ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLVII, nr 4 : 325—342
1978

Analysis of the variability of nine natural *Anthyllis vulneraria* s. l. populations*

Part I. Biometry of vegetative parts

K. ŁUKASZEWSKA, J. SZWEYKOWSKI **, Z. KACZMAREK

Institute of Plant Genetics, Polish Academy of Sciences

(Received: April 20, 1978)

Abstract

Variation of 11 vegetative traits has been studied in 9 natural populations of *Anthyllis vulneraria*. The analysis was performed in the field and then, for the second time, on samples of these populations grown in experimental garden in similar conditions. Three genetically different groups has been discovered: 1 — populations from the Tatra Mountains, 2 — from the Central Poland and Sudetes, 3 — from the Baltic Sea Coast.

INTRODUCTION

The variability observed in natural plant populations is a reflection both of genetic distinctness of the particular individuals and of the influence of the environment of their genotype. Genecological experiments are aimed at elimination of the effects of the differentiating influence by culture of population samples on experimental plots. It is then possible, by appropriate methods of sampling, transplantation and statistical analysis to evaluate the character of the differences between populations. The so far used method of classical genecological experiments takes into account but the little role of correct designing of field experiments and does not fully take advantage of modern statistical analysis. This is true, even for large scale studies carried out in the USA

* This study was supported by grant n° 09.7.—3.2.2. from the Polish Academy of Sciences.

** To whom the reprint requests should be adressed. Present adress: Department of Genetics A. Mickiewicz University, Dąbrowskiego 165, 60-594 Poznań, Poland.

by the Carnegie group (Clausen, Keck, Hiesey, 1934, 1940, 1948; Clausen 1951; Clausen, Hiesey 1958a,b).

The object of the present investigation is *Anthyllis vulneraria*, a plant common in Poland on open land, which exhibits so high a variability that particular populations of this plant have frequently been described as separate varieties and subspecies (Ascherson, Gräbner, 1898—1902; Ball-Chater, 1968, Beck v. Mannagetta, 1892; Becker, 1910; Gams, 1924; Rouy, 1897) or have been distinguished as independent species (Rothmaler, 1941). As a rule, however, these forms are of solely local significance (Juzepchuk, 1945; Sagorski, 1912). On the basis of comparison of the Scandinavian forms of *Anthyllis vulneraria* with Belgian and Spanish ones Jalas (1957) advanced the hypothesis that this high variability of *Anthyllis* is due to intensive crossing (associated with introgression) of several forms at the end of the glaciation era. Contemporary populations of this plant would be, according to this suggestion, relicts of a great postglacial hybrid swarm. Since in such a situation intensive evolutional processes may be expected, *Anthyllis* seems to be an appropriate material for genetic population studies.

MATERIAL AND METHODS

The analysis included 9 *Anthyllis vulneraria* populations from 4 different geographical regions of Poland: 1. Władysławowo, 2. Chłopy, 3. Mielno — coastline *(A. vulneraria* ssp. *maritima)*, 4. Kalatówki, 6. Skupniów Upłaz — Tatra *(A. vulneraria* ssp. *affinis)*, 5. Łężyce — Sudetes *(A. vulneraria* ssp. *polyphylla)*, 7. Rożnowo, 8. Gorzyń, 9. Międzychód — Wielkopolska Province Lowland *(A. vulneraria* ssp. *polyphylla)* (Kostrakiewicz, 1959).

From each population 60 randomly chosen plants were transferred to an experimental plot. The cultures were run in a completely randomized block design with 3 replications to ensure uniform distribution of the unavoidable differences in the test environment to all the individuals of the 9 samples. After cultivation for one year the plants were characterised in respect to 11 traits (Fig. 2): 1 — height to which they rise above the ground (in dependence on the stiffness of the shoot the plant may have an erect of slanting habitus), 2 — length of the generative stem, referred to for simplicity as the leader, 3 — number of inflorescences on the leader, 4 — number of leaf nodes on leader, 5 — length of first leaf on leader, 6 — length of petiole of this leaf, 7 — number of 1st order branchings of leader, 8 — sum of 1st order branchings of leader and 1st order branchings of the remaining generative stems, 9 — number of generative stems, 10 — number of inflorescences, 11 —

Fig. 1. Localization of the investigated 9 natural *Anthyllis vulneraria* s. 1. populations: 1. Władysławowo, 2. Chłopy, 3. Mielno, 4. Kalatówki, 5. Łężyce, 6. Skupniów Upłaz, 7. Rożnowo, 8. Gorzyń, 9. Międzychód

number of inflorescences on shortened 2nd and 3rd order axes (without leaf nodes). The same traits were investigated in plants growing on natural sites.

The data were statistically processed. In order to obtain possibly comprehensive data and to make adequate conclusions possible, statistical analysis was parallelly performed in two ways. Thus, beside the analysis of variance performed separately for each trait (ANOVA) and correlation coefficients (e.g. Freund, 1968) which are the measure of the degree of linear dependence between two traits, multivariate analysis of variance was also applied (MANOVA, e.g. Caliński, Kaczmarek, 1973) utilizing the Mahalanobis distance as the measure of the taxonomic distance between two populations (Anderson, 1961; Caliński, 1970; Caliński, Kaczmarek, 1973, 1975; Mahalanobis, 1936; Rao, 1948a) and canonical analysis (Anderson, 1961; Blackith, Reyment, 1971, Caliński, Czajka, Kaczmarek, 1975); Mor-

Fig. 2. Diagramme of single *Anthyllis plant* (1 — height to which the plants rise above ground, 2 — length of generative stem, 3 — leaf node on leader, 4 — first leaf on leader, 5 — 1st order branching of leader, 6 — inflorescence on shortened 2nd order axis)

rison, 1967, Sneath, Sokal, 1973; Sokal, Sneath, 1963). The discrimination power of the particular traits was also investigated (Caliński, Kaczmarek, 1973).

A graphic illustration of the results are the diagrammes of the position of the populations on the plane of the 1st and 2nd canonical axes, with a dendrite of the shortest distances between the populations, plotted on the basic of the distance of Mahalanobis. This dendrite is constructed on the points which are estimations of means of each of the 9 population traits analysed. The coefficients of correlation are presented according to Terentiev (1959) in the form of correlation pleiads.

