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# An electrophoretic and cytological study of hybridisation between *Aconitum napellus* ssp. *skerisorae* (2n=32) and *A. variegatum* (2n=16). I Electrophoretic evidence

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Abstract

The variability of six enzymes in pure and mixed populations of *Aconitum napellus* and *A. variegatum*, both from the Tatra Mountains was analysed by means of electrophoresis on starch and polyacrylamide gels. The enzymes differentiating the studied species are: glutamate dehydrogenase, isocitrate dehydrogenase, esterases and peroxidases. A group of plants was isolated with phenotypes intermediate between *A. napellus* and *A. variegatum*. Among them were most probably both $F_1$ and introgressive hybrids.

## INTRODUCTION

The genus *Aconitum* groups species with a great diversity of the chromosome number which is a multiple of the basic number x=8. The somatic numbers 2n=16 and 2n=32 occur most frequently, however, triploid, pentaploid up to decaploid numbers inclusively may be found (Darlington and Wylie 1955). In this chromosome diversity interspecies crossing played no doubt a considerable role. An example of this may be *A. stoerkianum*, a species of probably allopolyploid origin (Afify 1933). The latter species is at the same time an example of stabilisation of a hybrid without any increase in the degree of ploidy. Amphiploidisation may have occurred in these *Aconitum* species in which the chromosome number is a manifold multiple of x=8.

There are numerous reports on the sporadic occurrence of hybrids in *Aconitum*, among them between *A. napellus* and *A. variegatum*. (Pawłowski 1956, Götz 1967, Seitz 1969). Such hybrids if they are at least partly fertile can reproduce generatively not only by way of the mentioned stabilisation, or by polyploidisation, but also by means of back crosses with

the parent species (Anderson 1948, 1949, 1953, Heiser 1949, Alston and Hempel 1964, Carter and Brehm 1969, Whiffin 1973. Brophy and Parnel 1974, Hopper 1977a, b, 1978). Such backcrosses also described as introgression and the flow of genes connected with it are a particularly interesting problem, especially among such species as *A. napellus* and *A. variegatum* since they represent different ploidy levels. It is considered that, on account of the strong chromosome sterility barrier which occurs in triploid hybrids, they can play the role of a bridge for interspecific gene exchanges only in a slight degree (Heiser 1949). This leads to a limitation of introgression the consequences of which become hardly noticeable. Reports on this, however, are very scarce and rather controversial (Gajewski 1959, Zohary and Nur 1959, Hull 1974, Dancik 1975, Hunziker et al. 1975).

The occurrence of *Aconitum* hybrids in the Tatra mountains has been described by Pawłowski (1956). Szweykowski and Mendelak (1977) found in the Tatra Mts in a mixed *A. napellus* and *A. variegatum* population from the Dolina Kondratowa (valley) one triploid plant which was examined for the composition of phenolic compounds and peroxidase isoenzymes (Krzakowa and Szweykowski 1977, Szweykowski and Krzakowa 1977). The authors considered that this plant is an $F_1$ hybrid that is allotriploid of the above mentioned species. This plant was marked with the number 2413 and is referred to in the present paper under this symbol.

The frequency of occurrence of such $F_1$ hybrids and the possibility of introgressive crosses and gene flow remained an open question, therefore, it became the subject of further studies.

## MATERIAL

*Aconitum napellus* ssp. *skerisorae* (Gayer) Seitz (= *A. firmum* Rochel= = *A. callibotryon* (Rchb.), (Gayer)) (2n= 32) and *Aconitum variegatum* L. (2n= 16) occur both in the Tatra Mts. They show certain ecological differences: *A. napellus* grows to altitudes of 2400 m a.s.l. on both granite and calcareous substrate in most places, usually along streams, whereas *A. variegatum* reaches altitudes of 1600 m a.s.l. on a less moist calcareous substrate.

*A. napellus* and *A. variegatum*, in spite of these differences, form both one-species and mixed populations. In such mixed ones, in contrast to the one-species populations, the dates of flowering of both species coincide suggesting the possibility of interspecific crossing.

For the investigation 636 plants were taken both from one-species *A. napellus* and *A. variegatum* populations as well as from mixed ones (Fig. 1). A total of 21 samples was collected, including a most numerous one from the Kondratowa Valley (66 plants). In order to increase the probability of distinguishing the hybrids in the sample from Kondratowa, phenological observations were conducted in 1976 over a part of the vegetation season comprising the phase from the beginning to the end of flowering.

