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Variability of enzymatic systems in natural populations of *Anthyllis vulneraria* s.l. from three geographic regions of Poland. Part II. Geographic variability of enzymatic systems in six woundwort populations

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Abstract

Differences between populations of *Anthyllis vulneraria* s.l. from three distinct geographic regions of Poland for four enzyme systems showed different variability and various patterns of isoenzymes. The smallest differences between populations from similar habitats were found for two populations from dune. Data from statistical analysis of enzyme variation in the examined natural populations of *Anthyllis vulneraria* were correlated with data of statistical analyses of morphological features, features of flowers, and analyses of phenolic compounds.

INTRODUCTION

It has been shown in the first part that three woundwort populations originating from different geographic regions of Poland were characterized by different variability of enzymatic patterns. In order to support these observations it was necessary to perform analyses of enzymatic pattern in a higher number of populations, originating from different geographical regions and various habitats. Taking advantage of the results presented in Part I, analyses of the same individuals were made in three terms.

At attempt was made to analyse inter- and intra-population variability, with attention being paid to genetically determined variability. Modifying effect of environment upon plant phenotypes was eliminated due to the fact that the experiment was made in randomly selected blocks.

In order to obtain a full picture of variability and proper interpretation of the results, an equation for the coefficient of similarity and multivariate analysis of variance were used apart from biometric tests (the latter being described in Part I: polymorphism index, test for the significance of differences for PI, and chi-squared test for large and small samples).

MATERIAL AND METHODS

1. Plant material

Six populations of *Anthyllis vulneraria* s.l. from three geographic regions of Poland were studied: coastal region — Chłopy (A) and Mielno (B), mountain region — Kalatówki (C) and Skupniów Upłaz (D), and lowland region — Rożnowo (E) and Międzychód (F).

Fig. 1. Distribution of the populations under study in Poland.

Seeds were collected from each wild population and sown into flower pots in a green house. In spring plant seedlings were transplanted to an experimental field. Random blocks were used in the experiment, with three replications. Scheme of the experiment can be presented as follows:

D	B	E	F	C	A
C	F	A	E	B	D
A	D	C	B	F	E

One replication for each population consisted of 25 plants. Analyses of enzymatic pattern were made on 30 individuals, selected randomly from each population (10 for each replication). Analyses of enzymatic pattern in the same individuals were made in three terms (1st term — Aug. 14, 1975, 2dn term — Sept. 10, 1975, and 3rd term — Sept. 24, 1975).

2. Biochemical methods

Methods of extraction, separation and gel dyeing are given in Part I.

3. Statistical methods

Apart from statistical methods dealing with one variable (described in Part I: polymorphism index, ANOVA and chi-squared test), methods used for analyses of several variables were also adopted (multivariate analysis of variance — MANOVA, and analysis of canonical variates), the similarity between populations being defined beforehand.

In order to compare pairs of populations from general zymograms (see Part I), taking into account all bands present in a population (also the rarely occuring ones), the following equation (coefficient of similarity) was used:

$$S=\frac{a+d}{a+b+c+d}$$

(Sneath and Socal, 1973, ref. after Prus-Głowacki and Szweykowski, 1977).

OTU 1

OTU 2	+	−
+	a	b
−	c	d

— = lacking band
\+ = occuring band

Values of the coefficient of similarity were used for the preparation of dendrograms.

In order to define the significance of differences between populations, both with respect to the frequency, and position of bands upon zymograms, multivariate analysis of variance was performed (Ceranka et al., 1976). Successive bands (electrophoretic mobility — R_p) were taken as variables, whereas variable value was expressed by the frequency of the given band in the population. Frequency of bands was furthermore transformed with Freeman-Tukey method (square root transformation).

General hypothesis on the lack of differences between populations as regards frequency and position of bands upon zymograms and hypothesis, constituting contrasts (comparisions) between particular populations and groups of populations as regards all bands separately were verified by the use so-called simultaneous testing procedure (Caliński, Kaczmarek, 1973; Caliński et al., 1976).

In order to define differences between populations Mahalanobis distances were calculated taking into account all analysed bands. Dendrite of the shortest connections between populations was constructed taking advantage of these distances.

RESULTS

In order to obtain full picture of variability of populations under study, the results are discussed separately for particular enzymatic systems.

1. Malate dehydrogenase (MDH)

Quantitative variability within population is presented in Table 1. As it is seen, PI values are characterized by a rather large scattering. The lowest polymorphism is noted in lowland populations (E, F). Average PI values from the three terms, similary as PI from particular terms, were not significantly different for populations.