RESULTS

The significance of differences between the populations in respect to each of the traits was analysed by Tukey's test. Its results are graphically presented in the tables (Fig. 3). The sign "plus" denotes differences statistically significant, and "minus" indicates a lack of significant differences between the populations as regards the given trait. Confrontation of the results of calculations for natural sites and plants from the experimental plot reveals the elimination of a certain part of environmental variability in culture. This is manifested in a decrease in the number of significant differences, i.e. tables for the traits: length of

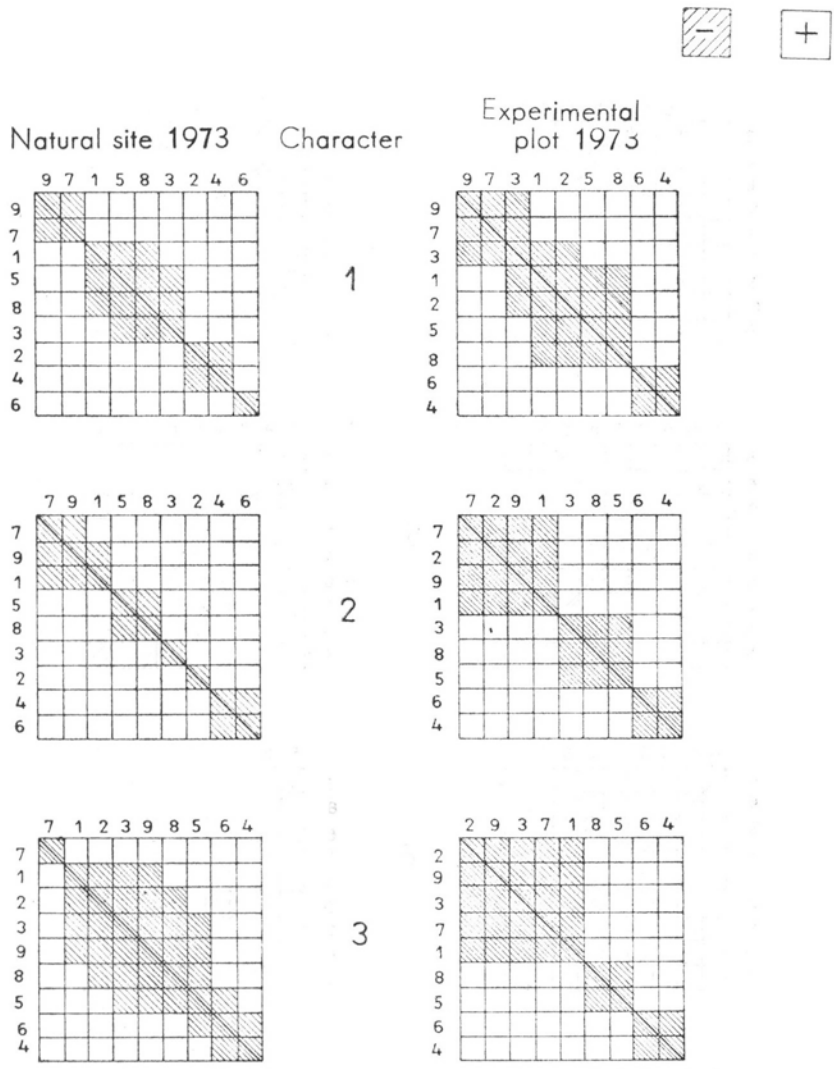

Fig. 3A

Explanations on page 331

1st leaf of leader (5), length of petiole (6), number of 1st order branchings on one plant (8), number of generative stems (9), number of inflorescences on one plant (10).

Analysis of material from natural sites demonstrated a clear distinctness of the Tatra plants. This distinctness persisted when they were cultivated. The trait which distinguished most the Tatra populations from the remaining ones seems to be the absence of side branchings characteristic only for these plants (cf. Tables for traits 7 and 8). The Tatra plants also exhibit the smallest length of the generative shoots

