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

Part II. Biometry of flowers

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Abstract

It was established on the basis of examination of nine natural *Anthyllis vulneraria* populations from various geographical regions of Poland that the type of variability differs in the coastal, Wielkopolska lowland and Tatra populations.

The results of the experiments support the idea of the taxonomic distinctness of the coastal, lowland and montane plants; the coastal populations belong to *Anthyllis vulneraria* ssp. *maritima*, Tatra populations to *Anthyllis vulneraria* ssp. *affinis* and populations from the Wielkopolska Lowland and Sudetes to *Anthyllis vulneraria* ssp. *polyphylla* (Kostrakiewicz, 1959).

INTRODUCTION

In the first part of this paper (Łukaszewska, Szweykowski and Kaczmarek, 1978) the results were presented of biometric analyses of vegetative traits. The next step in the studies on the variability of natural *Anthyllis vulneraria* populations is the analysis of floral material.

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MATERIAL AND METHODS

Plants of nine *Anthyllis* populations from 4 different geographical regions of Poland given in detail in Part I were characterized both on natural sites and after one-year's culture on an experimental plot (the culture was run in a completely randomised block design with 3 replications). Ten traits of the flowers were taken into account: 1 — length of

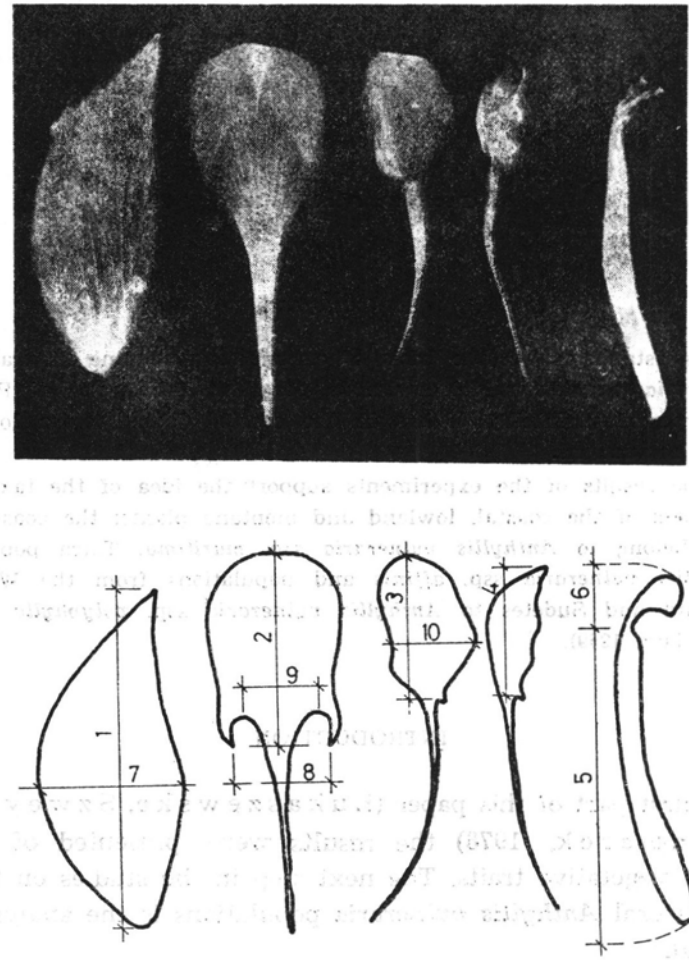

Photo 1 A, B. Elements of *Anthyllis vulneraria* flower: 1 — length of calyx, 2 — length of vexillum without unguis, 3 — length of wing without unguis, 4 — length of keel without unguis, 5 — length of androecium, coalesced part, 6 — length of free part of androecium, 7 — width of calyx, 8 — distance between dents of vexillum, 9 — width of vexillum at constriction under dents, 10 — width of wing at the site of its bulge