Table 1

Polymorphism index (PI) in six woundwort populations, in three terms, as an average for the three terms and upon synthetic zymograms for malate dehydrogenase

Populations	Terms 1	Terms 2	Terms 3	Mean PI from terms	PI from synthetic zymograms
Chłopy — A	0.063	0.100	0.174	0.112	0.158
Mielno — B	0.157	0.095	0.178	0.143	0.154
Kalatówki — C	0.000	0.156	0.119	0.092	0.158
Skupniów Upłaz — D	0.136	0.185	0.148	0.156	0.128
Rożnowo — E	0.195	0.116	0.046	0.119	0.046
Międzychód — F	0.113	0.000	0.060	0.058	0.080

As results from Table 2, populations from Tatra Mountains National Reserve (C and D) were characterized by the presence of band 0.19-d. The same band was found in Międzychód (F) population, although in a sample of 30 plants its frequency was rather low. 0.13-a was characteristic for population from Skupniów Upłaz (D); it was also found in two plants from mountain population (Kalatówki — C). Detail comparison of populations with respect to the frequency of defined bands of MDH is presented in Table 2, and in contrast table (Table 3).

Populations presented in dendrograms are distributed in two groups. One group is represented by mountain populations (Kalatówki — C, Skupniów Upłaz — D), the other — by the remaining populations.

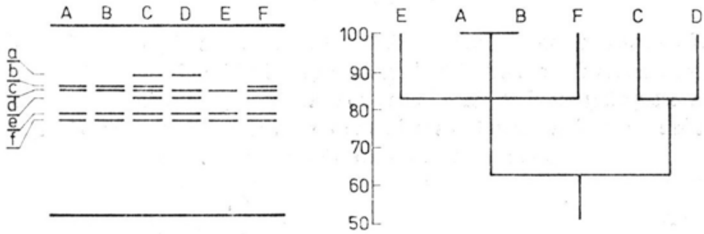

Fig. 2. General zymogram of malate dehydrogenase and dendrogram illustrating arrangement of populations.

Table 2

Frequency of identified bands of malate dehydrogenase upon synthetic zymograms (in per cent)

Bands \ Populations	A	B	C	D	E	F
0.13-a	0	0	7	80	0	0
0.16-b	50	33	27	0	0	60
0.17-c	100	100	100	100	100	100
0.19-d	0	0	73	73	0	20
0.23-e	33	80	87	90	100	100
0.25-f	80	63	43	73	83	100

Coastal populations (Chłopy — A, Mielno — B) occur jointly with lowland populations (Rożnowo — E, Międzychód — F).

Chi-squared test for small samples showed that the populations possessed characteristic spectra of isoenzymes of malate dehydrogenase:

$$\chi^2_{MDH}=195.089 \qquad \chi^2_{0.05}=43.773$$

Detail comparison of populations as regards frequency of particular MDH bands is presented in Table 3. It should be noted that there are no significantly different bands between coastal populations (Chłopy — A, Mielno — B). The highest number of significantly different bands in groups of populations was noted between coastal (A, B) and mountain (C, D) populations.

Upon dendrite (Fig. 3a) both coastal populations (A, B) occur jointly, the shortest distance between them being insignificant. In case of rectangular coordinates (Fig. 3b) both coastal populations are close to each other. Attention should be given to significant specificity of Tatra Mountains populations (C, D).

Table 3

Significance of contrasts upon the level $\alpha=0.05$ between pairs and groups of populations, as an average for three terms for malate dehydrogenase (a=significantly more frequent accurance in the population occupying the first place in the contrast, b=significantly more frequent occurance in the population occupying second place in the contrast, —=frequency of bands in contrast populations does not differ significantly)

Contrasts	Bands					
	a	b	c	d	e	f
A-B	—	—	—	—	—	—
A-C	a	—	b	b	—	—
A-D	—	b	—	b	a	b
A-E	—	b	—	—	a	—
A-F	—	b	—	—	—	—
B-C	a	—	b	b	—	—
B-D	—	—	—	b	a	b
B-E	—	—	—	—	a	—
B-F	—	b	—	—	—	—
C-D	b	b	a	a	a	b
C-E	b	b	a	a	a	—
C-F	b	b	a	a	b	—
D-E	—	—	—	a	—	a
D-F	—	—	—	—	b	a
E-F	—	b	—	—	b	—
(A+B)—(C+D)	a	—	b	b	a	b
(A+B)—(E+F)	—	b	—	—	a	—
(C+D)—(E+F)	—	b	a	a	—	a