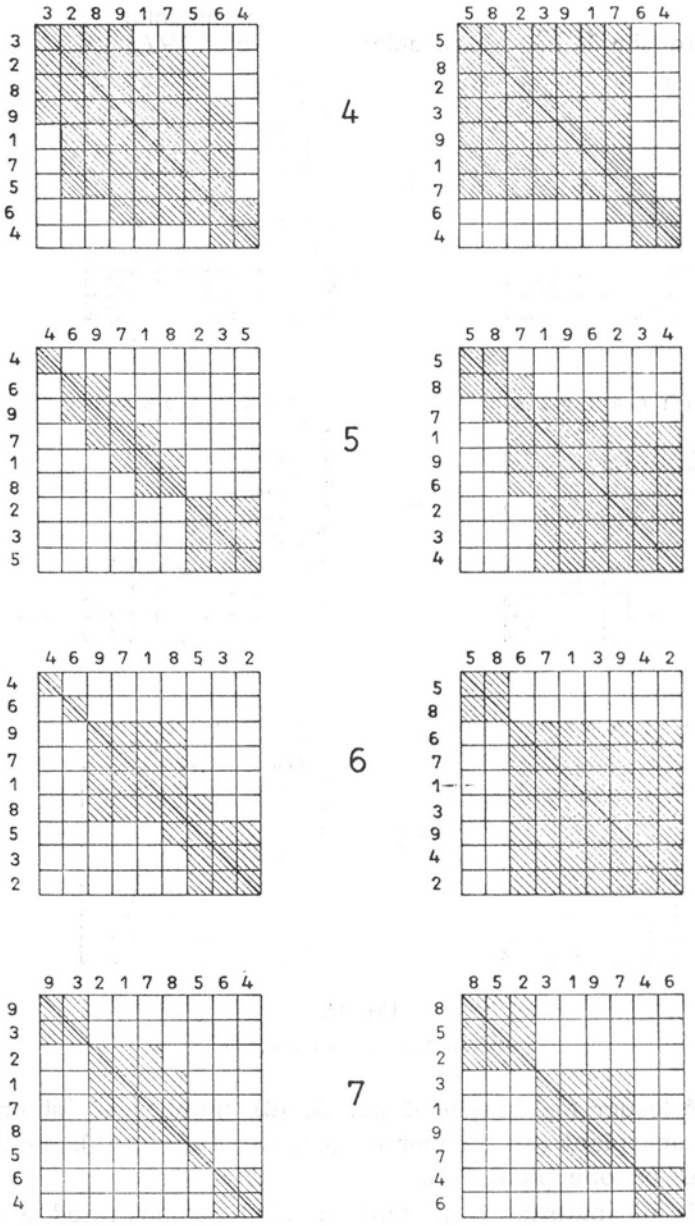

Fig. 3B

Explanations on page 331

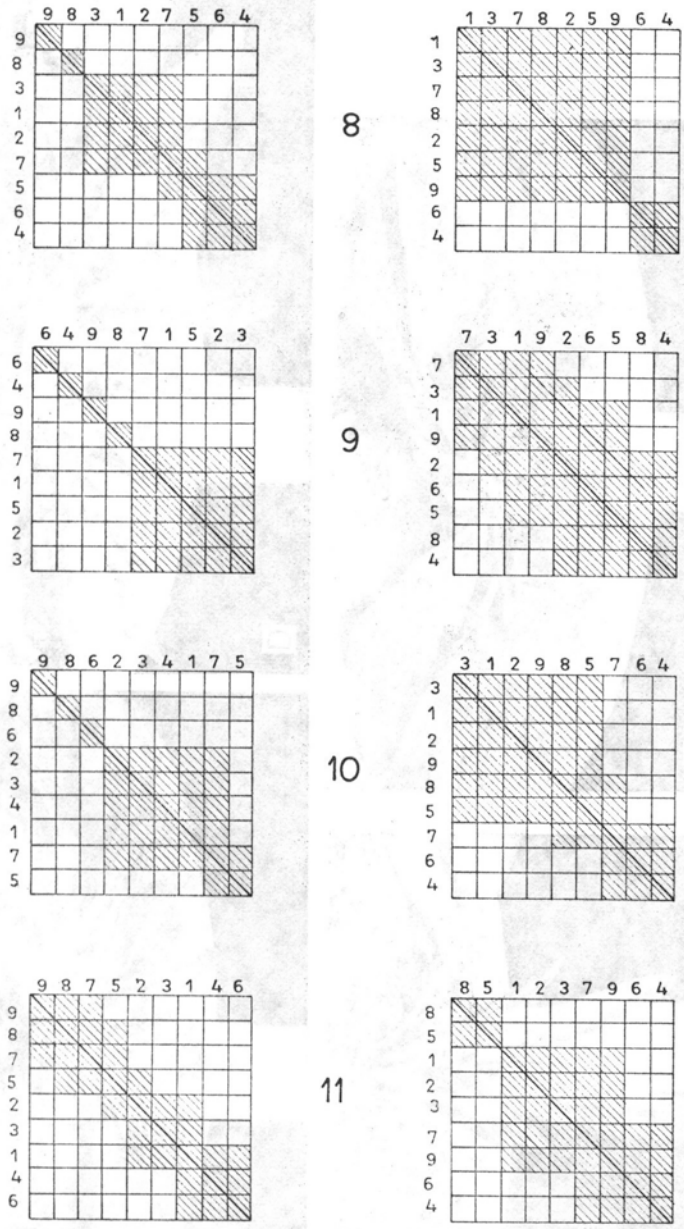

Fig. 3C

Fig. 3 A, B, C. Tables of significant differences between populations subjected to Tukey's test for 9 *Anthyllis vulneraria* s. l. populations from natural sites and from experimental plot, vegetative traits

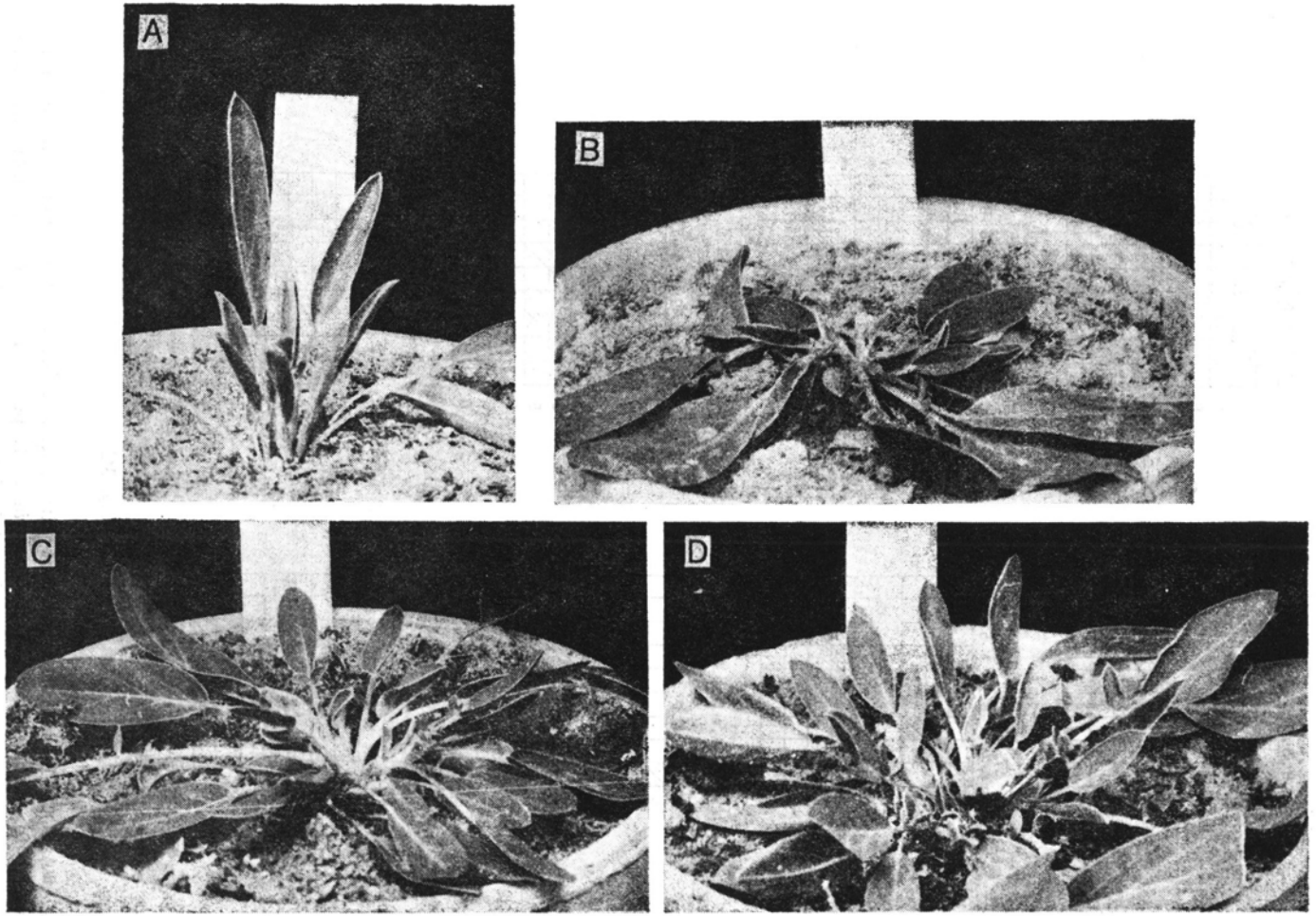

Photo 1. Randomly chosen individuals: from the coast (A), the Tatra (B), Wielkopolska (C), and Sudets (D) populations at the stage of rosette with several leaves

(trait 2) differing in this significantly both on natural sites and on the experimental plots from the remaining populations. As regards trait 9 (number of generative stems) the populations nos 4 and 6 showed a tendency to reduction of the number of shoots in culture, whereas all the other populations were characterized by an increased number of generative shoots on the experimental plants. These results suggest a genetic distinctness of the Tatra populations from Kalatówki and Skupniów Upłaz.