Five phases of flowering were distinguished: the beginning of *A. napellus* flowering, full bloom, end of flowering of *A. napellus* and the beginning of flowering of *A. variegatum* and the end of flowering of *A. variegatum*. The latter group consisted of plants which did not come into flower or had very few flowers. Several plants from each phase of flowering were permanently marked (most in the phase comprising the end of flowering of *A. napellus* and the beginning of flowering of *A. variegatum*). In the autum of 1976 their fleshy rhizomes were collected and brought to the experimental garden of the Department of Genetics of the A. Mickiewicz University in Poznań. The rhizomes (14-45) of the 20 remaining populations were taken randomly without regard to the flowering dates.

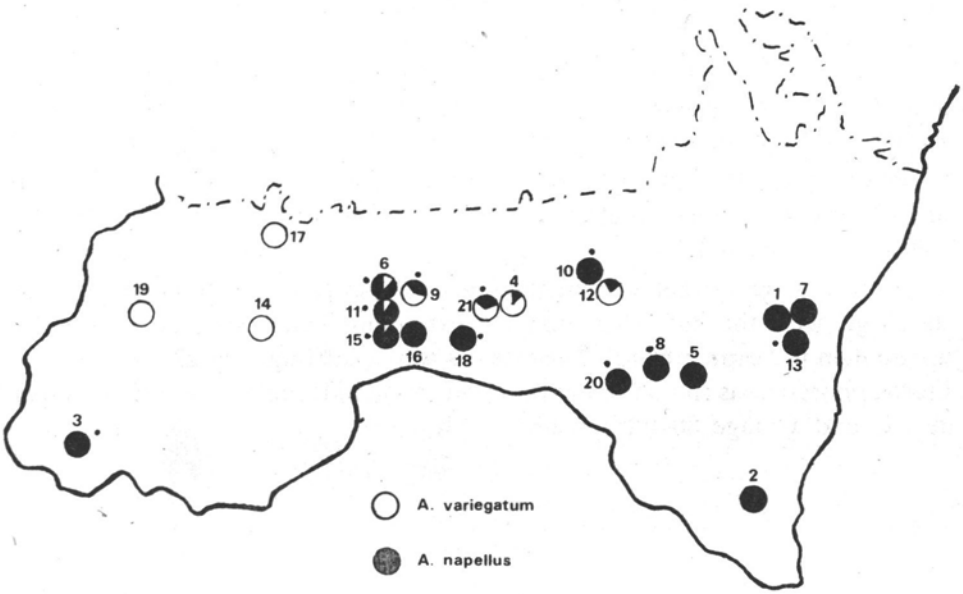



Fig. 1. Distribution of *Aconitum* populations in the Polish part of the Tatra Mountains: 1—Waksmundzka Polana (clearing), 1350 m a.s.l.; 2—Slope of Miedziane near Morskie Oko, 1400 m; 3—Dolina Jarząbcza (valley), 1420 m; 4—Kalatówki, 1200 m; 5—Czerwony Stawek (pond) in Dolina Pańszczycy (valley), 1650 m; 6—Wyżyna Równia Miętusia (tableland), 1300 m; 7—Gęsia Szyja, 1450 m; 8—Czarny Potok (stream) in Gąsienicowy Las (forest), 1450 m; 9—polana (clearing) in Dolina Małej Łąki (valley), 1200 m; 10—Boczań (stream), 1200 m; 11—Miętusi torrent source, 1400 m; 12—Boczań, 1250 m; 13—Waksmundzka Dolina (valley), 1350 m; 14—Hala Pisana (coomb), 1100 m; 15—Kobylarz, Żleb Wodniściak (gully), 1300 m; 16—Kobylarz (torrent), 1200 m; 17—Kiry, 800 m; 18—Piekło, 1700 m; 19—Olejarnia, 1000 m; 20—Hala Gąsienicowa (coomb), 1650 m; 21—Hala Kondratowa (coomb), 1350 m. Dots denote populations in which phenotype $PX^{ab}$ occurs