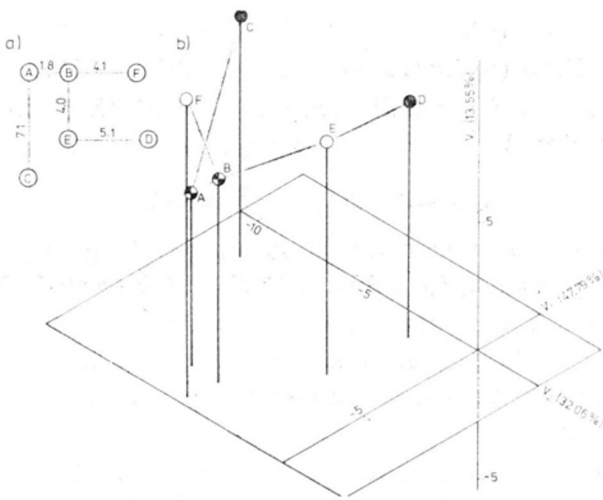

Fig. 3. a — Dendrite of the shortest distance between populations, constructed basing on the Mahalanobis distance, b — arrangement of six populations in rectangular coordinate system for the first three canonical variates for malate dehydrogenase.

2. Esterases (E)

Table 4 presents quantitative variability in the three terms, and average values for the populations and for synthetic zymograms. Average PI values for the populations were significantly different:

$$F_{calc.} = 9.21 \qquad F_{0.01} = 8.91$$

Tukey's test showed that coastal populations (A, B) were characterized by a significantly lower PI than lowland (E, F) and Skupniów Upłaz (D) populations.

As seen in Table 6, populations from dune (A, B) were distinguished by high frequency of bands 0.26-g and 0.30-i, and a lack of 0.32-j and 0.33-k. On the other hand, 0.14-d was characteristic for mountain populations (C, D), whereas 0.08-b occured rarely. Lowland populations (E, F) were distinguished by a lack of 0.21-e. Bands common for all populations, and characteristic for particular populations are presented in Table 6.

Dendrogram showed that the populations were separated into three groups, depending on their geographic origin, i.e. to coastal (A, B), Tatra

Table 4

Polymorphism index (PI) in six woundwort populations, in three terms, as an average for the three terms, and upon synthetic zymograms for esterases

Populations	Terms 1	Terms 2	Terms 3	Mean PI from terms	PI from synthetic zymograms
Chłopy — A	0.025	0.110	0.045	0.060	0.053
Mielno — B	0.059	0.094	0.060	0.071	0.039
Kalatówki — C	0.080	0.157	0.098	0.115	0.158
Skupniów Upłaz — D	0.140	0.167	0.159	0.155	0.150
Rożnowo — E	0.149	0.147	0.160	0.152	0.149
Międzychód — F	0.177	0.135	0.148	0.153	0.126

Table 5

Significance of differences between average PI values from three terms, for six populations for esterases

	A	B	C	E	F	D	
A		—	—	+	+	+	0.05
B	—		—	+	+	+	
C	—	—		—	—	—	
E	+	—	—		—	—	
F	+	—	—	—		—	
D	+	—	—	—	—		
0.01							

Table 6

Frequency of identified bands of esterases upon synthetic zymograms (in per cent)

Bands \ Populations	A	B	C	D	E	F
0.05-a	100	100	100	100	100	100
0.08-b	100	100	37	33	53	100
0.12-c	100	67	37	87	23	93
0.14-d	0	0	53	93	0	0
0.21-e	100	100	10	53	0	0
0.24-f	97	100	40	77	23	80
0.26-g	93	100	50	33	53	63
0.29-h	23	0	0	0	33	83
0.31-i	100	100	40	37	40	10
0.32-j	0	0	27	33	60	23
0.33-k	100	100	100	100	100	100
0.35-l	0	0	53	80	57	73
0.37-m	73	100	7	30	83	50
0.39-n	100	100	0	80	100	100
0.40-o	100	100	100	100	100	100
0.43-p	67	57	63	57	83	70
0.45-r	0	0	0	13	0	0

Mountains (C, D) and lowland (E, F) group. Similary as in case of malate dehydrogenase, lowland (E, F) and coastal (A, B) populations were directly connected, while mountain (C, D) populations were more distant. It should be added that similarity between the groups of populations from particular regions was rather small.

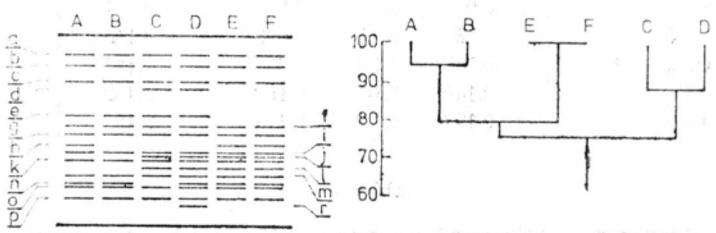

Fig. 4. General zymogram of esterases and dendrogram illustrating arrangement of populations.