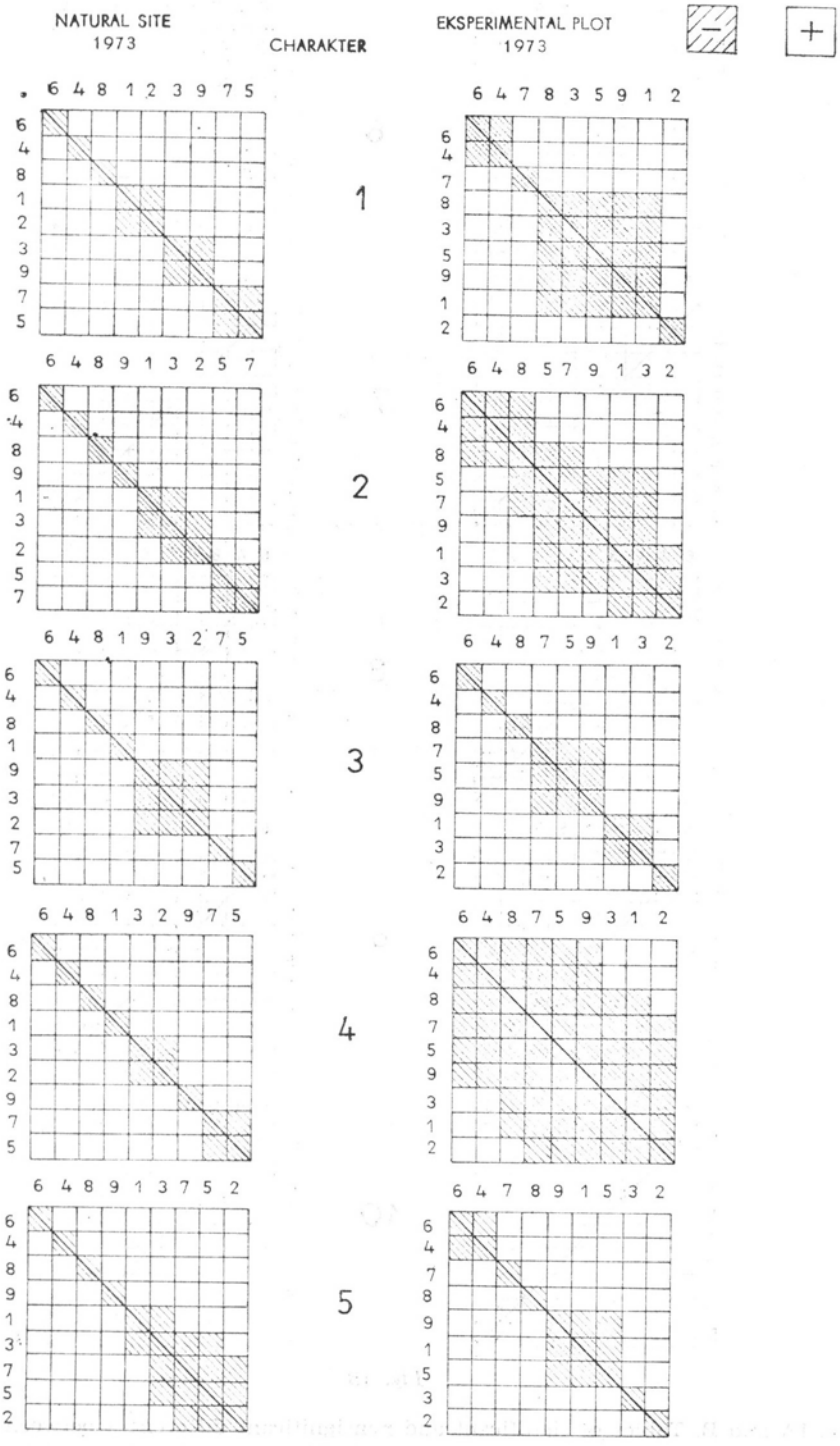

Fig. 1A

Explanations see p. 346

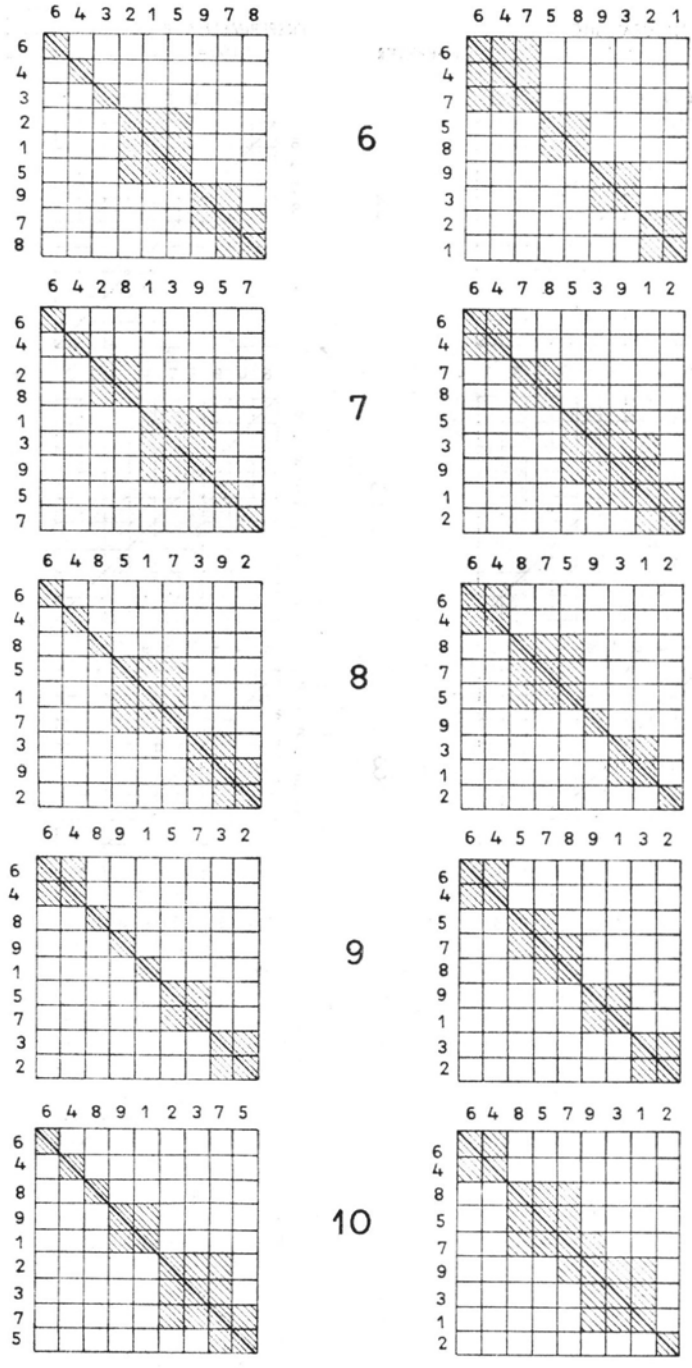

Fig. 1B

Fig. 1A and B. Tables of significant and nonsignificant differences between populations subjected to Tukey's test for 9 *Anthyllis vulneraria* s.l. populations from natural sites and from compartive cultures en experimental plots — floral traits

calyx, 2 — length of vexillum without unguis, 3 — length of wing without unguis, 4 — length of keel without unguis, 5 — length of androecium (coalesced part), 6 — length of androecium (free part), 7 — width of calyx, 8 — distance between dents of vexillum, 9 — width of vexillum at constriction under dents, 10 — width of wing at bulge. Measurements were taken on 180 flowers of each population on the prepared out particular elements of the flower (Photo 1).

For characterizing the variability of the floral traits, analysis of variance was applied for each trait and multivariate analysis of variance analogously as in Part I for the vegetative traits separately for population samples from natural sites and those of cultivated plants. Thus, besides, analysis of variance performed separately for each trait (ANOVA) and correlation coefficients (e.g. Freund, 1968), multivariate analysis of variance was also applied (MANOVA — e.g. Caliński, Kaczmarek, 1973) with calculation of the Mahalanobis distances as the measure of distinctness between two populations (Mahalanobis, 1936; Rao, 1948; Sokal, Sneath, 1963; Sneath, Sokal, 1973; Caliński, Kaczmarek, 1975) as well as of canonical analysis (Anderson, 1961; Blackith, Reyment, 1971; Caliński, Czajka, Kaczmarek, 1975; Morrison 1967; Sneath, Sokal, 1973; Rao 1964). The discrimination power of the traits was also investigated (Caliński, Kaczmarek, 1973).

RESULTS

The analysed floral traits characterize the size of the particular elements of the flower. One-way analysis of variance supplemented by Tukey's test allowed the estimation of the significance of differences between the populations in respect to each trait. The results of the test are shown graphically in the tables (Fig. 1). The sign "plus" denotes significant differences, while the sign "minus" indicates a lack of significant differences between the populations.

The plants of the investigated 9 populations of *Anthyllis* proved to differ more as regards the size of flowers than in respect to vegetative traits. The transplantation experiment and culture under uniform conditions on the experimental plot do not generally eliminate these differences, however, the somewhat chaotic picture of variability obtained in calculations from natural sites is somewhat better ordered here. A marked similarity of the populations after culture was observed for only two traits: vexillum length (2) and keel length (4). This may be explained by the elimination of the effects of the differentiating environmental modification. Comparison of the degree of reaction to equal habitat conditions seems to indicate a lower plasticity of the floral traits.