## METHODS

### PROTEIN SEPARATION

Esterases (EST), glutamate oxaloacetate transaminase (GOT), malic dehydrogenase (NADP-dependent) (ME) and isocitrate dehydrogenase (IDH) were separated on starch gel. Electrophoresis of the first three enzymes was run in a lithium-boric buffer system (Shaw and Prasad 1970). The gel buffer consisted of a mixture of the buffer TRIS-citric 0.2 M, pH 8.3 with the buffer lithium-boric 0.2 M, pH 7.6 (9:1). The same was used as electrode buffer. Isocitrate dehydrogenase was separated in gel TRIS-$H_3BO_3$-EDTA buffer consisting of 0.045 M TRIS. 0.025 M boric acid, 0.001 M EDTA, pH 8.6. The electrode buffer composed of 0.9 M TRIS, 0.5 M $H_3BO_3$, 0.02 M EDTA, pH 8.6 was diluted in the anode part fivefold and in the cathode part sevenfold (Bielayev 1977). Protein was extracted from young leaves by means of the buffer: 0.09 M TRIS, 0.27 M $H_3BO_3$, 0.004 M EDTA, pH 7.4 with 0.7 per cent mercaptoethanol added and PVP. Electrophoresis was run for about 4 h at 4°C and voltage 250 on a length of about 7.5 cm. The gel used was BDH 12 per cent.

Peroxidases (PX) and glutamate dehydrogenase (GDH) were separated in polyacrylamide gel in electrode buffer: 0.09 M TRIS, 0.27 M $H_3BO_3$, 0.004 M EDTA, pH 7.4.

Proteins were extracted from the leaves similarly as in electrophoresis on starch gel with the difference that the extracting buffer for peroxidases did not contain mercaptoethanol. The extracts were centrifuged at 22 000 g at 3°C. Electrophoresis was run on 10 per cent polyacrylamide plates in a vertical system at 4°C and voltage 80-100 for about 4 h.

### STAINING OF ENZYMES

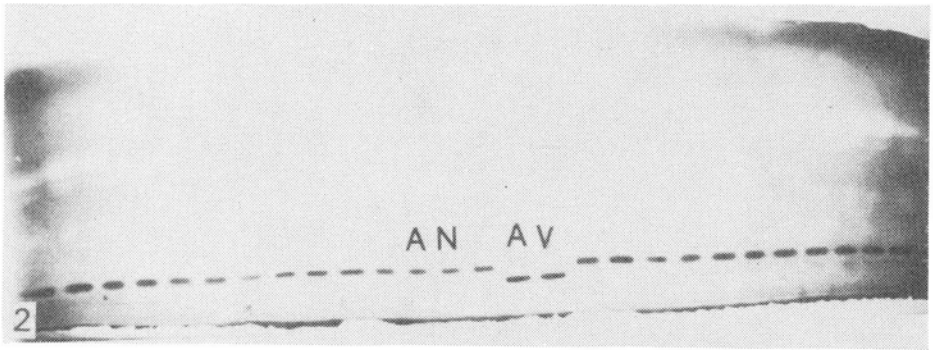
EST (EC 3.1.1.2.): α-naphthyl acetate 40 mg, FBRR 60 mg, in 100 ml phosphate buffer 0.1 M, pH 6.0.

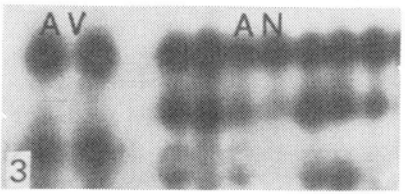
GOT (EC 2.6.1.1.): aspartic acid 150 mg, katoglutaric acid 100 mg, FBRR 60 mg, pyridoxal-5-phosphate 2 mg, in 100 ml TRIS-HCl buffer 0.1 M, pH 8.0.

ME (EC 1.1.1.3.): NADP 2 mg, PMS 2 mg, MTT 3 mg, malic acid 100 mg, $MgCl_2$ 20 mg, in 10 ml TRIS-HCl buffer 0.1 M, pH 8.0.

IDH (EC 1.11.1.7.): NADP 2 mg, PMS 1 mg, MTT 3 mg, DL-isocitric acid (trisodium salt) 100 mg, $MgCl_2$ 20 mg, in 10 ml TRIS-HCl buffer 0.1 M, pH 8.0

PX (EC 1.11.1.7.): 3-amino-9-ethylocarbazole 50 mg, $CaCl_2$ 0.1 M, 2 ml, $H_2O_2$ 3%, 0.5 ml, in 100 ml acetate buffer 0.05 M, pH 5.0.