Chi-squared test showed that each population possessed characteristic spectrum of esterases:

$$\chi^2_E = 195.879 \qquad \chi^2_{0.05} = 43.773$$

Detail comparison of populations with respect to the frequency of bands is given in Table 7. Bands 0.45-r, 0.43-p, and 0.32-j did not differ in the compared pairs and groups of populations. It should be, however, added that coastal populations (A, B) differed significantly by two bands, mountain (C, D) populations — by four bands, and low-land populations (E, F) by eight bands. Furthermore, there were nine significantly different bands between coastal (A, B) and mountain (C, D) populations, eight bands between coastal (A, B) and lowland (E, F) populations, and seven bands between lowland (E, F) and mountain (C, D) populations.

Table 7

Significance of contrasts upon the level $\alpha=0.05$ between pairs and groups of populations, as an average for three terms for esterases (a=significantly more frequent occurance in the population occupying the first place in the contrasts, b=significantly more frequent occurance in the population occupying second place in the contrast —=frequency of bands in contrast populations does not differ significantly)

Contrasts	Bands																
	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	r
A-B	—	—	—	—	—	—	—	—	—	—	—	—	a	—	a	—	—
A-C	—	—	a	a	a	b	—	—	a	—	—	—	a	b	a	a	—
A-D	—	—	a	a	—	b	—	—	—	—	—	—	a	b	—	a	—
A-E	—	—	—	—	—	b	a	—	—	—	—	—	a	—	a	a	a
A-F	—	—	a	—	—	b	—	—	a	b	—	—	a	—	—	—	—
B-C	—	—	a	a	a	b	—	—	a	—	—	—	a	b	a	a	—
B-D	—	—	a	a	—	b	—	—	a	—	—	—	a	b	b	a	—
B-E	—	—	—	—	b	b	a	—	a	b	—	—	a	—	—	a	a
B-F	—	—	a	—	—	b	—	—	a	b	—	—	a	—	b	—	—
C-D	—	—	a	b	—	—	—	—	—	—	—	—	b	—	b	—	—
C-E	—	—	—	b	b	—	a	—	—	b	—	—	a	a	—	—	a
C-F	—	—	a	b	—	—	—	—	—	b	b	b	a	a	b	b	—
D-E	—	—	b	b	b	—	a	—	—	b	—	—	a	a	a	—	a
D-F	—	—	—	b	—	—	—	—	—	b	b	b	a	a	—	b	—
E-F	—	—	a	—	a	—	b	—	—	b	—	b	—	—	b	b	b
(A+B)—(C+D)	—	—	a	a	a	b	a	—	a	—	—	—	a	b	—	a	—
(A+B)—(E+F)	—	—	a	—	—	b	a	—	a	b	—	—	a	—	—	a	a
(C+D)—(E+F)	—	—	—	b	b	—	—	—	—	b	b	—	a	a	—	—	a

Dendrite in Fig. 5a showed that all distances between populations were significant, the lowest ones being between coastal (A, B) and mountain (C, D) populations.

Populations plotted in a coordinate system (Fig. 5b) are close to each other (coastal — A, B, mountain — C, D, and lowland — E, F). Two coordinates differentiated the populations geographically (V_1, V_2), while the third one (V_3) reflected differences noted in populations from similar habitats.

3. Acid phosphatases (Ph)

Quantitative variability (PI) is presented in Table 8. As it results from this table, Chłopy (A) population was the least variable one,

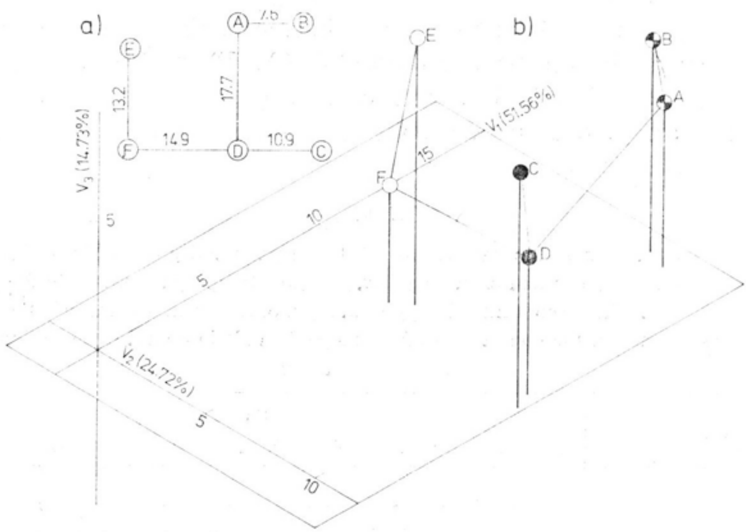

Fig. 5. a — Dendrite of the shortest distance between populations, constructed basing on the Mahalanobis distance, b — arrangement of six populations in rectangular coordinate system for the first three canonical variates for esterases.

while both lowland (E, F) populations — the most variable. Values given in Table 8 do not differ significantly.

