

Serological characteristics of some putative hybrid individuals from a *Pinus sylvestris* × *Pinus mugo* hybrid swarm population

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Abstract

The antigenic properties of some putative hybrid individuals from a hybrid swarm population were studied. All the plants were to a major or lesser degree intermediate between three pure taxa, i.e. *P. sylvestris*, *P. mugo* and *P. uliginosa*. The plants are, however, not intermediate in general, but are similar to one species in part of their antigenic properties (enzymatic systems) and to the other one in the rest. It means that there is no common trend in the variation of the antigenic characters (proteins) and they behave independently.

INTRODUCTION

Individuals from an interesting population of possible *Pinus sylvestris* × *P. mugo* hybrid swarm character were compared serologically with four different taxa. Two of them, *Pinus sylvestris* and *P. mugo* are the putative parents of the extant population. The third taxon, *Pinus uliginosa* Neumann, is a critical tree form similar in its morphological and anatomical characters to the dwarf mountain pine (*P. mugo*). This pine grows on wet peat bogs in the Central European mountains. The fourth taxon used for comparison was *P. nigra*, a South European species. It does not grow in Poland, but it is known to hybridize with *P. sylvestris* in natural conditions.

As many individuals of the population in question are intermediate in their morphological, anatomical and chemical characters (Szweykowski, 1969; Szweykowski et al., 1976 a and b; Szweykowski, Bobowicz, 1977 and unpublished data) it seemed desirable to characterize them in as many aspects as possible. In this paper the

results of the investigation of their antigenic properties are given. Papers published so far were devoted either to the putative parental species (Prus-Głowacki, Szweykowski, 1979) or to hybrid swarm individuals (Prus-Głowacki et al., 1978). In the latter case the intensity of the precipitin reaction was used. In this paper we give the results of particular band identification.

MATERIAL AND METHODS

Needles were taken from 16 individuals of each of the following five populations.

1. *Pinus sylvestris*: Piekielna Góra Mt., Kotlina Kłodzka Valley, woj. Wałbrzych, alt. ca 450 m.

2. *Pinus uliginosa*: Wielkie Torfowisko Batorowskie (peat bog) in Góry Stołowe Mts, woj. Wałbrzych, alt. ca 720 m.

3. *Pinus mugo*: Kopa Magury Mt. Tatra Mts., woj. Nowy Sącz, alt. ca 1600 m.

4. *Pinus nigra*: artificial stand in Zielonka Forest near Poznań, alt. ca 100 m.

5. Hybrid swarm population: Czarne Bagno (peat bog) by Zieleniec village in Kotlina Kłodzka Valley, woj. Wałbrzych, alt. ca 700 m.

The trees were marked with permanent metal labels, their field numbers are listed in Table 1.

Protein extraction, preparation of antisera, immunodiffusion analyses, staining procedures and interpretation of precipitin patterns were the same as in our previous publication (Prus-Głowacki et al., 1978; Prus-Głowacki, Szweykowski, 1979), except for the precipitin arcs identification where Ouchterlony's criteria were used. (Ouchterlony, 1967).

To get more reliable results for comparison with the particular hybrid swarm individuals, the four "pure" taxa were used as "synthetic comparison units". For this purpose, 0.5 ml of protein extract of each of the 16 individuals of a "pure" species population, e.g. *Pinus sylvestris*, were mixed together and the mixture was used in analyses as, in this case, the *Pinus sylvestris* sample. Extracts of plants from the hybrid swarm were, of course, used singly, i.e. were not mixed.

The simple matching coefficients (Sneath, Sokal, 1973) were computed, transformed into taxonomic distances and used for dendrite construction. They were used, in addition, for comparative scatter diagrams; for these, two orthogonal axes were scaled according to the taxonomic distance values of two taxa. Then the individuals of the hybrid swarm were plotted on the dendrite. The distances from the two axes show the character of each individual plant: those lying on

Table 1

Morphological characteristics of investigated individuals from the hybrid swarm population

Individual	Field-number	Habitus	Stem height m	Age years	Needle colour	Colour of bark in upper part of the trunk	Periderm in lower part of the trunk
H1	5223	monocormic tree	10	60	green	reddish-gray	deeply fissured
H2	2105	monocormic tree	11	60	bluish-green	reddish	deeply fissured
H3	2106	monocormic tree	20	70	bluish-green	reddish	deeply fissured
H4	2093	monocormic tree	4	25	bluish-green	reddish-gray	deeply fissured
H5	3097	monocormic tree	10	60	green	black	black, thin scales
H6	3092	oligocormic bush 3 trunks	8	90	green	black	black, thin scales
H7	2100	monocormic tree	10	100	green	black	superficially fissured
H8	2475	monocormic tree	12	100	bluish-green	reddish-gray	superficially fissured
H9	4498	oligocormic bush 3 trunks	14	75	yellowish-green	black	black, thin scales
H10	4496	monocormic tree	18	85	bluish-green	reddish	deeply fissured
H11	2099	monocormic tree	10	70	bluish-green	reddish-gray	superficially fissured
H12	4494	monocormic tree	20	50	bluish-green	reddish-gray	deeply fissured
H13	2473	monocormic tree	5.5	35	bluish-green	gray with reddish spots	deeply fissured
H14	2110	monocormic tree	3	17	bluish-green	black	deeply fissured
H15	4497	monocormic tree	18	90	bluish-green	reddish-gray	deeply fissured
H16	3091	monocormic tree	13	70	green	gray with reddish tinge	superficially fissured

one or the other axis are typical for the respective "pure" species, others, situated on the line bisecting right angle are exactly intermediate.

The matrices of similarity coefficients were then sent to the Computing Centre of the Agricultural University in Poznań where the principal components were extracted from them.

RESULTS

Immunodiffusiograms of some selected individuals from the hybrid swarm population are shown in Fig. 1. The serological similarities depend on the antigen used. They are shown on the dendrites (Fig. 2-6) and will be discussed separately.

Protein stained with amido-black

As easily seen, most of the putative hybrid individuals are connected mainly with *Pinus mugo* and, to a lesser extent, with *P. uliginosa*, *P. sylvestris* is situated in the marginal part of the whole group, joining *P. uliginosa* only (Fig. 2).