The population from Łężyce in the Sudetes (no 5) on its natural site does not show any special similarities, whereas on the experimental plot it shows close relationship with population no. 8 from Gorzyń. These two populations are also related by their earlier flowering on the experimental plot as compared with other Wielkopolska and coastal plants.

The populations from the coast do not form such a distinct group as do the Tatra ones. Although they show the greatest resemblance to one another, frequently the boundary between the coastal and the Wielkopolska populations is obliterated. It is so for instance in the ordering of the population samples for Tukey's test (they are arranged according to diminishing means).

The lowland populations from Wielkopolska are most diversified. Beside the already mentioned population from Gorzyń (no. 8), the one from Rożnowo (no. 7) shows a certain individuality. In the natural habitat *Anthyllis* grows densely and reaches extreme mean values as regards the traits: length of leader (2) and number of inflorescences on it (3). As shown by cultivation, the reason for these differences lies in the environment. Under the equal conditions of the experimental plot this population loses its individuality.

Interesting results were obtained in the analysis of the material for the number of inflorescences on one plant (trait 10). In natural conditions no correlation of this trait is observed with the geographic position of the plants. The experiments on the plot demonstrated, however, that the mountain populations are characterized by the lowest values, mean ones fall to the Wielkopolska and Sudetes groups, and the highest are exhibited by the coastal populations. Such a geographically ordered gradation of this trait may indicate perhaps the existence of a cline of the inflorescence number. Further studies are required to confirm this conclusion.

The transplantation experiments also revealed that, at the stage of vegetative development from a rosette of several leaves to the formation of generative stems, the *Anthyllis* plants form groups characteristic for the geographical regions from which they originate. The coastal populations differ in general from the remaining ones by the erect stiff growth of the seedlings (Photo 1A). The plants from the Tatra form in this period spreading rosettes adhering to the ground (Photo 1B), while