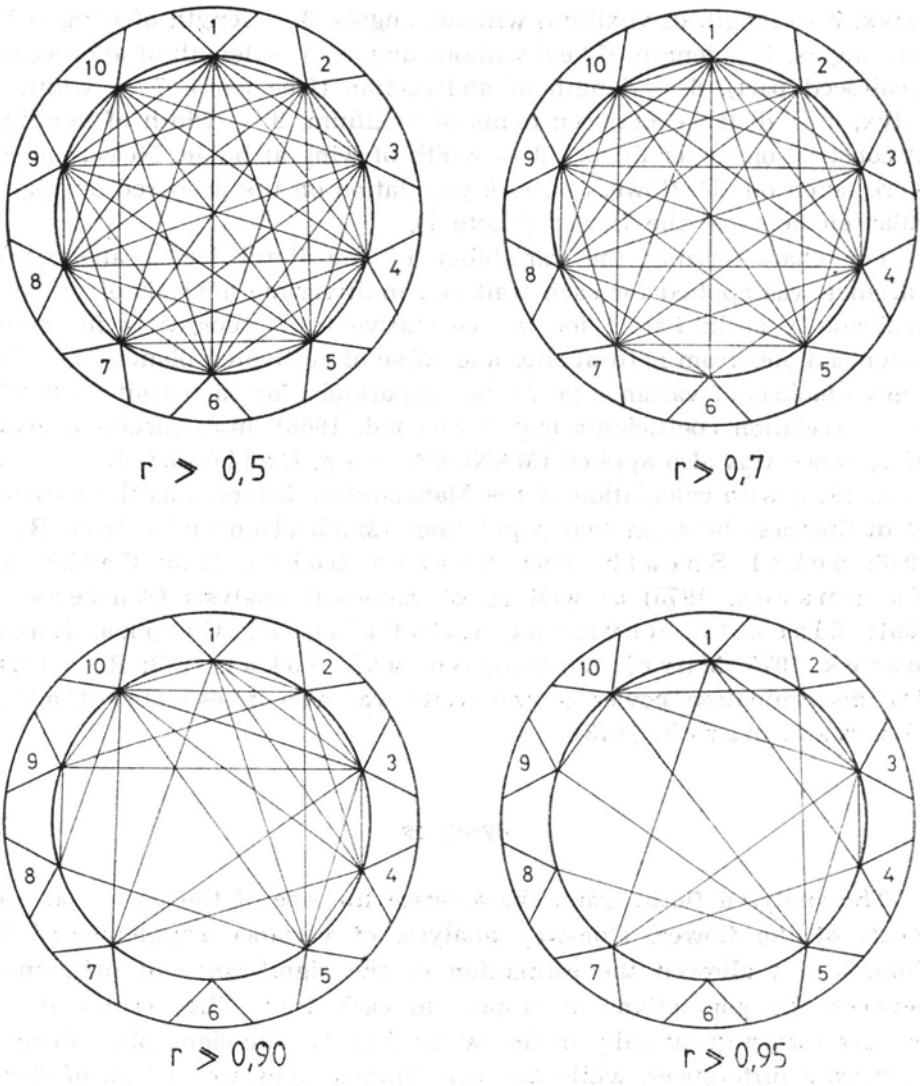

Fig. 2. Correlation pleiads of floral traits for data from natural sites

The elimination of a certain range of environmental variability may, however, be observed within population groups bound with the geographical region from which they originated. This is particulary true for Tatra populations (nos 4 and 6) which differed in natural conditions significantly in respect to 9 traits (of the 10 analysed), whereas in comparative culture they proved to be highly homogeneous group with the exception of trait 3 (length of wing) and showed no significant differences.

The population from the coast (nos 1, 2, 3), although mixed for field observation with those from Wielkopolska form in culture the group