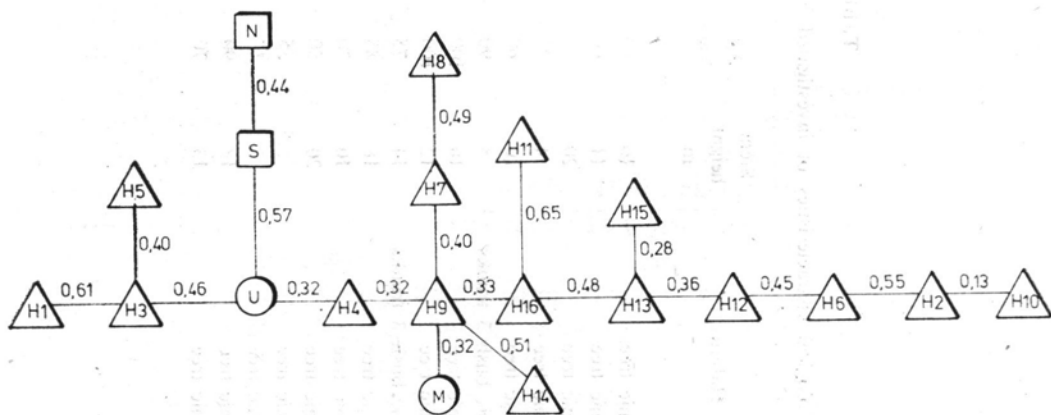


Fig. 2. Dendrite showing the serological similarity (on the basis of proteins stained with amido black) of individuals from the hybrid swarm population to *P. sylvestris* (S), *P. mugo* (M), *P. uliginosa* (U) and *P. nigra* (N). Based on average results with three sera: antisylvestris, antimugo and antiuliginosa

Malate dehydrogenase

The dendrite based on the MDH analysis (Fig. 3) shows a different pattern. The putative hybrid individuals form two different clusters joined through *P. uliginosa*. The first group (H3, H4, H6, H8, H11, H12,

A

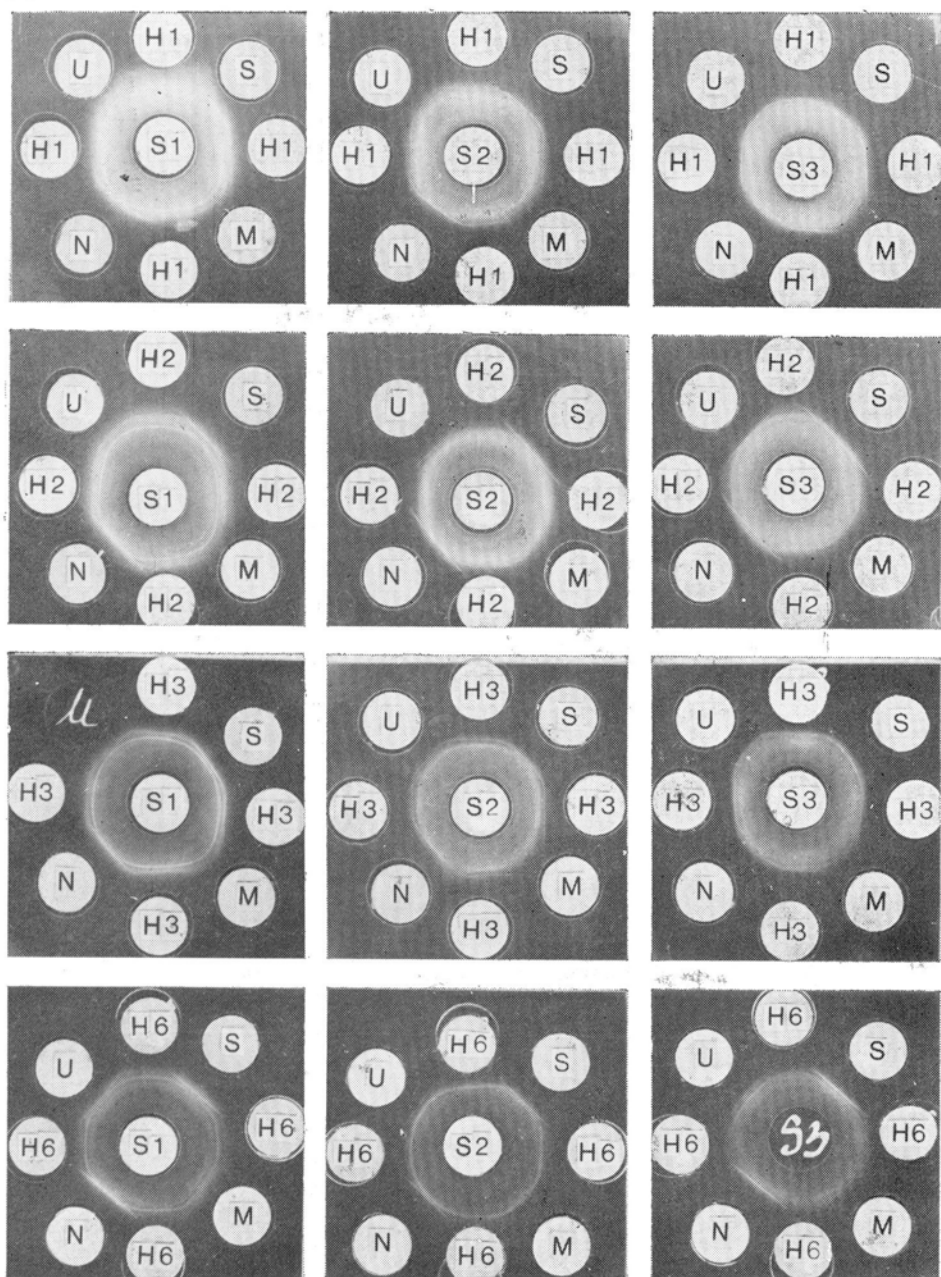
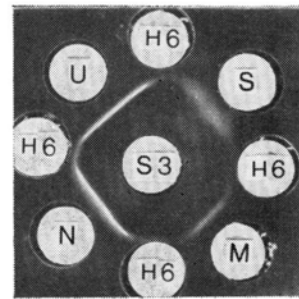
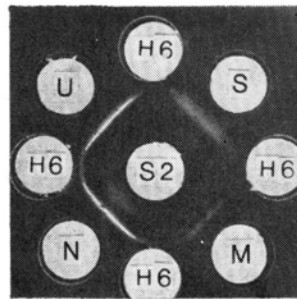
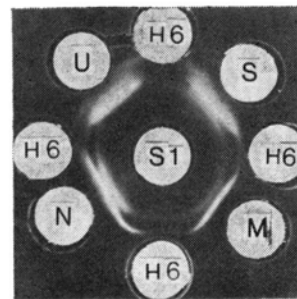
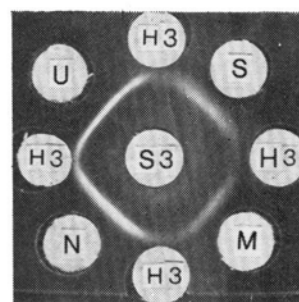
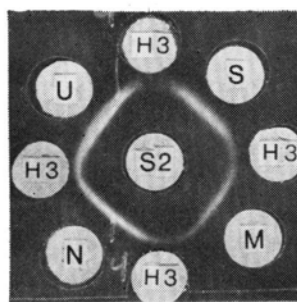
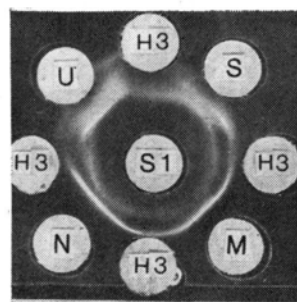
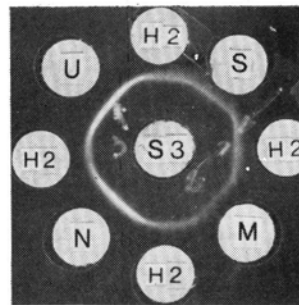
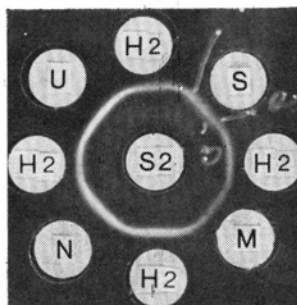
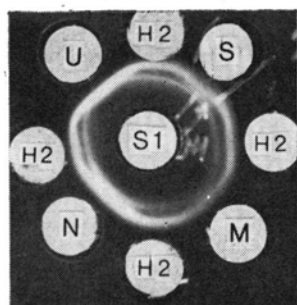
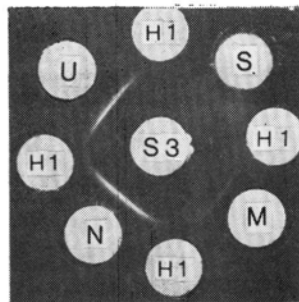
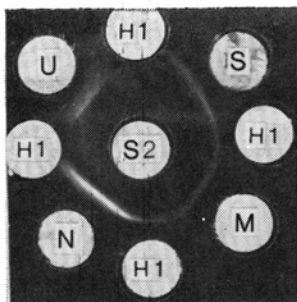
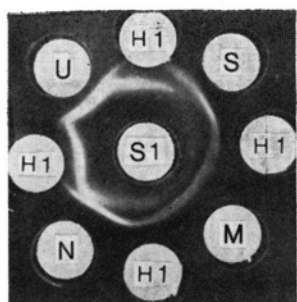