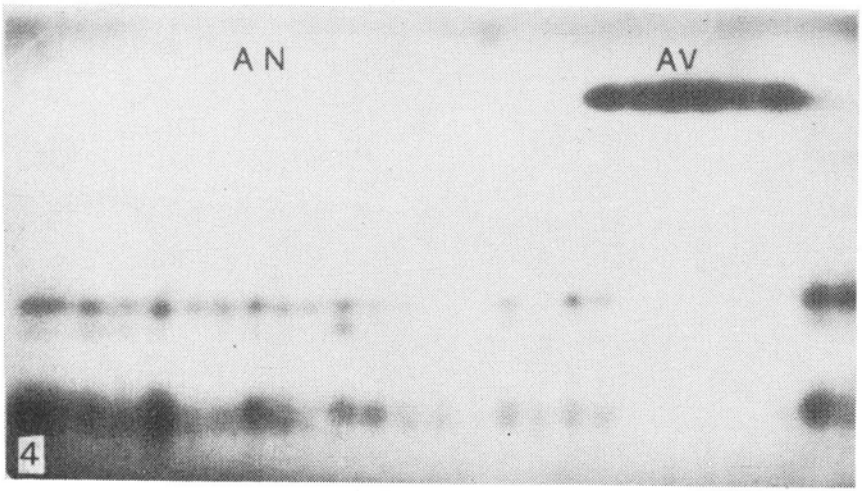
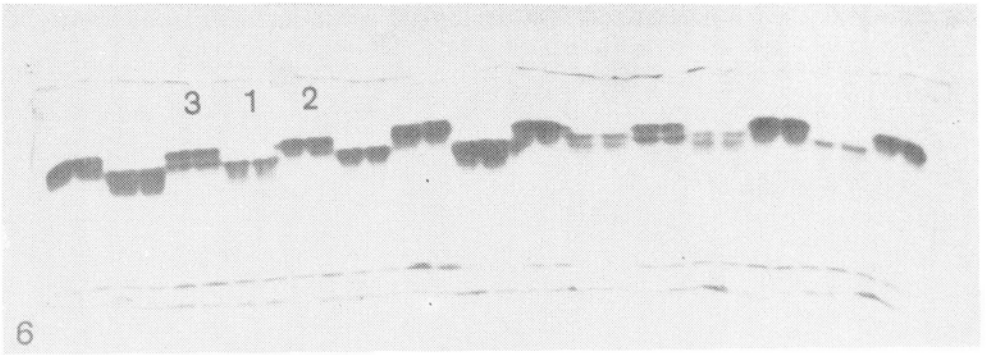
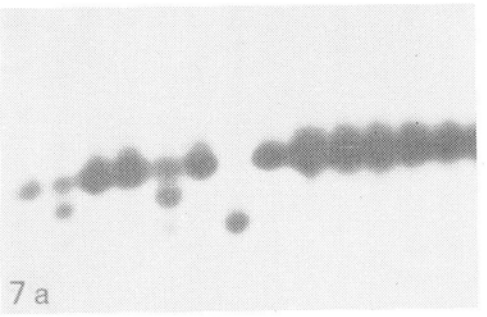




Fig. 2. Glutamate dehydrogenase in *A. napellus* (AN) and *A. variegatum* (AV)
Fig. 3. Isocitrate dehydrogenase in *A. napellus* (AN) and *A. variegatum* (AV)
Fig. 4. Esterases in *A. napellus* (AN) and *A. variegatum* (AV)



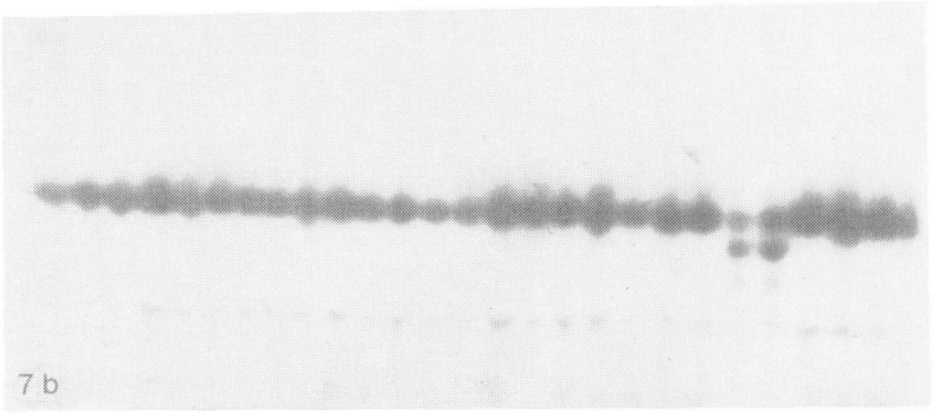




Fig. 6. Peroxidases in *A. napellus* and *A. variegatum*, 1—$PX^b$, 2—$PX^a$, 3—$PX^{ab}$

Fig. 7a, b. Glutamate oxaloacetate transaminase: a—phenotypes (from left side of gel): $GOT^a$, $GOT^{ab}$, $GOT^b$; b—phenotypes (from left side of gel): $GOT^a$, $GOT^{ab}$

GDH (EC 1.4.1.2.): NAD 20 mg, PMS 3 mg, MTT 20 mg, glutamic acid 4 g, in 100 ml TRIS-HCl buffer 0.1 M, pH 8.0

For detection of ME and IDH activity 1.5 per cent agar film (agar over-lay) with staining mixture was used.