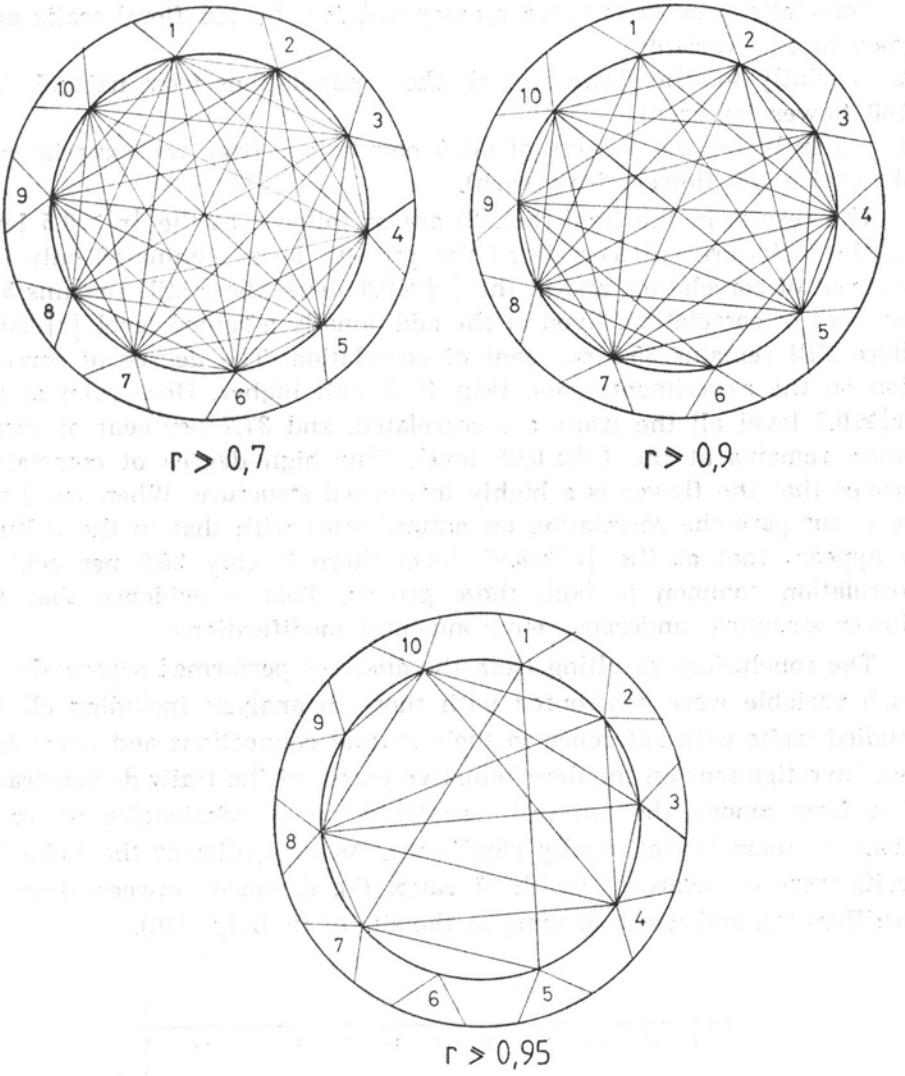

Fig. 3. Correlation pleiads of floral traits for data from experimental plots

with smallest flowers. It should be stressed here that after culture a distinct tendency to reduction of the flower size is observed from south northward in the pattern of the variability of floral traits of all the 9 populations. A similarly geographically ordered character of variability was noted earlier in the analysis of vegetative traits for the number of inflorescences on one plant (trait 10). In both these cases the correlation of the gradual changes in the trait with the geographical gradient suggests a clinal character of the variability. This, however, requires confirmation after examination of a larger number of populations in the south-north transect.

Correlation coefficients were computed for the ten floral traits analysed in two variants:
A — jointly for the flowers of all the 9 populations from natural sites (180 flower for each),
B — jointly for the flowers of all 9 populations from the experimental plots (also 180 flowers from each).

The results in the field (Fig. 2) are as follows: at the $|r| \geqq 0.5$ level all the traits are still correlated, the $|r| \geqq 0.7$ level eliminated only 11.2 per cent of correlation, and at the $|r| \geqq 0.9$ level there still remains 58.9 per cent of correlation. Even at the additionally assumed level $|r| \geqq 0.95$ there still remains 35.5 per cent of correlation. The degree of correlation on the experimental plot (Fig. 3) is still higher. Here even at the $|r| \geqq 0.7$ level all the traits are correlated, and 37.7 per cent of correlation remains at the $|r| \geqq 0.95$ level. This high degree of correlation proves that the flower is a highly integrated structure. When we, however, compare the correlation on natural sites with that in the culture, it appears that at the $|r| \geqq 0.95$ level there is only 26.9 per cent of correlation common to both these groups. This is evidence that the flower structure undergoes environmental modifications.

The conclusions resulting from the analyses performed separately for each variable were confronted with those in analysis including all the studied traits with reference to their mutual connections and dependences. Investigations on the discriminative power of the traits demonstrated that from among the ten traits analysed the discriminative power of seven of them is statistically significant. As unsignificant the following traits were disregarded: width of calyx (7), distance between dents of vexillum (8), and width of wing at the site of its bulge (10).