Fig. 1. Immunodiffusion plates of proteins from needles of some individuals from a hybrid swarm population and of particular taxa: S — *P. sylvestris*, M — *P. mugo*, U — *P. uliginosa*, N — *P. nigra*, S1 — antisylvestris serum, S2 — antimugo serum, S3 — antiuliginosa serum. The outer walls were filled with 30 μ l of antigens (concentration of proteins 8 mg/ml)

A — plates stained with amido black, B — plates stained for esterase activity, C — plates with peroxidase activity



H13, H15 and H16) is situated between *P. sylvestris* and *P. uliginosa*, the second one (H2, H5, H7, H9, H10, H14) is connected with *P. mugo*. Interestingly enough, one individual (H1) which is a typical tree morphologically intermediate between *P. uliginosa* and *P. sylvestris* clusters with *P. sylvestris* without showing any similarity to the rest of the hybrid swarm individuals.

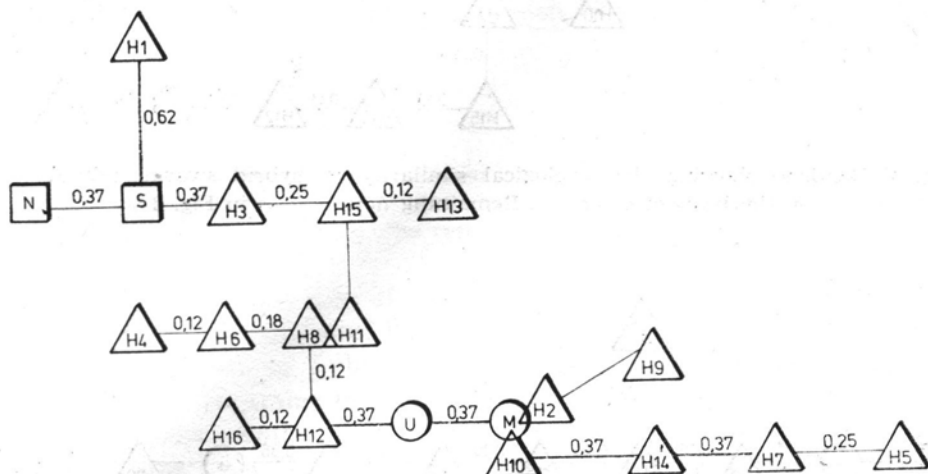


Fig. 3. Dendrite showing the serological similarity of individuals from the hybrid swarm population on the basis of malate dehydrogenase. Remaining notations as in Fig. 2

Esterases

According to the esterase patterns, most of the putative hybrid individuals are similar to *P. mugo* which is situated inside the group. This time, *P. sylvestris*, although situated marginally, is connected very closely with the hybrid tree H3 which is, again, morphologically intermediate between *P. uliginosa* and *P. sylvestris* (Fig. 4, see also Table 1).

Peroxidases

The similarity pattern (Fig. 5) based on peroxidase analysis is rather different as far as the position of *P. sylvestris* is concerned. This species is, in this respect, clearly very different from all the rest: it joins *P. mugo*, but the distance between these two taxa is the longest one in the whole dendrite.

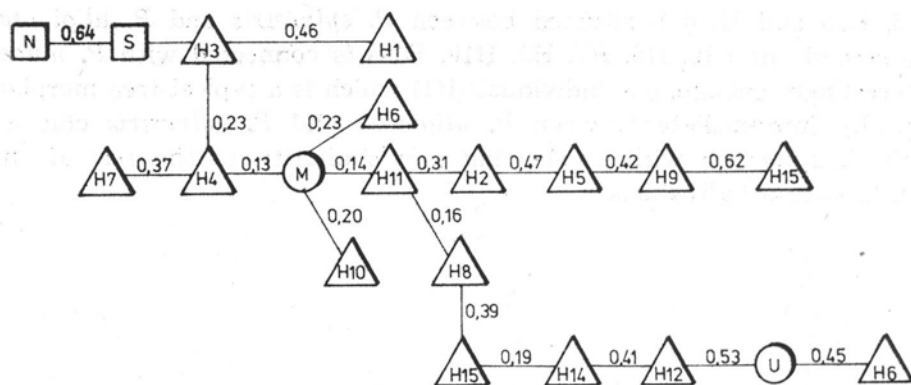


Fig. 4. Dendrite showing the serological similarity of hybrid swarm individuals on the basis of esterases. Remaining notations as in Fig. 2