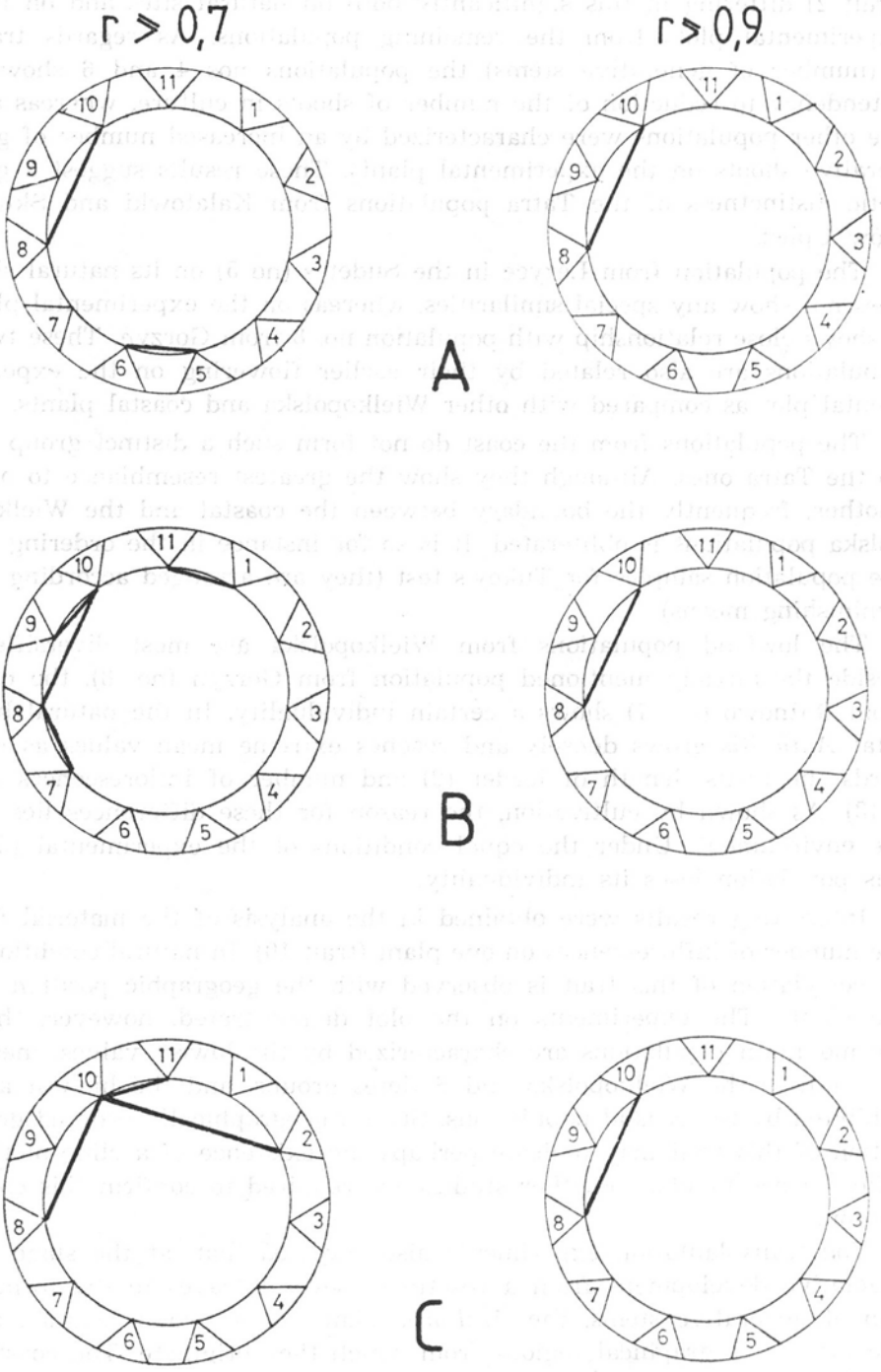

Fig. 4. Correlation pleiads: A — Władysławowo, B — Chłopy, C — Mielno

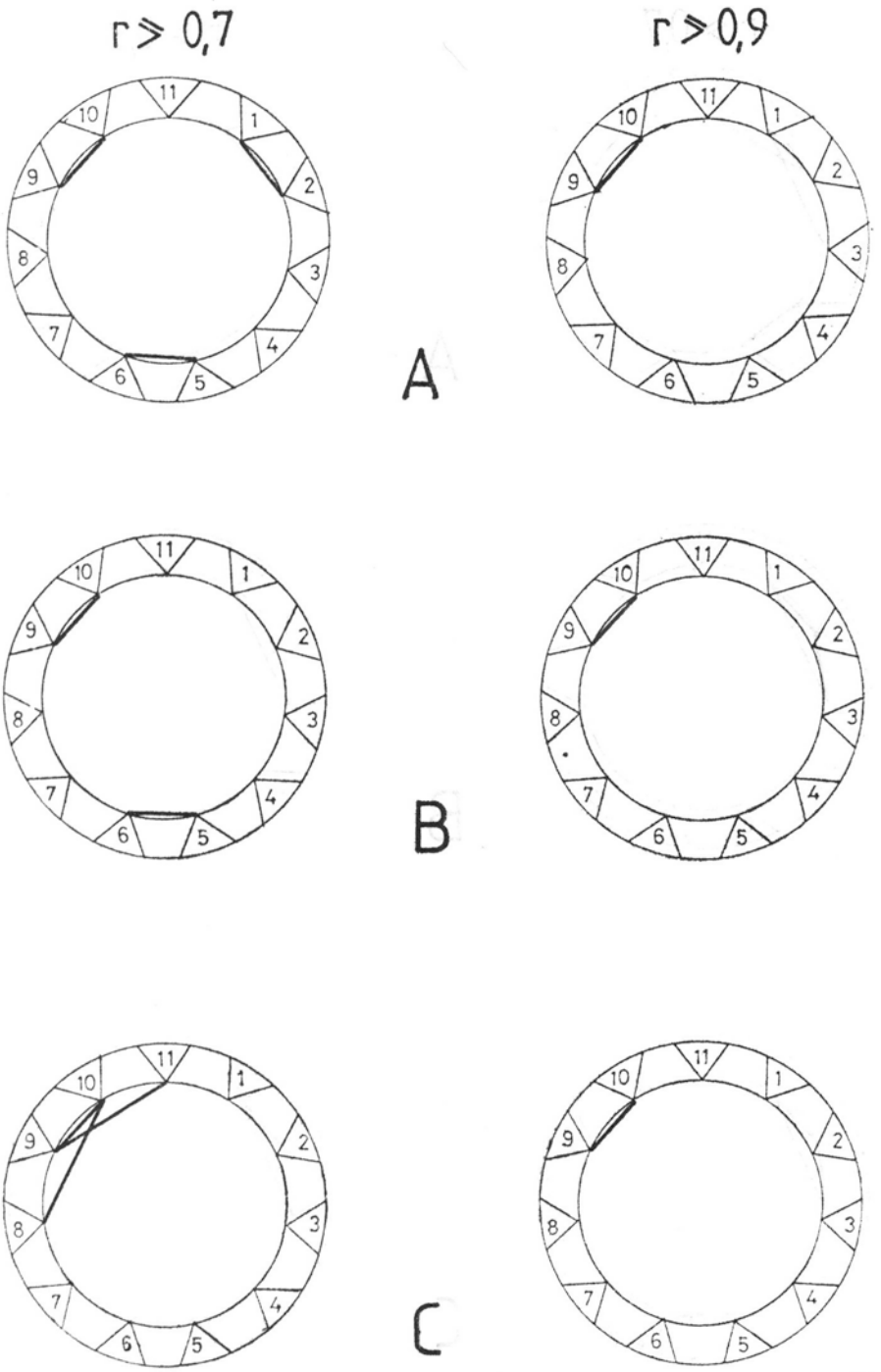

Fig. 5. Correlation pleiads: A — Kalatówki, B — Skupniów Upłaz, C — Łężyce

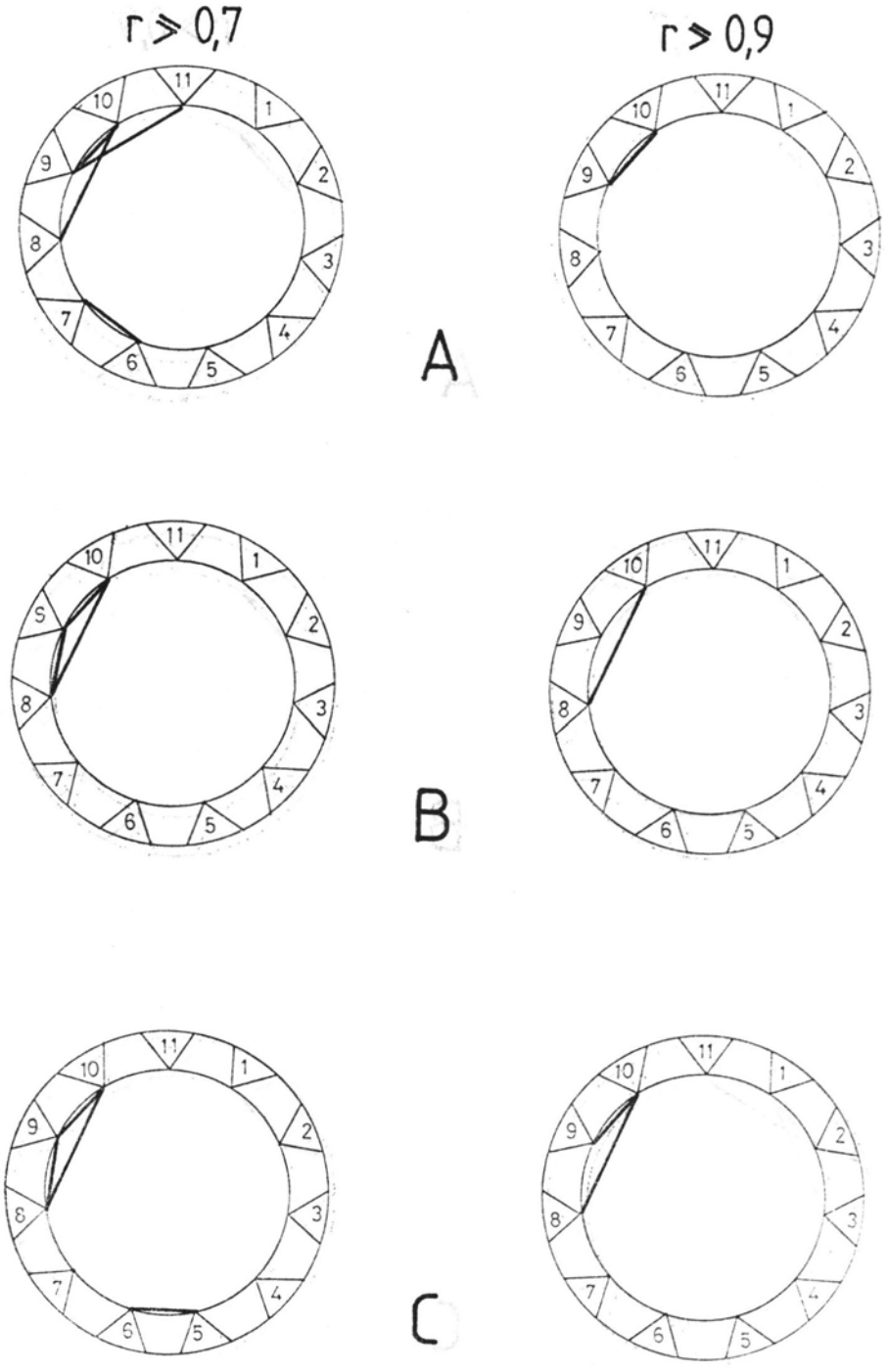

Fig. 6. Correlation pleiads: A — Rożnowo, B — Gorzyń, C — Międzychód

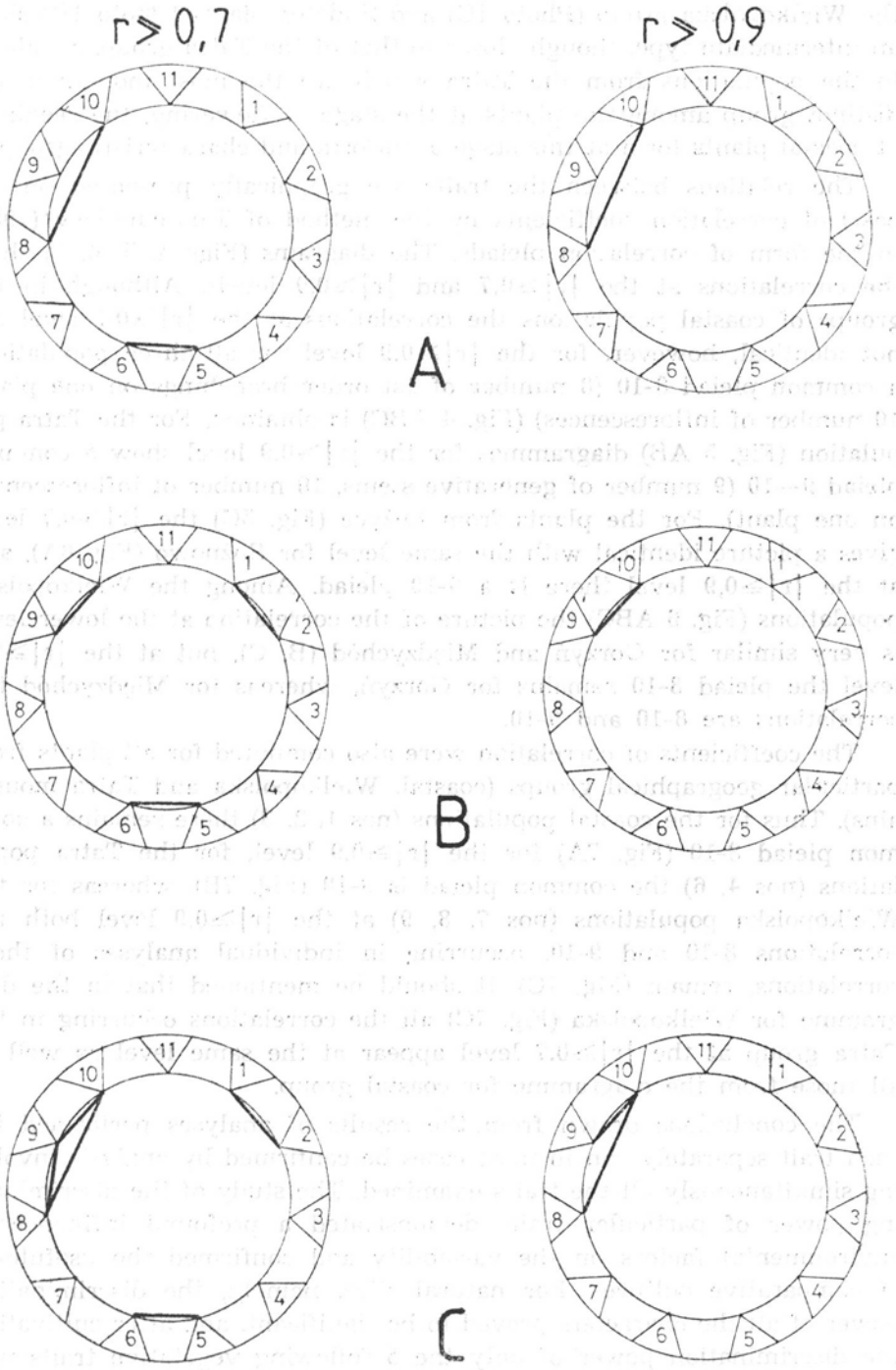

Fig. 7. Correlation pleiads: A — coastal populations, B — Tatra populations, C — Wielkopolska populations

the Wielkopolska group (Photo 1C) and Sudetes plants (Photo 1D) show an intermediate type, though closer to that of the Tatra group. Similarly to the populations from the Tatra which are the most individual and distinct group among the plants at the stage of flowering, the seedlings of coastal plants form at this stage a uniform and characteristic group.

The relations between the traits are graphically presented on the basis of correlation coefficients by the method of T e r e n t i e v (1959) in the form of correlation pleiads. The diagrams (Figs 4, 5, 6, 7) show the correlations at the $|r| \geqq 0{,}7$ and $|r| \geqq 0{,}9$ levels. Although in the groups of coastal populations the correlations at the $|r| \geqq 0{,}7$ level are not identical, however, for the $|r| \geqq 0{,}9$ level for all three populations a common pleiad 8-10 (8 number of 1st order branchings on one plant, 10 number of inflorescences) (Fig. 4 ABC) is