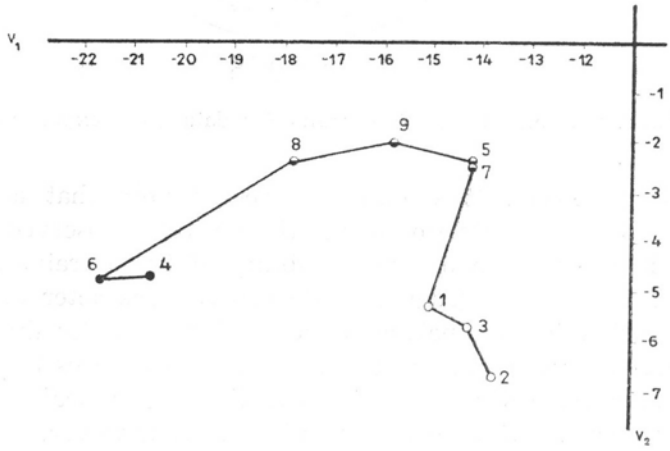

Fig. 4. Scatter diagramme of populations in the system of 1st and 2nd canonical axes for data from natural sites

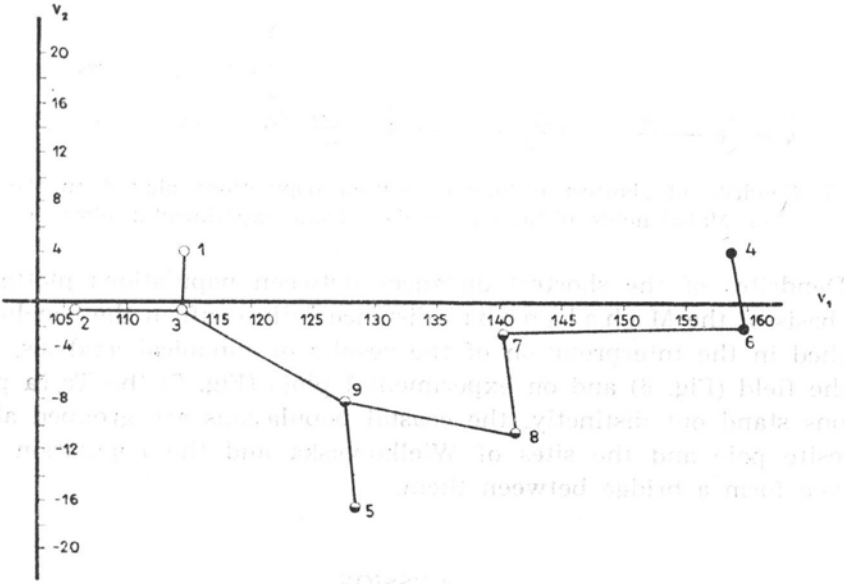

Fig. 5. Scatter diagramme of populations in the system of 1st and 2nd canonical axes for data from experimental plot

The canonical axes allowed, in this part of the investigations, the reduction of the ten-dimensional space to the plane described by the coordinates V_1 and V_2 with a loss of information for the field experiments of 19,26 per cent and for the experimental plot 8.76 per cent only. A graphic illustration of the results of canonical analysis is given in the scatter diagrammes. It results from these diagrammes that both in the field (Fig. 4) and in culture (Fig. 5) the populations form 3 groups according to the geographical regions from which they originate: the coastal group (nos 1, 2 and 3), the Tatra group (nos 4 and 6) and the intermediate group including the Wielkopolska (nos 7, 8 and 9) and Sudetes sites (no. 5). These groups, particulary the Wielkopolska and coastal ones, are much more homogeneous as regards floral traits than it was indicated by analysis of the vegetative traits.

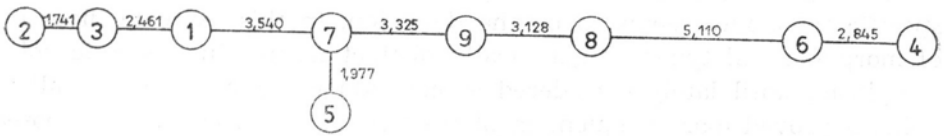

Fig. 6. Dendrite of shortest distances between population plotted on the basis of Mahalanobis distances for data from natural sites

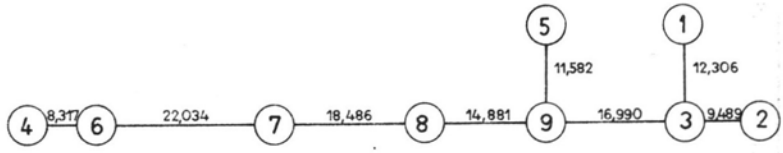

Fig. 7. Dendrite of shortest distances between populations plotted on the basis of Mahalanobis distances for data from experimental plots

Dendrites of the shortest distances between populations plotted on the basis of the Mahalanobis distance fully confirm the conclusions reached in the interpretation of the results of canonical analysis. Both in the field (Fig. 6) and on experimental plots (Fig. 7) the Tatra populations stand out distinctly, the coastal populations are grouped at the opposite pole and the sites of Wielkopolska and the population from Łężyce form a bridge between them.