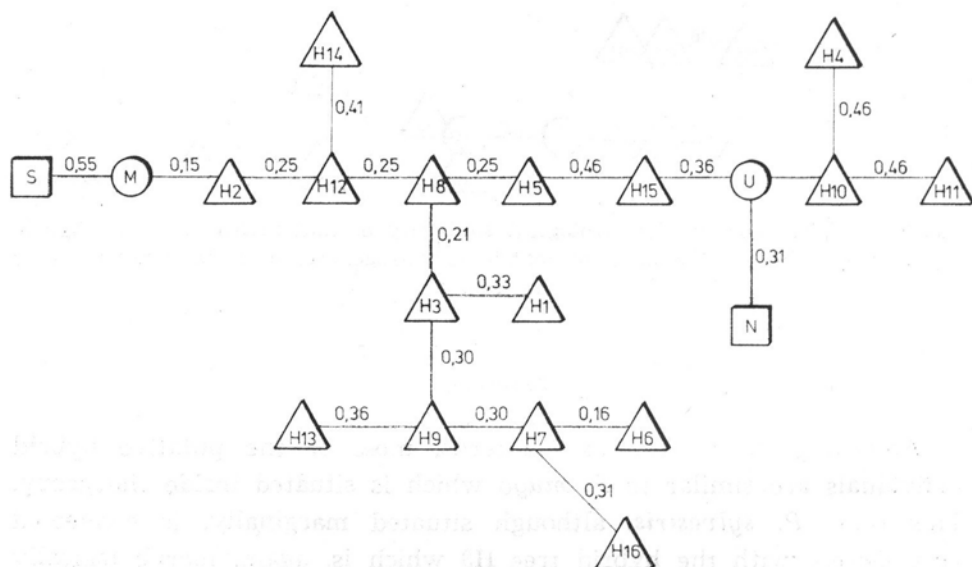


Fig. 5. Dendrite showing the serological similarity of hybrid swarm individuals on the basis of peroxidase. Remaining notations as in Fig. 2

Overall serological similarity

On the basis of averaging the four similarity matrices described above (a through d) the dendrite shown in Fig. 6 is constructed. This time, *P. mugo* is situated in the central part of the hybrid individuals cluster, *P. sylvestris* and *P. uliginosa* being rather loosely connected with it.

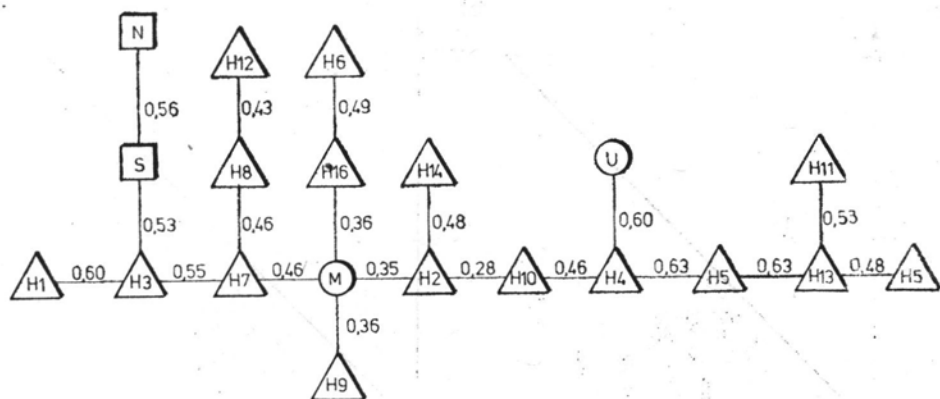


Fig. 6. Dendrite showing the serological similarity of hybrid swarm individuals on the basis of average results for three enzymes and proteins stained with amido black. The rest of notations as in Fig. 2

Graphical comparison of particular hybrid swarm individuals is shown on scatter diagrams, Figs 7 through 11.

1. Comparison of hybrid swarm individuals with *Pinus sylvestris* and *P. mugo* (diagrams marked with A). Most of the hybrid individuals show a greater similarity to *P. mugo*. There are, however, plants very similar to or even identical with *P. sylvestris* (e. g. H1 in the case of MDH — Fig. 8, or, H3 in the case of esterases — Fig. 10).

2. Comparison of hybrid swarm individuals with *P. sylvestris* and *P. uliginosa* (diagrams marked with C). In the case of esterases (Fig. 10) most of the hybrids are exactly intermediate, whereas according to the protein stained with amido black pattern (Fig. 7) and to the overall similarity (Fig. 11) they are closer to *P. uliginosa* and according to the results of peroxidase analyses more of them are *Pinus sylvestris*-like. Especially interesting are the diagrams based on the MDH similarities (Fig. 8). All the hybrid swarm individuals form two rather distinct clusters, one of them related to *P. uliginosa*, the second one to *Pinus sylvestris*.

3. Comparison of hybrid swarm individuals with *Pinus mugo* and *P. uliginosa* (diagrams marked with B). The plants in question are mostly exactly intermediate which is particularly well marked in the case of the protein stained with amido-black similarity pattern (Fig. 7). Peroxidases, however, show two different clusters (Fig. 9): a bigger one comprising individuals closely related to *P. mugo* and a smaller one which contains plants similar to *P. uliginosa*.

It is worth mentioning that, after averaging the similarity matrices obtained in all four series of analyses (i.e. stained with amido-black protein, peroxidases, MDH, esterases), most hybrid individuals show