Table 9 shows that Chłopy (A) population had two characteristic bands (0.23-h and 0.34-k), which were also found in other populations but only in a few specimens. Acid phosphatases constituted enzymatic system which only slightly showed geographic variability of the populations under study. Specificity of each population is underlined in Table 8.

Upon the Ph dendrogram the populations were arranged in two groups (Fig. 6). The first one consisted of dune populations (A, B)

Table 8

Polymorphism index (PI) in six woundwort populations, in three terms, as an average for the three terms, and upon synthetic zymograms for acid phosphatases

Populations	Terms 1	Terms 2	Terms 3	Mean PI from terms	PI from synthetic zymograms
Chłopy — A	0.026	0.040	0.111	0.059	0.047
Mielno — B	0.117	0.108	0.073	0.099	0.092
Kalatówki — C	0.112	0.098	0.119	0.110	0.103
Skupniów Upłaz — D	0.059	0.068	0.114	0.080	0.061
Rożnowo — E	0.144	0.088	0.124	0.119	0.147
Międzychód — F	0.157	0.092	0.138	0.129	0.140

Table 9

Frequency of identified bands of acid phosphatases upon synthetic zymograms (in per cent)

Bands \ Populations	A	B	C	D	E	F
0.04-a	100	100	100	100	100	100
0.08-b	70	20	87	100	43	70
0.10-c	100	43	63	83	50	10
0.13-d	0	0	33	13	0	37
0.15-e	70	100	100	100	100	87
0.19-f	100	27	10	93	23	40
0.21-g	100	100	100	100	20	100
0.23-h	100	10	0	0	0	13
0.25-i	0	0	7	3	0	0
0.30-j	100	87	93	97	73	77
0.34-k	100	30	57	63	63	33

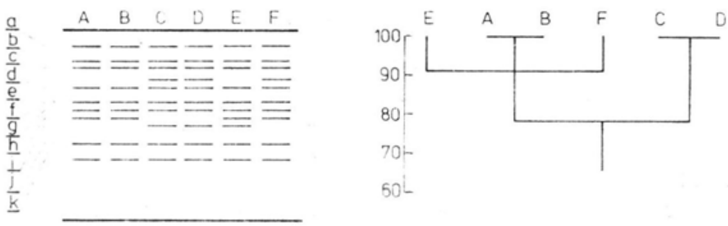

Fig. 6. General zymogram of acid phosphatases and dendrogram illustrating arrangement of populations.

together with lowland populations (E, F). The other group embraced mountain populations (C, D), the latter being similar in 100%.

Chi-squared test showed that, similary as before, the populations were characterized by specific patterns of isoenzymes of acid phosphatases:

$$\chi^2_{Ph}=195.157 \qquad \chi^2_{0.05}=43.773$$

Significance of differences in the frequency of particular bands is given in Table 10. Only 0.25-i and 0.04-a did not differ in the compared pairs of populations. As results from Table 10, coastal (A, B) and lowland (E, F) populations, as well as coastal (A, B) and mountain (C, D) populations differed significantly by four bands. Lowland (E, F) and mountain (C, D) populations differed only by one band.

Connection of populations with the shortest Mahalanobis distance is presented in a dendrite (Fig. 7a). As it is seen, populations from Tatra Mountains (C, D) are directly connected, while coastal (A, B) populations are connected with Rożnowo (Lowland, E) population. All distances between the populations are significant.

Table 10

Significance of contrasts upon the level $\alpha=0.05$ between pairs and groups of populations, as an average for three terms for acid phosphatases (a=significantly more frequent occurance in the population occupying the first place in the contrast, b=significantly more frequent occurance in the population occupying second place in the contrast, —=frequency of bands in contrast populations does not differ significantly)

Contrasts	Bands										
	a	b	c	d	e	f	g	h	i	j	k
A-B	a	—	—	a	—	—	b	—	a	—	—
A-C	—	—	—	a	—	—	b	b	a	—	—
A-D	—	—	—	a	—	—	b	—	a	b	—
A-E	—	—	—	a	a	—	b	—	a	—	—
A-F	a	—	—	a	—	—	b	b	a	—	—
B-C	—	—	—	—	—	—	—	b	—	b	—
B-D	—	—	—	—	—	b	—	—	—	b	—
B-E	—	—	—	—	—	—	—	—	—	—	—
B-F	—	—	—	—	—	—	a	b	—	b	—
C-D	—	—	—	—	—	b	—	—	—	—	—
C-E	—	—	—	b	a	—	—	a	—	—	—
C-F	—	—	—	—	—	—	—	—	a	—	—
D-E	—	a	—	b	a	a	—	—	—	a	—
D-F	—	—	—	—	—	—	a	b	—	—	—
E-F	—	—	—	a	b	—	—	b	—	—	—
(A+B)—(C+D)	—	—	—	a	—	—	b	b	—	b	—
(A+B)—(E+F)	—	—	—	a	—	—	—	b	a	b	—
(C+D)—(E+F)	—	—	—	—	—	—	—	—	—	a	—