obtained. For the Tatra population (Fig. 5 AB) diagrammes for the $|r| \geqq 0{,}9$ level show a common pleiad 9—10 (9 number of generative stems, 10 number of inflorescences on one plant). For the plants from Łężyce (Fig. 5C) the $|r| \geqq 0{,}7$ level gives a picture identical with the same level for Rożnowo (Fig. 6A), and at the $|r| \geqq 0{,}9$ level there is a 9-10 pleiad. Among the Wielkopolska populations (Fig. 6 ABC) the picture of the correlation at the lower level is very similar for Gorzyń and Międzychód (B, C), but at the $|r| \geqq 0{,}9$ level the pleiad 8-10 remains for Gorzyń, whereas for Międzychód the correlations are 8-10 and 9-10.

The coefficients of correlation were also computed for all plants from particular geographical groups (coastal, Wielkopolska and Tatra mountains). Thus for the coastal populations (nos 1, 2, 3) there remains a common pleiad 8-10 (Fig. 7A) for the $|r| \geqq 0{,}9$ level, for the Tatra populations (nos 4, 6) the common pleiad is 9-10 (Fig. 7B), whereas for the Wielkopolska populations (nos 7, 8, 9) at the $|r| \geqq 0{,}9$ level both the correlations 8-10 and 9-10, occurring in individual analyses of these correlations, remain (Fig. 7C). It should be mentioned that in the diagramme for Wielkopolska (Fig. 7C) all the correlations occurring in the Tatra group at the $|r| \geqq 0{,}7$ level appear at the same level as well as all those from the diagramme for coastal group.