## RESULTS

**Glutamate dehydrogenase.** Two one-band phenotypes $GDH^a$ and $GDH^b$ were detected (Table 1, Fig. 2). Since in *A. napellus* $GDH^a$ was observed exclusively and in *A. variegatum* $GDH^b$ only this enzyme may serve as taxonomic marker. The analysed zone of GDH activity is probably coded by a single locus and the phenotypes *a* and *b* found are alleles of this locus.

Table 1

List of the analysed enzymes: GDH, IDH, EST, PX, GOT and ME and the detected polymorphism in *A. napellus* and *A. variegatum*

| Enzyme | Kind of phenotype | Phenotypes and their Rm |
|---|---|---|
| GDH | S | a=0.23; b=0.16 |
| IDH | D | a=0.83/1.00; b=0.73/1.00 |
| EST | S or null | a=null; b=0.90 |
| PX | S or D | a=0.78 |
| | | b=0.68 |
| | | ab=0.78/0.68 |
| | | ic=0.72/0.64 |
| | | ad=0.78/0.80 |
| | | ae=0.76/0.78 |
| | | f=0.75 |
| | | g=0.63 |
| | | h=0.62 |
| GOT | S, D, T, | a=0.49 |
| | | b=0.38 |
| | | ab=0.49/0.44/0.38 |
| | | ac=0.49/0.52 |
| | | cd=0.49/0.52/0.55 |
| | | ae=0.49/0.44 |
| ME | S | a=0.36 |

S—enzyme coded by 1 locus=1-band phenotype.
D—enzyme coded by 2 loci=2-band phenotype.
T—hybrid band.
Rm—electrophoretic mobility of a band.

**Isocitrate dehydrogenase.** In *A. napellus* exclusively $IDH^a$ was noted and in *A. variegatum* $IDH^b$ (Table 1, Fig. 3). Both phenotypes give two bands and are probably coded by two loci, of which locus 1 giving a faster migrating monomorphic band, while locus 2 giving a slower migrating band is polymorphic (Rm 0.83 and 0.73).

**Esterases.** Only the faster migrating esterase zone was analysed which sustainedly distinguished the two species (Table 1, Fig. 4). *A. napellus* showed no enzymatic activity in this zone ($EST^a$—null), whereas *A. variegatum* gave one monomorphic band ($EST^b$).

**Peroxidases.** Nine peroxidase phenotypes were detected (Table 1, Figs. 5,6). Each kind was represented by the most frequent phenotype and rare ones. In *A. napellus* $PX^{a}$ was observed in 96.4 per cent of plants, the rare phenotypes $PX^{ad}$, $PX^{ae}$ in 3.6 per cent. In *A. variegatum* $PX^{b}$ occurred in 88.3 per cent of plants, the rare phenotypes $PX^{ic}$, $PX^{f}$ and $PX^{g}$ in 10.5, 0.6 and 0.6 per cent, respectively (Table 2).