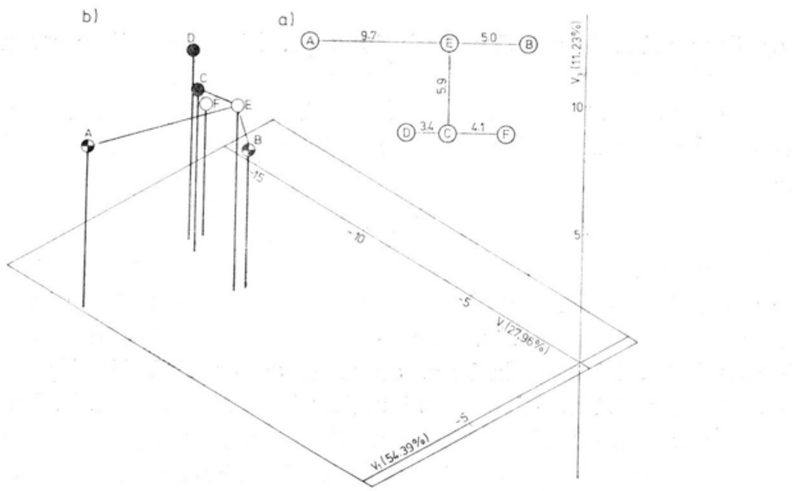

Fig. 7. a — Dendrite of the shortest distance between populations, constructed basing on the Mahalanobis distance, b — arrangement of six populations in rectangular coordinate system for the first three canonical variates for acid phosphatases.

In rectangular coordinate system Tatra Mountains populations (C, D) are close to each other, whereas Chłopy (A) population is the most distant one. Lowland (E, F) populations are between coastal (A, B) and mountain (C, D) ones.

4. Peroxidases (PX)

Quantitative variability of the six populations under study is presented in Table 11. This table shows that Chłopy (A) population is the least polymorphic with respect to peroxidases. Average PI values for the six populations were significantly different:

$$F_{calc.} = 8.19 \qquad F_{0.05} = 6.03 \qquad F_{0.01} = 8.91$$

Tukey's test also showed that Chłopy (A) population had significantly lower PI compared to other populations (Table 12).

Table 11

Polymorphism index (PI) in six woundwort populations, in three terms, as an average for the three terms, and upon synthetic zymograms for peroxidases

Populations	Terms 1	2	3	Mean PI from terms	PI from synthetic zymograms
Chłopy — A	0.000	0.066	0.000	0.022	0.058
Mielno — B	0.105	0.159	0.111	0.125	0.096
Kalatówki — C	0.104	0.068	0.121	0.098	0.031
Skupniów Upłaz — D	0.121	0.192	0.154	0.157	0.117
Rożnowo — E	0.146	0.133	0.147	0.142	0.152
Międzychód — F	0.080	0.150	0.155	0.128	0.062

Table 12

Significance of differences between average PI values from three terms for six populations for peroxidases

	A	C	B	F	E	D	0.05
A	\	+	+	+	+	+	
C	—	\	—	—	—	—	
B	—	—	\	—	—	—	
F	—	—	—	\	—	—	
E	+	—	—	—	\	—	
D	+	—	—	—	—	\	
0.01							

Basing only on the frequency of bands, analysed upon synthetic zymograms (Table 13), it was shown that there are no characteristic bands for populations from similar geographic regions. Frequency of bands was characteristic for particular populations. Attention should be given to the direct connection of Chłopy (A) and mountain (D) populations, although the difference between them was small. Furthermore, lowland (E, F) populations were characterized by high per cent of similarity with Kalatówki (C) population.

Table 13

Frequency of identified bands of peroxidases upon synthetic zymograms (in per cent)

Bands \ Populations	A	B	C	D	E	F
0.08-a	0	0	0	100	0	0
0.10-b	0	57	0	33	0	100
0.12-c	100	100	100	100	100	100
0.15-d	100	0	93	53	73	53
0.28-e	63	0	0	17	0	0
0.32-f	100	100	100	90	77	100
0.35-g	0	0	10	0	37	7

Specificity of each population with respect to peroxidases pattern was defined with chi-squared test:

$$\chi^2_{PX}=196.712 \qquad \chi^2_{0.05}=43.773$$

Fusion of population upon the dendrite (Fig. 9a), their position in rectangular coordinate system (Fig. 9b), and comparison of pairs of populations given in the contrast table (Table 14) showed that each population was characterized by specific peroxidase pattern.