The conclusions drawn from the results of analyses performed for each trait separately can in most cases be confirmed by analysis involving simultaneously all the traits examined. The study of the discriminating power of particular traits demonstrated a profound influence of environmental factors on the variability and confirmed the usefulness of comparative cultures. For natural sites, namely, the discrimination power of all the characters proved to be significant, and after cultivation the discrimination power of only the 5 following vegetative traits was significant: height of plants above ground (1), length of leader (2), number of generative stems (9), number of inflorescences on shortened axes of 2nd and 3rd order (11).

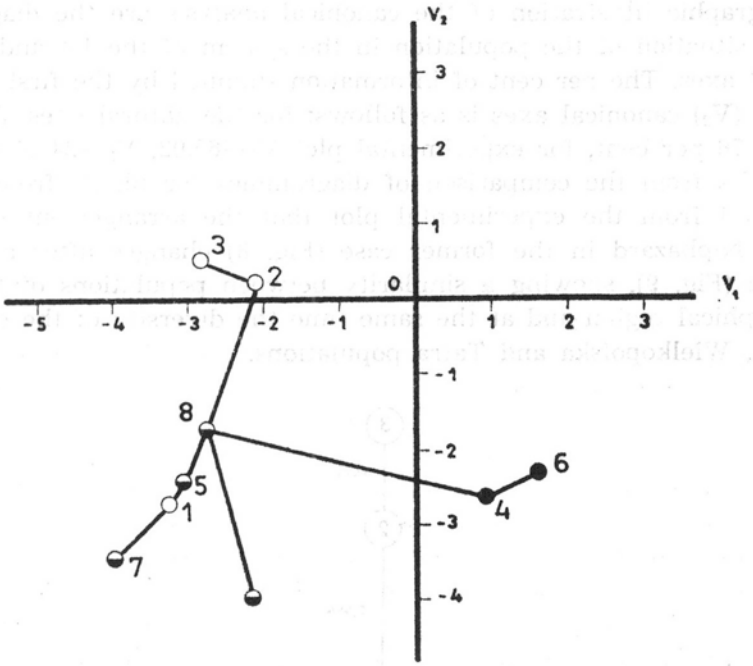

Fig. 8. Diagramme of position of the populations in the system of the 1st and 2nd canonical axes for data from natural sites

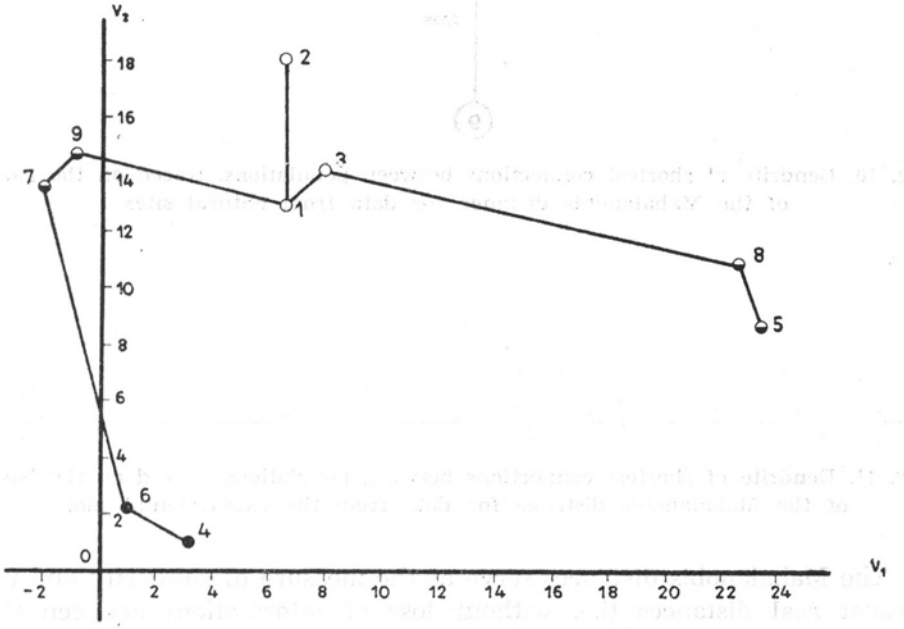

Fig. 9. Diagramme of position of the populations in the system of the 1st and 2nd canonical axes for data from the experimental plot

A graphic illustration of the canonical analysis are the diagrammes of the situation of the population in the system of the 1st and 2nd canonical axes. The per cent of information supplied by the first (V_1) and second (V_2) canonical axes is as follows: for the natural sites $V_1=50{,}41$, $V_2=21{,}76$ per cent, for experimental plot $V_1=65{,}02$, $V_2=24{,}24$ per cent. It results from the comparison of diagrammes for plants from natural sites and from the experimental plot that the arrangement of points rather haphazard in the former case (Fig. 8) changes after a year of culture (Fig. 9), showing a similarity between populations of the same geographical region and at the same time the diversity of the groups of coastal, Wielkopolska and Tatra populations.

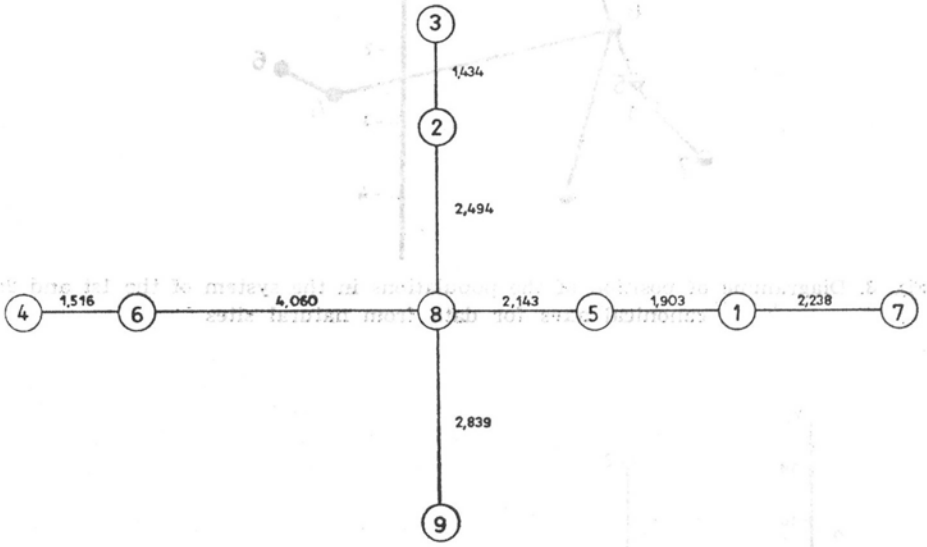

Fig. 10. Dendrite of shortest connections between populations, traced on the basic of the Mahalanobis distance for data from natural sites