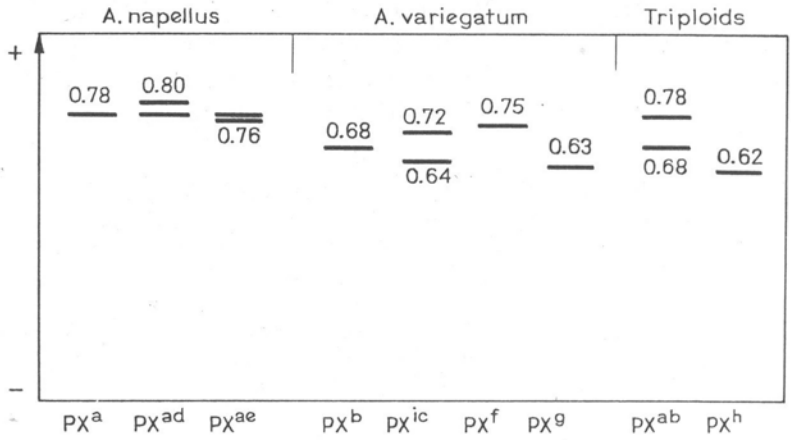



Fig. 5. Peroxidase phenotypes in *A. napellus* and *A. variegatum*

The detection of band phenotypes 1 and 2 exclusively seems to indicate that they represent homo- or heterozygotic genotypes. The former ones $Px^{aa}$ and $Px^{bb}$ are most frequent and the latter ($Px^{ad}$, $Px^{ae}$ and $Px^{ic}$) are much rarer. Peroxidase in this zone of activity is coded by locus 1, and, as regards structure, seems to be a monomer. Beside the here mentioned phenotypes two further ones have been detected: $PX^{ab}$ and $PX^{h}$. Phenotype $PX^{ab}$ is composed of two bands of the same electrophoretic mobility (Rm 0.78 and 0.68) as the most frequent phenotypes of *A. napellus* ($PX^{a}$) and ($PX^{b}$). These phenotypes therefore, represent heterozygotes of interspecific character. Phenotype $PX^{ab}$ appeared in the triploid hybrid 2413 and in 28 other plants. It occurred most numerously, however, in the population of *A. napellus* from Piekło (37%). It was also found in most of the mixed populations (in 5 of the 7 examined) and in more than half of the *A. napellus* populations (in 6 of 11 analysed). Phenotype $PX^{h}$ was found exclusively in plant no. 692 derived from a mixed population (source of Potok Miętusi (stream)).

**Glutamate oxaloacetate transaminase.** Mostly one monomorphic band ($GOT^{a}$) was observed which in *A. napellus* occurred with a frequency of about 97 per cent and in *A. variegatum* 88.3 per cent (Table 3, Fig. 7). Five rare phenotypes were also detected one of which was present only in *A. napellus* ($GOT^{ae}$), while two others only in *A. variegatum* ($GOT^{ab}$ and $GOT^{b}$). The two remaining rare phenotypes ($GOT^{ac}$ and $GOT^{ed}$) appeared both in

Table 2
Frequency of peroxidase phenotypes in *A. napellus*, *A. variegatum* and their hybrids

| Species, phenotype | Plants, % | Number of plants |
|---|---|---|
| *A. napellus* | | |
| $PX^{a}$ | 96.4 | 428 |
| $PX^{ad}$ | 3.4 | 15 |
| $PX^{ae}$ | 0.2 | 1 |
| *A. variegatum* | | |
| $PX^{b}$ | 88.3 | 143 |
| $PX^{ic}$ | 10.5 | 17 |
| $PX^{f}$ | 0.6 | 1 |
| $PX^{g}$ | 0.6 | 1 |
| Hybrids | | |
| $PX^{ab}$ | 4.6 | 29 |
| $PX^{h}$ | 0.1 | 1 |

Hybrids: $PX^{ab}$—triploid hybrid no. 2413 or probably introgressive hybrids, $PX^{h}$—triploid hybrid no. 692.

*A. napellus* and *A. variegatum*. The hybrid 2413 and the remaining plants with heterozygotic phenotype ($PX^{ab}$) showed, like the majority of plants of both species, $GOT^{a}$. The detected variability of GOT is probably coded by one locus. The most frequent allele. $Got^{a}$ occurred in the analysed plants above all in homozygotic form. The few three-band phenotypes observed in *A. napellus* and *A. variegatum* are interpreted as heterozygotic, the presence of an additional (hybrid) band suggesting that GOT in *Aconitum* is a dimer. Both homozygotes $Got^{aa}$, heterozygotes $Got^{ab}$ and homozygotes $Got^{bb}$ were detected in *A. napellus*. The next heterozygote in *A. napellus* is $Got^{cd}$ (Rm 0.49, 0.52, 0.55). The homozygote with Rm 0.55 was not found, however, perhaps because of its low frequency. The phenotypes $GOT^{ac}$ and $GOT^{ae}$ probably also represent heterozygotic genotypes, however, on account of the small difference in the mobility of the bands, the hybrid band was not noted.

**Malic dehydrogenase NADP-dependent.** In all the studied plants the presence of one monomorphic band $ME^{a}$ was observed (Table 1).

If we analyse jointly the four enzymes distinguishing the investigated *Aconitum* species we find that the phenotype of *A. napellus* consists of: $GDH^{a}$, $IDH^{a}$, $EST^{a}$, $PX^{a}$ (plus the rare phenotypes PX) and the phenotype of *A. variegatum* comprises: $GDH^{b}$, $IDH^{b}$, $EST^{b}$, $PX^{b}$ (plus the rare phenotypes PX) (Table 4). This rule, however, is not confirmed by the group of plants (4.7%) in which intermediate phenotypes were observed. To these belong:

1. The triploid 2413 from a mixed population in the Dolina Kondratowa.

Table 3
Frequency of glutamate oxaloacetate transaminase phenotypes in *A. napellus*, *A. variegatum* and their hybrids

| Species, phenotype | Plants, % | Number of plants |
|---|---|---|
| *A. napellus* | | |
| $GOT^a$ | 96.4 | 429 |
| $GOT^{ac}$ | 1.8 | 8 |
| $GOT^{ae}$ | 0.2 | 1 |
| $GOT^{cd}$ | 1.4 | 6 |
| *A. variegatum* | | |
| $GOT^a$ | 88.3 | 143 |
| $GOT^b$ | 0.6 | 1 |
| $GOT^{ab}$ | 5.5 | 9 |
| $GOT^{ac}$ | 3.1 | 5 |
| $GOT^{cd}$ | 2.5 | 4 |
| Hybrids | | |
| $GOT^a$ | 4.7 | 30 |

This hybrid has at the same time enzymes characteristic for *A. napellus* ($GDH^a$ and $IDH^a$) and *A. variegatum* ($EST^b$). Peroxidase gives additionally the heteozygotic phenotype ($PX^{ab}$). The over-all phenotype thus is as follows: $GDH^a$, $IDH^a$, $EST^b$ and $PX^{ab}$.

2. Plant no. 692 (source of Potok Miętusi). Like in the triploid 2413, GDH and IDH are present in the *A. napellus* type and EST in *A. variegatum* with the further difference that instead of the heterozygotic peroxidase phenotype we find the nowhere noted $PX^h$. The over-all phenotype is: $GDH^a$, $IDH^a$, $EST^b$ and $PX^h$.
3. The group of 28 plants derived from mixed populations as well as exclusively from the *A. napellus* one. In these plants only peroxidase gives the heterozygotic phenotype, the remaining enzymes are of the *A. napellus* type. The over-all phenotype is as follows: $GDH^a$, $IDH^a$, $EST^a$, $PX^{ab}$.

## DISCUSSION

The earlier detected triploid no. 2413 (Szweykowski and Mendelak 1977), in view of the intermediate peroxidase phenotype and the intermediate composition of phenolic compounds between *A. napellus* and *A. variegatum*, is considered as their hybrid $F_1$ (Krzakowa and Szweykowski 1977, Szweykowski and Krzakowa 1977). It was found at present that the hybrid peroxidase phenotype gives two bands one of which is the most frequent phenotype in *A. napellus* ($PX^a$, 96.4%) and the second one the most frequent phenotype in *A. variegatum* ($PX^b$, 88.3%). It may, therefore, be expected that in $F_1$ hybrids this phenotype will appear above all, although

Table 4

Frequency of *A. napellus*, *A. variegatum* and their hybrids in the particular populations on the basis of the 4 differentiating enzymatic systems

| No. of population (no. of plants) | *A. napellus*(1) | | *A. variegatum*(2) | | *A. napellus*(1') and *A. varieg.*(2') | | Hybrids $F_1$ (3) | | Introgressive hybrids (4) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | % | no. | % | no. | % | no. | % | no. | % | no. |
| 1 (45) | 100.0 | 45 | | | | | | | | |
| 2 (28) | 100.0 | 28 | | | | | | | | |
| 3 (29) | 79.3 | 23 | | | 13.8 | 4 | | | 6.9 | 2 |
| 4 (20) | 15.0 | 3 | 85.0 | 17 | | | | | | |
| 5 (19) | 100.0 | 19 | | | | | | | | |
| 6 (32) | 87.5 | 28 | 9.4 | 3 | | | | | 3.1 | 1 |
| 7 (32) | 100.0 | 32 | | | | | | | | |
| 8 (31) | 90.3 | 28 | | | 6.5 | 2 | | | 3.2 | 1 |
| 9 (19) | 31.6 | 6 | 52.6 | 10 | 10.5 | 2 | | | 5.3 | 1 |
| 10 (31) | 87.1 | 27 | | | 3.2 | 1 | | | 9.7 | 3 |
| 11 (32) | 84.4 | 27 | 6.3 | 2 | 3.1 | 1 | 3.1 | 1 | 3.1 | 1 |
| 12 (25) | 24.0 | 6 | 72.0 | 18 | 4.0 | 1 | | | | |
| 13 (31) | 87.1 | 27 | | | 3.2 | 1 | | | 9.7 | 3 |
| 14 (14) | | | 100.0 | 14 | | | | | | |
| 15 (28) | 85.7 | 24 | 3.6 | 1 | 3.6 | 1 | | | 7.1 | 2 |
| 16 (35) | 94.3 | 33 | | | 5.7 | 2 | | | | |
| 17 (27) | | | 92.6 | 25 | 7.4 | 2 | | | | |
| 18 (27) | 63.0 | 17 | | | | | | | 37.0 | 10 |
| 19 (32) | | | 62.5 | 20 | 37.5 | 12 | | | | |
| 20 (33) | 87.9 | 29 | | | 3.0 | 1 | | | 9.1 | 3 |
| 21 (66) | 39.4 | 26 | 50.0 | 33 | 7.6 | 5 | 1.5 | 1 | 1.5 | 1 |

