xx 1.88 131.461	xx 1.99 147.37	1.19 73.939	1.43 105.786		
12	x 2.59 119.16	xx 3.12 155.485	2.23 74.737	x 2.66 118.165	x 2.75 126.095	xx 4.06 278.313	xx 3.79 229.583	2.32 86.054	xx 3.57 231.461	xx 3.01 165.102	xx 3.5 190.776	
	51	55	56	57	58	60	61	62	63	64	11	12

$T^2_{0.01} = 127.8097$
 $T^2_{0.05} = 115.8823$

The analyses carried out in this study have demonstrated that the similarity of a given cross to a given parental species or the absence of such a similarity is determined mainly by the number of resin canals in the needles. All hybrids, except those of nos. 55 and 64, have differed significantly with respect to this trait from either of the parents (Table 2). On the other hand, regarding Marcet’s coefficient, all the crosses have proven significantly different from *P. montana* and from *P. sylvestris* (Table 2). Marcet’s coefficient and the number of resin canals represent the best anatomical markers, which differentiate *P. montana* from *P. sylvestris*. The remarkable lack of similarity between an F_1 progeny and its parents in such traits may be explained mainly by the intragenic recombination process in hybrid genes, inherited from their ancestors (for discussion see Bobowicz et al. 2000). The arising novel genes and the cumulative type of determining quantitative traits of needle anatomy, typical of cumulative gene inheritance, may provide the hybrids with the ability to adjust

to new ecological niches, usually unsuitable for their parent species.

CONCLUSIONS

- 1. Parents of the controlled F_1 hybrids, *Pinus montana* var. *rostrata* and *Pinus sylvestris*, are highly significantly different in regard to the applied complex of anatomical needle traits.
- 2. Calculated Mahalanobis distances have permitted to detect significant differentiations among the examined hybrid families; those which are similar to the parental species, hybrids of intermediate type and crosses different from both parents.
- 3. The number of resin canals and the thickness of epidermis on the flat side of the needle represent the main traits, which determine the taxonomic position of a given hybrid.

Fig. 4. Scatter diagram for ten families of F₁ hybrids and for their parental species with respect to resin canals (trait 1) and Marcet's coefficient (trait 8).

4. The number of resin canals and the Marcet's coefficient represent the most variable traits both in parents and in the hybrids. Marcet's coefficient represents the trait, which best discriminates parental species.
5. The width and the height of the needle represent the least variable traits both in parents and in their hybrids. These traits are also the most strongly positively correlated traits.
6. The distinct character of some crosses from the others and from the parental species results presumably from the presence of novel combinations of genes originated from parents.

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GENETYCZNA ZMIENNOŚĆ MIESZAŃCÓW F₁ Z KONTROLOWANEGO KRZYŻOWANIA
MIĘDZY *PINUS MONTANA* VAR. *ROSTRATA* I *PINUS SYLVESTRIS* L.
W CECHACH ANATOMII IGIEŁ

STRESZCZENIE

Z kontrolowanych krzyżówek między *Pinus montana* var. *rostrata* i *Pinus sylvestris* L. wyprowadzono 10 rodów mieszańców w uprawie doświadczalnej. 117 osobników reprezentujących te rody zbadano pod względem 8 cech anatomii ich dwuletnich igieł. Dane biometryczne uzyskane w wyniku pomiarów cech anatomicznych użyto do przeprowadzenia wielozmiennych analiz statystycznych z analizą zmiennych dyskryminacyjnych i odległościami Mahalanobisa jako metodami głównymi. Wykryto, że wśród rodów mieszańców można wyróżnić osobniki reprezentujące każdy z typów rodzicielskich, a więc typ Montana i typ Sylvestris oraz mniej liczne formy pośrednie. Wyniki otrzymane z badań anatomicznych bardzo dobrze korespondują z wynikami otrzymanymi z badań izoenzymatycznych i białek antygenowych (Prus-Głowacki and Stephan 1998).

SŁOWA KLUCZOWE: *Pinus montana*, *Pinus sylvestris*, kontrolowane mieszańce F₁, anatomia igieł, wielozmienna analiza statystyczna.