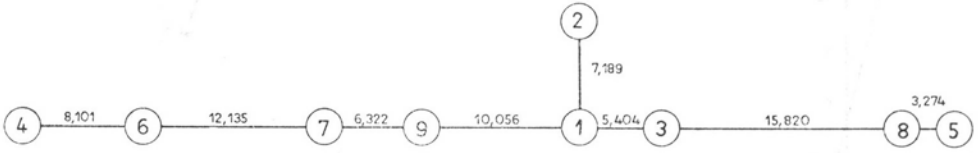

Fig. 11. Dendrite of shortest connections between populations, traced on the basic of the Mahalanobis distance for data from the experimental plot

The Mahalanobis distances serve at the measure of similarity and represent real distances (i.e. without loss of information) between the points characterizing the objects examined, lying in an n-dimensional space. Similarly as in the case of the canonical axes, the dendrite of

the shortest distances between the populations is rather little differentiated for natural sites, whereas after cultivation it groups the populations according to the geographical regions from which they originate (Figs 10, 11). These results confirm once more the usefulness of comparative culture under uniform conditions of the test environment.

The variability picture obtained indicates unequivocally that there exist genetically conditioned differences between the populations of the three different geographical regions: the Tatra, the Baltic coast and the Wielkopolska Lowland. The consistent differences become noticeable only on the experimental plot, in natural conditions the variability of the environment largely obliterates them. It will only be possible to rech final conclusions after studying the generative traits.

REFERENCES

Anderson T. W., 1961. An Introduction to Multivariate Statistical Analysis. New York, John Wiley.

Ascherson P., Gräbner P., 1898-1902. Synopsis der mitteleuropäischen Flora II. Leipzig, Engelman.

Ball P. W., Chater A. O., Fergusson J. K., 1968. Flora Europaea.

Beck v. Mannageta G., 1892. Flora von Nieder-Österreich II. Wien.

Becker W., 1910. Bearbeitung der *Anthyllis* — Section *Vulneraria* DC. Beih. Bot. Centralbl. 27, Abt. II: 256-287.

Blackith R. E., Reyment R. A., 1971. Multivariate Morphometrics. London, New York.

Caliński T., 1970. Wielozmienna analiza wariancji i pokrewne metody wielowymiarowe. I Colloquium Metodologiczne z Agro-Biometrii, Warszawa 1970: 3-51.

Caliński T., Czajka S., Kaczmarek Z., 1975. Analiza składowych głównych i jej zastosowanie. Roczniki AR w Poznaniu, R. LXXX, Algor. Biom. Statyst. 4: 159-186.

Caliński T., Kaczmarek Z., 1973. Metody kompleksowej analizy doświadczenia wielocechowego. III Colloquium Metodologiczne z Agro-Biometrii, Warszawa 1973: 258-320.

Clausen J., 1951. Stages in the evolution of plant species. Cornell University Press.

Clausen J., Hiesey W. M., 1958a. Experimental studies on the nature of species. IV. Genetics structure of ecological races. Carnegie Inst. of Washington Publication 615.

Clausen J., Hiesey W. M., 1958b. Phenotypic expression in contrasting environments. Rep. Scottish Plant Breeding Stat. 1958: 41-51.

Clausen J., Keck D. D., Hiesey W. M., 1934. Experimental Taxonomy. Yearb. Carneg. Inst. 33: 173-187.

Clausen J., Keck D. D., Hiesey W. M., 1940. Experimental studies on the nature of species. I. Effect of varied environments on western North American plants. Carnegie Inst. of Washington Publication 520.

Clausen J., Keck D. D., Hiesey W. M., 1948. Experimental studies on the nature of species. III. Environmental responses of climatic races of *Achillea*. Carnegie Inst. of Washington Publication 581.

Freund J. E., 1968. Modern Elementary Statistics. PWE Warszawa.
Gams H., 1924. Leguminoseae, in: Hegi G., Illustrierte Flora von Mittel-Europa, IV: 1113-1748. München.
Heslop-Harrison J., 1964. Forty Years of Genecology. Advances in Ecological Research 2: 159-247.
Jalas J., 1957. Rassentaxonomische Probleme in Bereich des *Anthyllis vulneraria* L. Komplex in Belgien. Bulletin du Jardin botanique de l'etat Bruxelles, vol. Jubilaire. Walter Robyns, XXVII fasc. 3: 405-416.
Juzepchuk S. W., 1945. *Anthyllis* L., Komarov W. A., Flora URSS, XI: 262-280, 292-294. Leningrad 1945.
Mahalanobis P. C., 1936. On the generalized distance in statistics. Proc. Nat. Inst. Sci. India 2: 49-55.
Morrison D. F., 1967. Multivariate Statistical Methods. McGraw-Hill, New York.
Rao C. R., 1948. The utilization of multiple measurements in problems of biological classification. J. Roy Statist. Soc. Ser. B 10: 159-203.
Rothmaler W., 1941. Westmediterrane Arten der Section Vulneraria DC der Gattung *Anthyllis*. Rep. Spec. Nov. Regni Vegetab. 50: 177-192, 233-245.
Rouy G., 1897. Flore de France IV: 313. Tours.
Sagorski F., 1912. Über *Anthyllis polyphylla* Kit. in Tirol und über einige andere *Anthyllis* Formen. Allgem. Botan. Zeitschrift 17: 37-40, 57-59, 69-75.
Sneath P. H., Sokal R. R., 1974. Principles of Numerical Taxonomy. San Francisco, London.
Sokal R. R., Sneath P. H. 1963. Principles of Numerical Taxonomy. Freeman and Comp. San Francisco.

Authors' address:
Mgr Krystyna Łukaszewska, Prof. Jerzy Szweykowski, Dr Zygmunt Kaczmarek
Institute of Plant Genetics, Polish Academy of Science;
Strzeszyńska 30/36, 60-479 Poznań, Poland

Analiza zmienności dziewięciu naturalnych populacji Anthyllis vulneraria s.l. Cz. I. Biometria części wegetatywnych

Streszczenie

Dla dziewięciu naturalnych populacji przelotu z różnych regionów geograficznych Polski wykonano analizę zmienności cech wegetatywnych na stanowiskach naturalnych w terenie i po rocznej kulturze na polu doświadczalnym. Wszystkie zastosowane w tym celu jedno- i wielozmienne metody statystyczne potwierdziły celowość kultury porównawczej, a uzyskany obraz zmienności wskazuje na istnienie genetycznie uwarunkowanego zróżnicowania między populacjami trzech regionów geograficznych: Wybrzeża, Wielkopolski i Tatr. Konsekwentnie różnice uwydatniają się dopiero w kulturze — zmienność środowiskowa występująca w naturze zaciera je w dużej mierze.