DISCUSSION

Anthyllis vulneraria used to be a plant of high economic value (Stebler, Schröter, 1889). In Poland it was only cultivated on poor soils on which more fertile species of perennial legumes did not grow well. The mentions concerning this species are scarce in the literature and mostly based on observations from agricultural practice and not on strict genetic-populational experiments (e.g. Agababian; 1951; Becker-Dillingen, 1928; Ivanov, 1929; Kossowski, 1963; Robinson, 1947; Simon, 1956).

In wild state, the species *Anthyllis vulneraria* has a very wide geographical range. It occurs in nearly the whole of Europe up to 70° of northern latitude in Scandinavia, Iceland, Finland and northern USSR, in the East it reaches to the Caucasus and Asia Minor, to the south to Africa up to the Sahara and Abissinia (Gams, 1924). Within this species a large number of subspecies and varietes have been distinguished (see Introduction). Their identification, however, is a problem because the morphological traits of diagnostic character are few and frequently masked by the high plasticity of the plant (Gams, 1924; Jalas, 1957; Juzepchuk, 1945; Marsden-Jones, Turrill 1933; Sagorski, 1912). Therefore, the only way leading to the ordering of the intraspecific taxonomy seems to be the detection, in this extreme diversity of morphological types, of genetically distinct forms, since genetic polymorphism, until lately considered as common phenomenon above all in animals proved more frequent in plants than it was previously supposed (Jones, Wilkins, 1971). A part of the investigations on the genetic structure of populations is analysis of variance concerning the effects of genetic polymorphism at various levels (individual polymorphism, and

polymorphism of chromosomes and chemical compounds). Analysis of variance was performed for 9 *Anthyllis* populations from various geographical regions of Poland.

Marsden-Jones and Turrill (1933a, b) were the first to proceed beyond the purely taxonomic aspect of studies in the analysis of the complicated intraspecific differentiation of *Anthyllis*. These authors analysed the generation obtained from seeds collected of wild single plants of know origin. They investigated the results of controlled self-polination of selected standard plants representing the majority of English varieties of these species. They also analysed in detail 4 populations from various regions of England. The stress laid on the usefulness of comparative cultures in analysis of variance and taxonomical studies is no doubt an achievement in these studies, the methodical aspect, however, arouses at present some reservations. The authors applied no criteria for random sampling, and the consequence of this may be a false picture of the differences between the populations (Harberd, 1957; 1958; Willkins, 1959; Greig-Smith, 1964; Heslop-Harrison, 1964). Neither was statistical analysis applied, only detailed discriptions are given of the morphological types with mention that the colour of the flowers is an important polymorphic trait, a fact not observed in studies on the Polish *Anthyllis* populations. Marsden-Jones and Turrill also noted a marked readiness of spontaneous hybrids formation within the species: the introduction of a foreign type var. Amaranth Purple into the population in Par Sands (S. Cornwall) produced hybrids with phenotypes completely different from those which arose when this type was absent. On this basis the authors affirm that the wide diversity of phenotype within the species *Anthyllis vulneraria* is due to intensive crossing with a limited number of original different types derived probably from mutations.

The studies of Coudrec and Gonnet (1972, 1973) are an attempt of gaining some knowledge on the genetic basis of the wide diversity of *Anthyllis* forms with the application of biochemical criteria. The authors tried to solve the taxonomical problems by way of analysis of flavone compounds, and they demonstrate that these compounds previously known as taxonomic markers at the level of higher taxonus may give interesting results as well at the intraspecific level. They, namely, found a wide diversity in the distribution of flavone compounds in the investigated taxons. Since the latter compounds are markers of genetic differentiation (Harborn, 1967) one may speak of genetic differentiation within the French populations of the species *Anthyllis vulneraria*. However, on account of the sensitivity of this method, which causes an apparent disaccord with classical taxonomy, the biochemical data, in the authors' opinion, should be confronted and integrated with those supplied by other taxonomic methods.