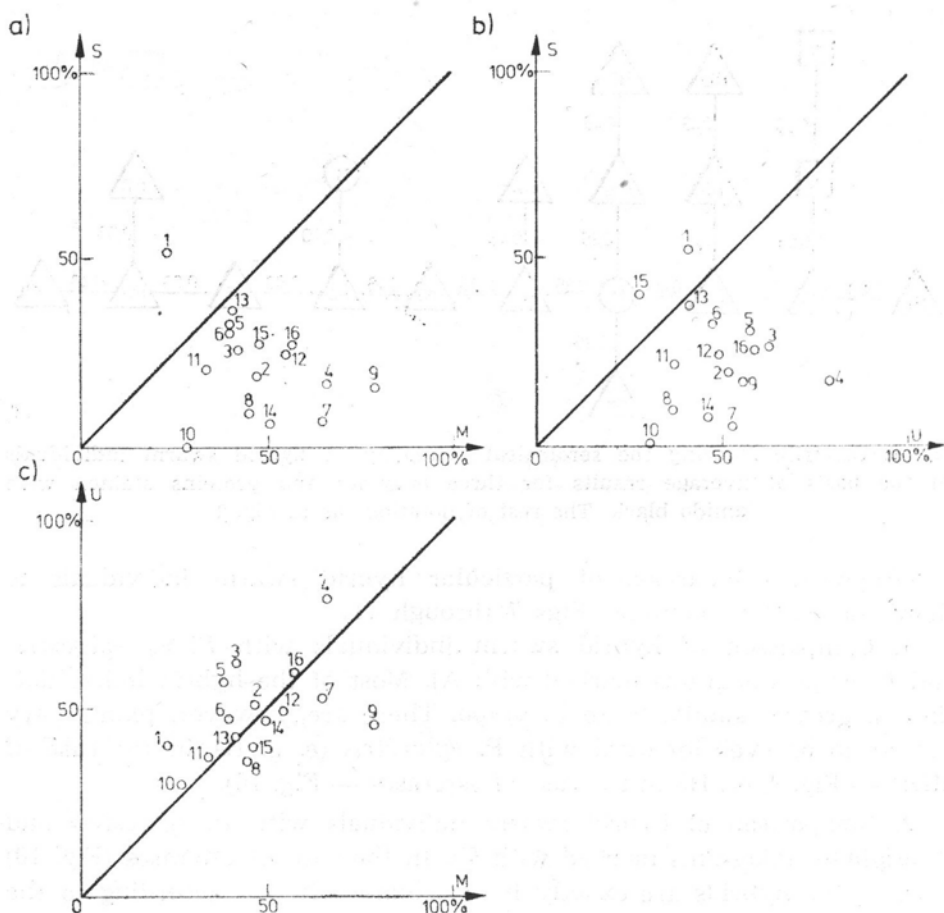


Fig. 7. Diagrams showing the antigenic similarity of hybrid swarm individuals (on the basis of proteins stained with amido black) to: a — *P. sylvestris* (S) and *P. mugo* (M), b — *P. uliginosa* (U) and *P. mugo* (M), c — *P. sylvestris* (S) and *P. uliginosa* (U), based on taxonomic distances.

a pronounced intermediate character. This, and, at the same time, the rather heterogeneous character of the group investigated is particularly well seen on three dimensional graphs where the hybrid swarm individuals are compared with the three "pure" species simultaneously (Fig. 12).

Particular individuals of the hybrid swarm population were characterised by their similarity coefficients to each of the four "pure" species. In addition, Anderson's hybrid index based on the results of our present serological investigations was computed for each of them. The results are diagrammed in Fig. 13. The most interesting fact that emerges from the analysis of these results is that one and the same

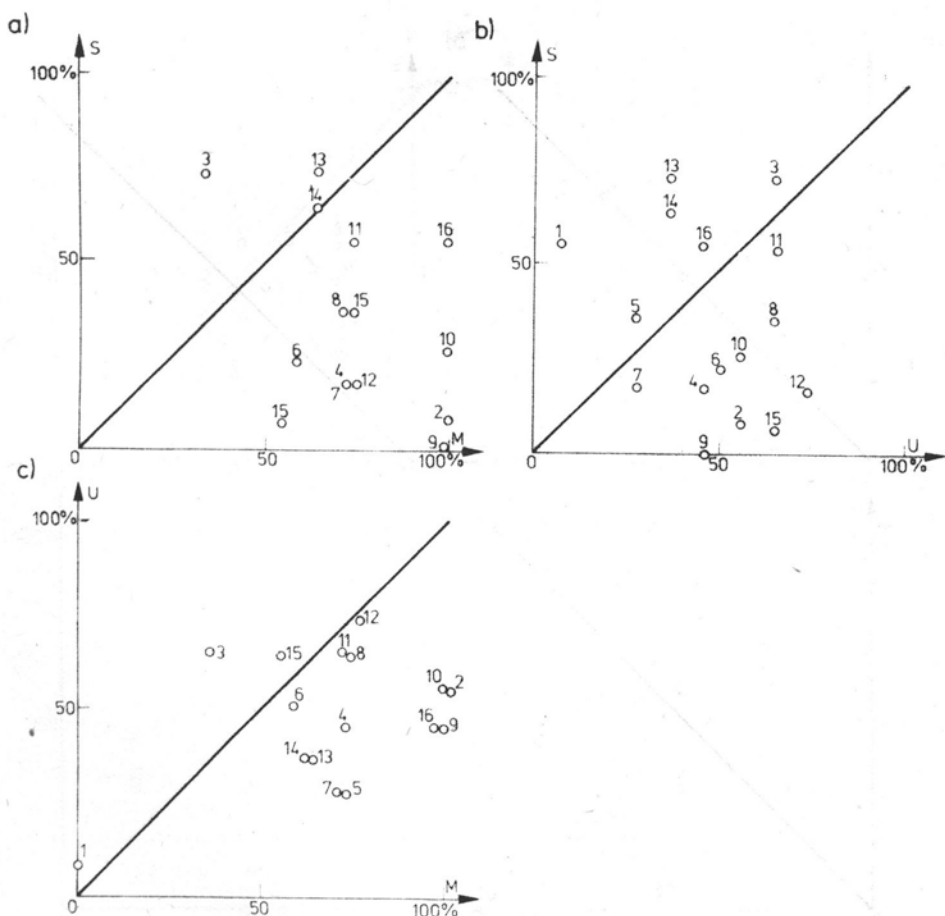


Fig. 8. Diagrams showing the antigenic similarity of malate dehydrogenase of hybrid swarm individuals to three pure species. The rest of notations as in Fig. 7

individual can show rather different relations to the pure species depending on the antigen used. For instance, the individual H1 is rather close to *P. sylvestris* according to the MDH pattern (hybrid index value = -54.8 , pure *P. sylvestris* = -100), whereas it is related to *P. mugo* as far as its peroxidase pattern is concerned (hybrid index = $+22.6$, pure *P. mugo* = $+100$). Individual H9 is very close to *P. mugo* in its MDH pattern (hybrid index = $+99.92$) whereas its esterase pattern shows an intermediate character.

The results of the principal component analysis are shown on two-dimensional scatter diagrams (Fig. 14) and in three-dimensional space (Fig. 15). Individuals from the hybrid swarm population form a rather