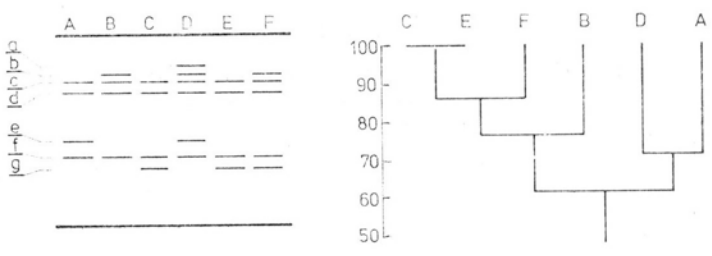

Fig. 8. General zymogram of peroxidases and dendrogram illustrating arrangement of populations.

5. Synthetic approach to all enzymatic systems

All populations under study were characterized by high variability. As regards the index of polymorphism dune populations (A, B), and especially Chłopy (A) population, were quite distinguishable due to rather low PI values.

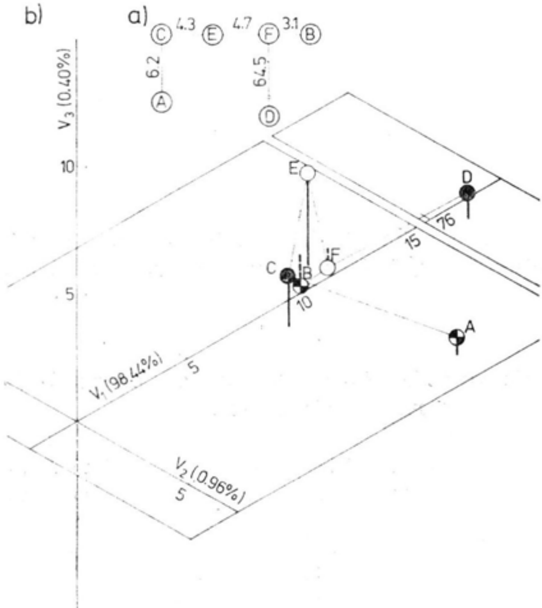

Fig. 9. a — Dendrite of the shortest distance between populations, constructed basing on the Mahalanobis distance, b — arrangement of six populations in rectangular coordinate system for the first three canonical variates for peroxidases.

Table 14

Significance of contrasts upon the level $\alpha=0.05$ between pairs and groups of populations, as an average for three terms for peroxidases (a=significantly more frequent occurance in the population occupying the first place in the contrast, b=significantly more frequent occurance in the population occupying second place in the contrast, —=frequency of bands in contrast populations does not differ significantly)

Contrasts	Bands: a	b	c	d	e	f	g
A-B	—	—	a	—	b	b	—
A-C	—	—	a	—	b	—	—
A-D	—	a	a	—	—	b	b
A-E	b	a	a	—	—	—	—
A-F	—	a	a	—	—	b	—
B-C	—	—	—	b	—	a	—
B-D	—	—	—	—	—	b	b
B-E	b	—	—	b	—	a	—
B-F	—	—	—	—	—	—	—
C-D	—	—	—	—	—	b	b
C-E	b	—	—	—	—	—	—
C-F	—	—	—	—	a	b	—
D-E	b	—	—	—	—	a	a
D-F	—	—	—	—	—	—	a
E-F	a	—	—	—	—	b	—
(A+B)—(C+D)	—	—	—	b	—	—	b
(A+B)—(E+F)	b	a	a	—	—	—	—
(C+D)—(E+F)	b	—	—	—	—	—	a

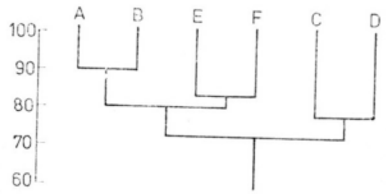

Fig. 10. Dendrogram constructed basing on the average coefficient of similarity between populations for four enzymatic systems.