Biometric elaboration of the variability od Polish *Anthyllis* populations also suggest a genetic distinctness of the populations from different geographical regions. The samples of coastal, lowland and Tatra populations are grouped in different areas of the dendrite of shortest distances and in the diagramme of dispersion on the plane of the 1st and 2nd canonical axes, according to their geographical origin. The picture is more distinct for the flowers which undergo less readily the modifying influence of the environment. The Tatra population form the most distinct group (greatest Mahalanobis distances), and least homogeneous within their group are the Wielkopolska populations, particularly as regards the vegetative traits.

After investigating the biology of flowering, Coudrec and Gonnet (l.c.) established that natural *Anthyllis vulneraria* populations in France (in contrast to the earlier discussed English populations — Marsden-Jones, Turrill, 1933) are well isolated genetically by the "precocity of autogamy". They further affirm on this basis that "... ces populations, au départ identiques, évolueraient chacune indépendamment et essentiellement par mutations, cela pouvant expliquer le nombre considérable de formes et variétés décrites, mais qui restent très voisines". The diversity noted in the distribution of flavone compounds in various populations seems to be, in the opinion of these authors, an argument supporting this hypothesis.

In trials of intra- and interpopulational crosses of the Polish *Anthyllis* populations it was observed that the pollen ripens and is shed in very small flowers which are still closed. This causes considerable technical difficulties in the artificial production of crosses. The test for selfpollination (Apolinarska, unpublished data) demonstrated, moreover, a high per cent of seed setting (for the coastal populations 77%, for the Wielkopolska ones 50% and for the Tatra ones 45% on the averaje). It seems, therefore, that interpopulational crosses are difficult in nature owing to precocity of selfpollination (chasmogamic flowering). This, however, does not rule out the possibility of spontaneous hybrid production. Since a good marker is lacking, observations of the progeny of the studied populations does not allow to establish whether there is cross pollination. Neither was the process of fertilization been analysed, hence protandry and the eventual role of insects cannot be completely excluded, in only on account of the vivid yellow-orange colour of the flowers.

Another attempt of taxonomic ordering of the species *Anthyllis vulneraria*, was the study of Jalas (l.c.), based on the elucidation of the evolutionary history of this species. The author, on the basis of confrontation of Scandinavian forms of *Anthyllis* (1950, 1952) with Spanish and Belgian forms (1957), advanced the following hypotheses: "*Anthyllis vulneraria* hat sich offenbar schon früh in zahlreiche mehr oder minder deutlich geschiedene geographische Rassen aufgespaltet.

Diese haben sich dann später in vielen Gegenden sicherlich u.a. als Folge zahlreich eingetretenen Klimaschwankungen, offentlich aber zumal infolge ihrer starken Neigung zum Apophytischen Auftreten sowie zur anthropochoren Ausbreitung weitgehend vermischt, unter teilweiser oder völliger Verwischung sowohl ihrer geographischen als morphologisch-systematischen Grenzen. Mancheorts begegnet man heute an Stelle der ehemaligen klar umrissenen Rassen einem taxonomisch schier hoffnungslosen Hybridkomplex ("complex hybrid swarm"). So dürfte es sich z.B. in den alten Kulturgegenden Mittel- und Westeuropas verhalten". According to this argumentation J a l a s tried to find out to what extent the Belgian material may be considered as an intermediate stage between the material from North Europe and that from the Pyrenees. It is a pity that he did not deal with this material from the genetic and population aspect and largely based it on herbary material, since in such a case his work might have contributed much more to the elucidation of evolutional processes within this species.

In the present investigations of Polish material many conclusions may be reached analogous to the views of J a l a s. The tendency to diminution of the flower size, increase of the number of inflorescences and increasing stiffness of the stem at the stage of seedling with a dozen leaves or so, when progressing from south towards north, is an argument supporting the hypothesis which stresses the role of intensive crossing in the past in eliciting the extreme intraspecific variability of *Anthyllis*. The intermediate character of the Wielkopolska populations may be interpreted as a succesive step in the changes caused by the climatic gradient. These suggestions require, however, confirmation in more extensive investigations in the north-south transect.

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Analiza zmienności dziewięciu naturalnych populacji Anthyllis vulneraria s.l. Cz. II. Biometria kwiatów

Streszczenie

Biometryczna analiza kwiatów potwierdziła wniosek, że układy zmienności w populacjach nadmorskich, wielkopolskich i tatrzańskich są różne.

Kultura prób na polu doświadczalnym dowiodła, że różnice wymienione wyżej są uwarunkowane genetycznie.

Ponadto wykryto kierunkowe zmiany niektórych cech, co sugeruje, że istnieje u przelotu zmienność typu klinowego.