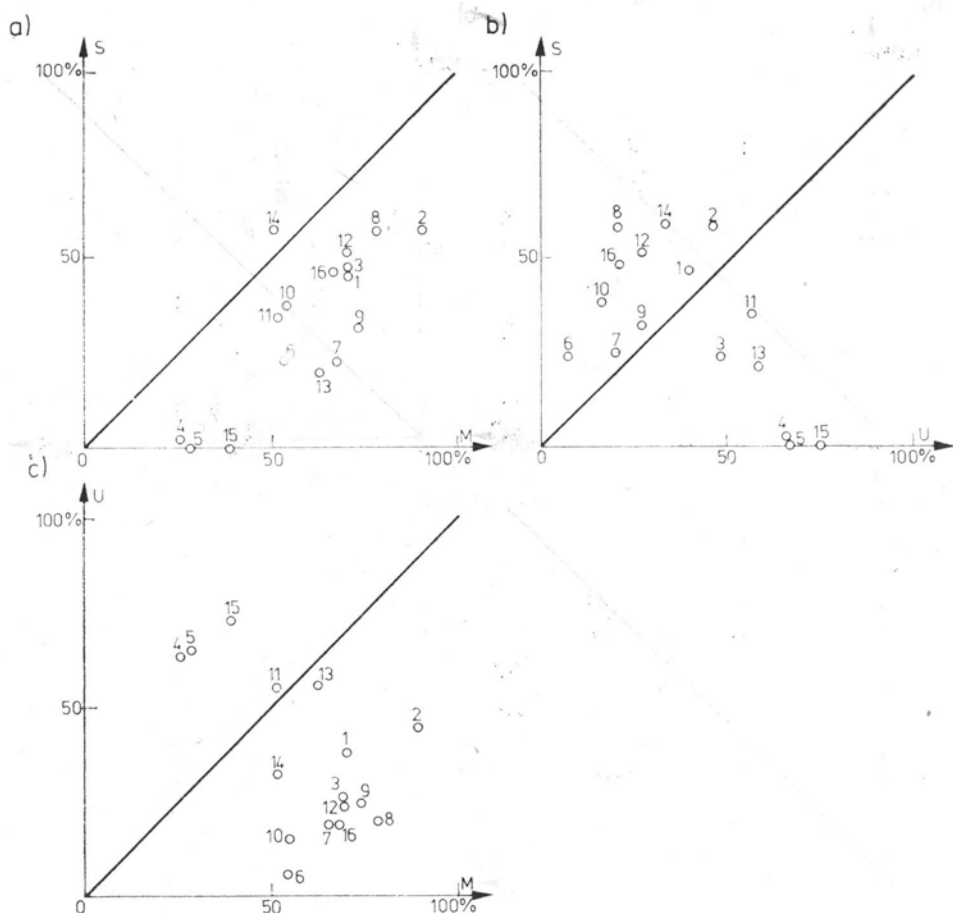


Fig. 9. Diagrams showing the antigenic similarity of peroxidases of hybrid swarm individuals to three pure species. The rest of notations as in Fig. 7

compact cluster situated between the pure species, showing, however, most close relationship to *P. mugo*.

DISCUSSION

An interesting population of probably hybrid swarm character is situated on a big peat-bog complex called Torfowiska Zielenieckie in Góry Bystrzyckie Mts. (central Sudetes) at an altitude of ca. 720 m. The population which formed in the remote past by hybridization of a relic *Pinus mugo* population with the surrounding *P. sylvestris* forest (Szweykowski, unpublished data) consists of very heterogeneous elements: from low, polycormic bushes resembling typical *P. mugo*,

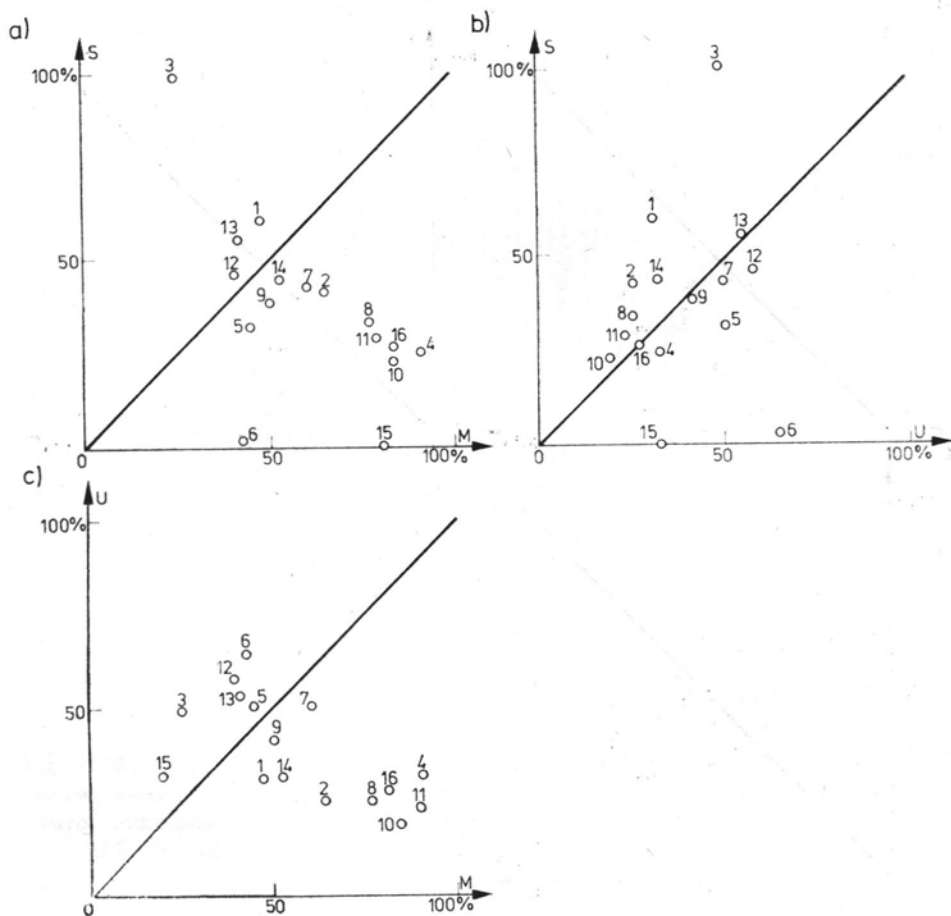


Fig. 10. Diagrams showing the antigenic similarity of esterases of hybrid swarm individuals to three pure species. The rest of notations as in Fig. 7

through various forms of higher bushes (up to 4-5 m. high) and oligocormic trees (3-5 trunks growing from a common base) to monocormic trees of highly variable appearance. In a previous publication (Prus-Głowacki et al., 1978), serological characteristics are given for 22 individuals of this population taken randomly along a transect.

In the present paper we publish new results obtained by more detailed serological investigations and performed on 16 specially chosen treelike plants. Fourteen of them were monocormic with quite stright trunks, the remaining two (H6 and H9) having the character of big, oligocormic bushes. On the basis of external appearance all these plants show an intermediate character between *Pinus sylvestris* and *Pinus mugo*