Table 15

Average PI values in six populations from three terms, and average for four enzymatic systems from synthetic zymograms

Populations	Mean PI for enzymatic systems from terms	Mean PI for enzymatic systems from synthetic zymograms
Chłopy — A	0.063	0.079
Mielno — B	0.110	0.095
Kalatówki — C	0.103	0.113
Skupniów Upłaz — D	0.137	0.114
Rożnowo — E	0.133	0.124
Międzychód — F	0.117	0.102

As it is seen from the dendrogram based on average similarity of the four enzymatic systems, populations under study are arranged in three groups, reflecting their geographic origin: coastal (A, B), lowland (E, F), and Tatra Mountains (C, D). Mountain populations were most distinct. At the same time they were characterized by the lowest similarity in the compared pairs od populations. Lowland (E, F) and coastal (A, B) populations were directly connected.

Upon dendrograms, dendrites, and in coordinate systems, populations were divided into groups in case of all enzymatic systems, with the exception of peroxidases. These groups were directly related to geographic origin of the populations. At the same time it was shown that the populations possessed specific patterns of enzymatic systems (chi-squared test for large samples):

$$\chi^2_{\text{enz. syst.}} = 191.721 \qquad \chi^2_{0.05} = 24.996$$

It should be pointed out that the lowest differences between pairs of populations from the same region were noted in case of coastal populations (A, B). Geographic character of variability in the populations under study was most clearly reflected by esterases pattern. On the other hand, peroxidases showed specificity of each population. The remaining two enzymatic systems (MDH and Ph) reflect both, geographic variability and specificity of populations.

DISCUSSION

Analyses of the variability of biochemical features, and most, of all electrophoresis of enzymatic proteins, are widely used in studies on population genetics. They are complementary to the analyses of morphological features and result in more comprehensive picture of genetic variability.

The results obtained so far in studies on variability of vegetative features and features of flowers, as well as of phenolic compounds, undertaken in natural populations of *Anthyllis* showed that these features are usually highly variable (Łukaszewska, et al., 1978; Kalinowski and Bartkowiak, 1979). The same was noted in case of enzymatic proteins. It should be underlined that variability noted in the present work was determined genetically since all plants under study were grown in similar conditions. This fact has already been stressed in the previous studies on woundwort, as well as in part I of this paper. Studies by Marsden-Jones and Turrill (1933) on natural populations of woundwort in England, and on the progeny of single plants, showed that colour of flowers is also subject to high polymorphism. The authors were of the opinion that it resulted from plant hybridization, although they also stated that the occurance of rare plant types may result from mutation. On the other hand, Couderc and Gonnet (1972), and Couderc (1973), who studied variability of flavone aglucone in *Anthyllis* populations in France, were of the opinion that mutation was the main reason of variability. This statement was based on observations of woundwort blooming. According to these authors, early maturation of pollen closed in the flower protects the plants from allogamy. Consideration of mutations as the main reason of variability noted in woundwort populations is in contradiction to Jalas' hypothesis (1957), which states, among others, that populations occuring at present result from hybridization. This problem has been discussed by Łukaszewska et al. (1978, part II), who are of the opinion that insects may play a certain role in plant hybridization due to intensive colour of woundwort flowers. The present paper showed that within the populations under study enzymatic proteins are characterized by high polymorphism. This can point to the fact that wounwort populations were panmictic.

It was shown that variability of woundwort populations is connected with geographic regions. This fact support previous results (Łukaszewska et al., 1978; Kalinowski and Bartkowiak, 1979), which agree also with the classification presented by Kostrakiewicz (1959). In case of esterases it was clearly shown that there exist three groups of populations related to geographic regions. It should be added that only for this enzymatic system populations from dune (Chłopy — A, Mielno — B) were characterized by insignificant distance upon the dendrite. Furthermore, they were characterized by the lowest polymorphism (PI). As regards malate dehydrogenase and acid phosphatases, particular populations were divided into groups according to the geographic origin, whereas in case of peroxidase each population appeared to be different. It should be also noted that mountain populations (Kalatówki — C, and Skupniów Upłaz — D) were more differentiated than the remaining ones. These results agree with the results of analyses of morphological features and features of flower, as well as of phenolic compounds. They point to genetic specificity of populations originating from various geographic regions of Poland, and allow for an assumption that the populations under study constituted panmictic systems.

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Zmienność układów enzymatycznych w naturalnych populacjach Anthyllis vulneraria s.l. z trzech regionów geograficznych Polski. Cz. II. Geograficzna zmienność układów enzymatycznych w sześciu populacjach przelotu

Streszczenie

Analizy czterech układów enzymatycznych w sześciu populacjach *Anthyllis vulneraria* s.l. z trzech regionów geograficznych Polski wykazały istnienie różnej zmienności. Każda z badanych populacji miała charakterystyczne spektrum rozdzielonych białek enzymatycznych. Najmniejsze różnice między populacjami z podobnych siedlisk były między wydmowymi. Wyniki analiz białek enzymatycznych były zgodne z wynikami analiz cech morfologicznych i cech kwiatu oraz z analizami związków fenolowych.