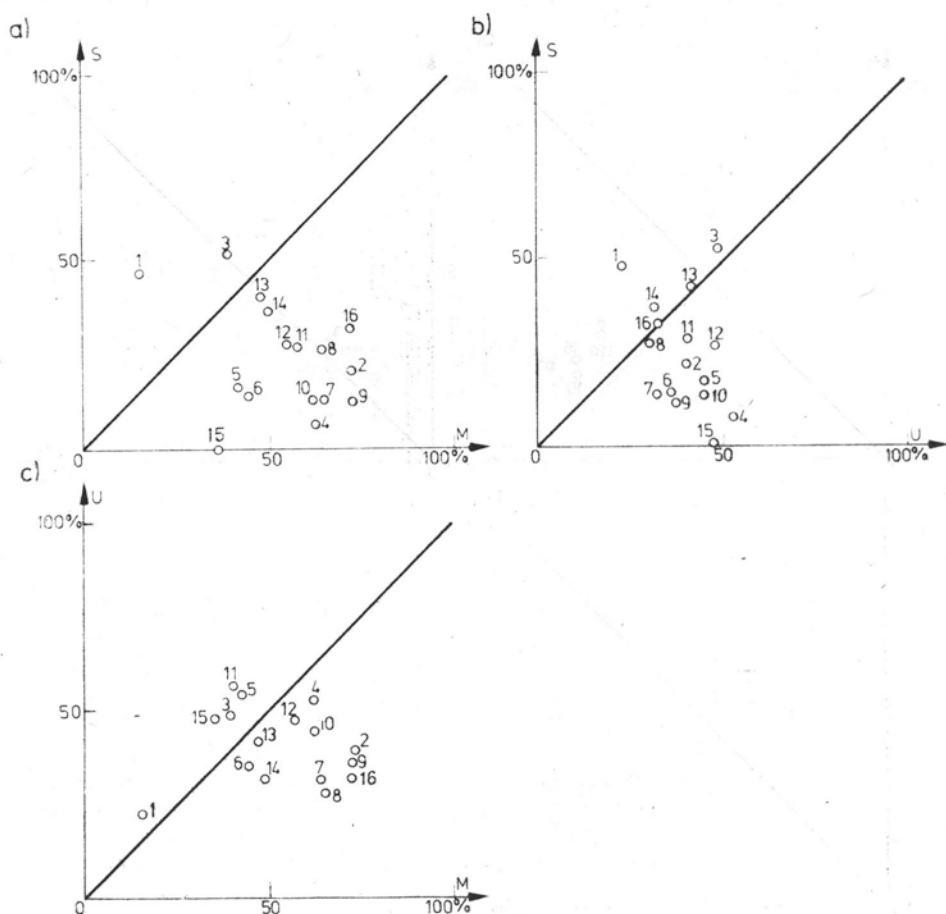


Fig. 11. Diagrams showing the antigenic similarity based on average results for malate dehydrogenase, peroxidase, esterase and proteins stained with amido black. The rest of notations as in Fig. 7

(or its tree-like form called *Pinus uliginosa*). The object of this study was to find whether the plants in question are also intermediate as regards their serological attributes.

As shown in the results, all the studied plants were to a major or lesser degree intermediate between these three taxa. But this intermediacy is of quite interesting nature. The plants are, namely, not intermediate in general but, they are rather, similar to one species in part of their antigenic properties and to an other one in the rest. Thus, there is no common trend in variation of the antigenic properties, but they behave, at least to some extent, rather independently. This result

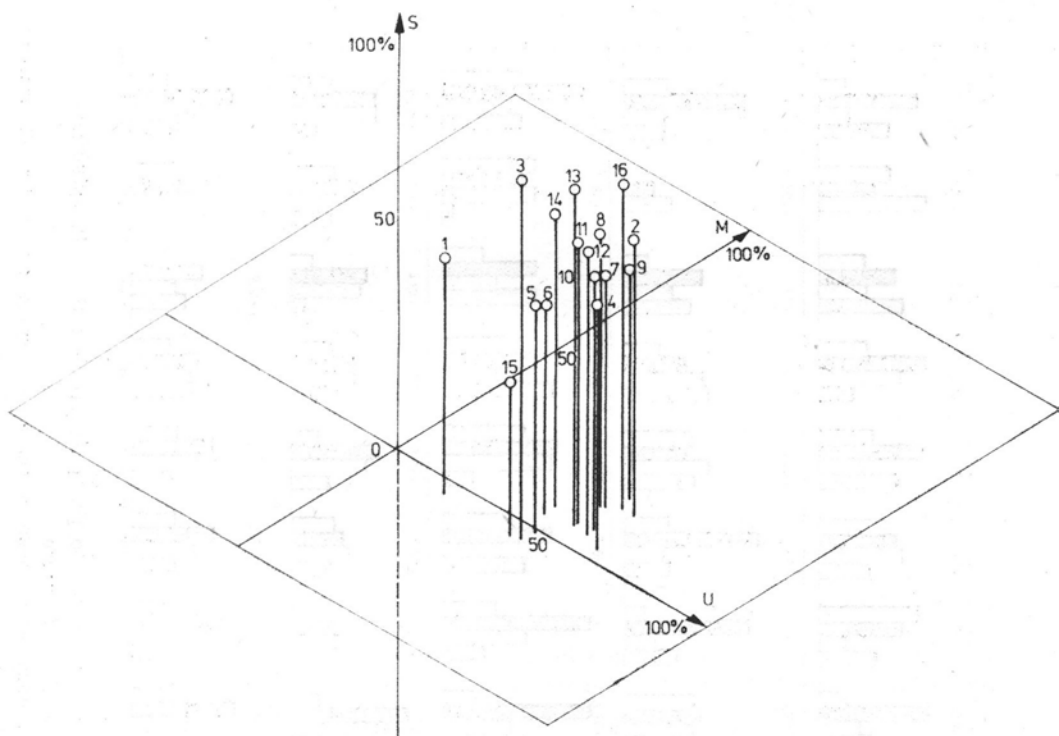


Fig. 12. Space diagram of antigenic similarity (average results for malate dehydrogenase, esterases, peroxidases and proteins stained with amido black), of hybrid swarm individuals to the three pure species: S — *P. sylvestris*, M — *P. mugo*, U — *P. uliginosa*. Percent of similarity is based on taxonomic distances

is in good agreement with the results obtained in our previous work using electrophoretic separation of needle proteins (Prus-Głowacki, Szwejkowski, 1977).

Such independent behaviour of particular characters is, after all, nothing peculiar in respect to strong recombinational opportunities in a hybrid swarm population. It would be interesting, however, to see whether there is some general trend in the variation of particular groups of proteins. In other words, it would be interesting to know if all combinations of these protein groups are equally frequent. We will try to answer this question in a future publication.

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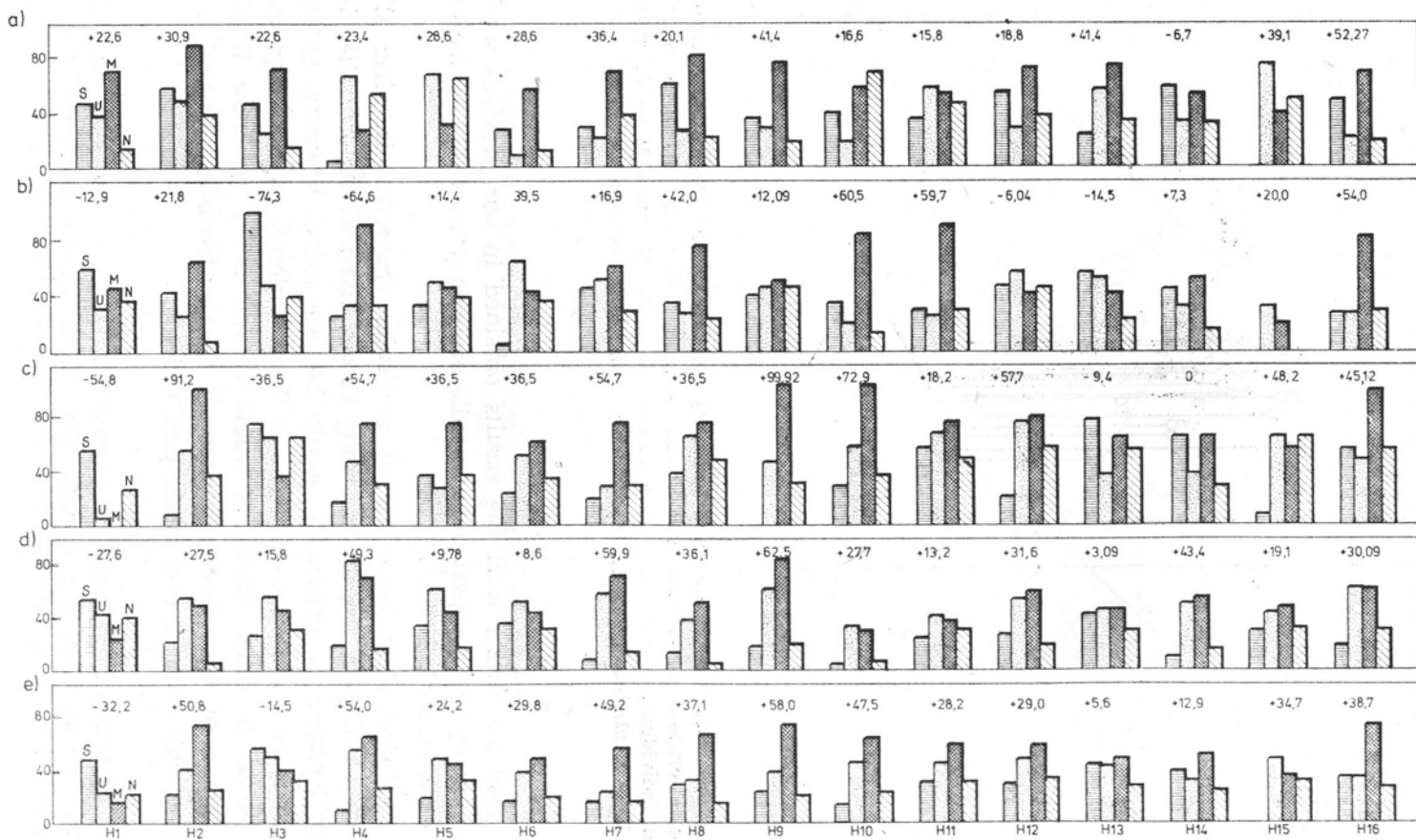


Fig. 13. Histogram showing (in per cent) the similarity of particular individuals to the pure species: S — *P. sylvestris*, U — *P. uliginosa*, M — *P. mugo*, N — *P. nigra*
 a — peroxidases, b — esterases, c — malate dehydrogenase, d — protein stained with amido black, e — average results for all analyses preformed

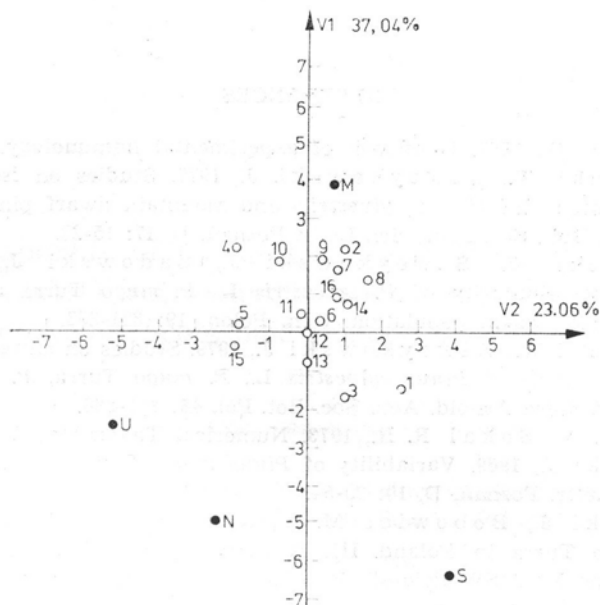


Fig. 14. Diagram showing the distribution of the 16 hybrid swarm individuals on the plane of the two first principal components axes. Points marked with letters S, M, U, N represent pure species populations (*P. sylvestris*, *P. mugo*, *P. uliginosa* and *P. nigra*, respectively)

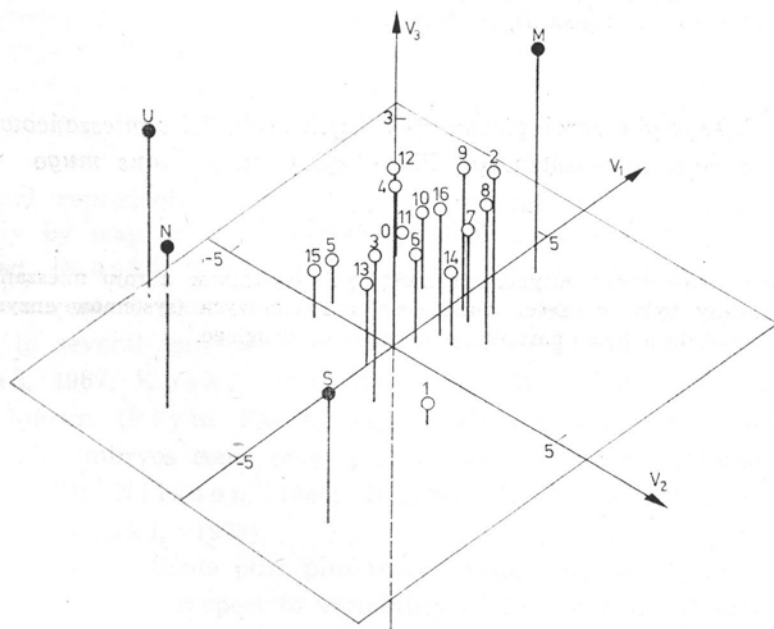


Fig. 15. Three-dimensional diagram showing the distribution of the hybrid swarm individuals in the three first principal components space. Points marked with letter S, M, U, N denote the populations of pure species as in the Fig. 14

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*Charakterystyka serologiczna niektórych osobników mieszańcowych
z roju mieszańcowego Pinus sylvestris × Pinus mugo*

Streszczenie

Badano właściwości antygenowe niektórych osobników z roju mieszańcowego. Badane rośliny były w części właściwości antygenowych (systemów enzymatycznych) podobne do jednego gatunku, a w części do drugiego.