*A. napellus*(1): $GDH^a$, $IDH^a$, $EST^a$, $PX^a$; *A. napellus*(1'); instead of $PX^a$ there is $PX^{ad}$ and $PX^{ae}$;
*A. variegatum*(2'): $GDH^b$, $IDH^b$, $EST^b$, $PX^b$; *A. variegatum*(2'); instead of $PX^b$ there is $PX^{ic}$, $PX^g$ and $PX^f$;
$F_1$ hybrids (3'): no. 2413. $GDH^a$, $IDH^a$, $EST^b$, $PX^{ab}$;
no 692. $GDH^a$, $IDH^a$, $EST^b$, $PX^h$;
Probable introgressive hybrids (4'): $GDH^a$, $IDH^a$, $EST^a$, $PX^{ab}$

owing to the existence of polymorphism other phenotypes cannot be ruled out in $F_1$ hybrids, and the more so in those of further generations. The hybrid character of the triploid no. 2413 was also confirmed by the analysis of the successive enzymes. It proved that for *A. napellus* the characteristic set of enzymes is: $GDH^a$, $IDH^a$, $EST^a$, $PX^a$ (with the rare phenotypes PX) and for *A. variegatum*: $GDH^b$, $IDH^b$, $EST^b$ and $PX^b$ (with the rare phenotypes PX). The triploid here discussed has the following set: $GDH^a$, $IDH^a$, $EST^b$ and $PX^{ab}$.

The analysed plants in populations *A. napellus* and *A. variegatum* and in the mixed population mostly have phenotypes corresponding either to *A. napellus* or *A. variegatum*. Sporadically intermediate phenotypes were detected (4.7%) which may indicate the hybrid character of these plants. In one of the latter from a mixed population source of Potok Miętusi (no. 692) the phenotype of several enzymes resembles that of the above described $F_1$ hybrid and is as follows: $GDH^a$, $IDH^a$, $EST^b$, $PX^h$. The only difference is the occurrence here of $PX^h$, a phenotype not met in any of the examined plants instead of $PX^{ab}$. This plant is also the only one which might be a potential $F_1$ hybrid.

It is interesting that in both the triploid hybrids (nos 2413 and 692, Zieliński 1982) only partial supplementation of the parent phenotypes occurs, instead of their simultaneous appearance. In the triploid no. 2413 only peroxidase gives the intermediate phenotype, whereas GDH and IDH are of the *A. napellus* type. In the case of esterase, because of the $EST^a$-null in *A. napellus*, one cannot tell whether the genotype $Est^{ab}$ or $Est^{bb}$ is present here. In the second triploid (no. 692) none of the differing enzymes gives a phenotype intermediate between *A. napellus* and *A. variegatum* on the contrary GDH and IDH give exclusively *A. napellus* phenotypes. The lack of appearance of $GDH^b$ and $IDH^b$ may be evidence of the effect of the gene dose in triploid systems, or of the selective suppression of some genes. Thus, it is a circumstance which makes detection and characterization of hybrids difficult.

The remaining 28 plants have only a peroxidase intermediate phenotype and may be introgressive hybrids, although part of them were found in mixed populations and part in *A. napellus* populations exclusively.

The occurrence in introgression between *A. napellus* and *A. variegatum* is potentially possible, mainly because the triploid hybrids nos 2413 and 692 produce viable pollen in about 13 per cent (Zieliński 1982). Gene flow between species with a different degree of ploidy has been described even in such cases when pollen viability on the $F_1$ hybrids did not exceed 1 per cent (Gajewski 1959, Moore 1959).

It is further necessary to perform a cytological analysis of all the plants with intermediate enzymatic phenotypes in order to confirm, if possible, in this way their hybrid origin.

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## *Elektroforetyczne i cytologiczne badania mieszańców między Aconitum napellus i A. variegatum. I Elektroforeza*

Streszczenie

Za pomocą elektroforezy na żelu skrobiowym i poliakrylamidowym analizowano zmienność sześciu ,enzymów w populacjach czystych oraz mieszanych *Aconitum napellus* i *A. variegatum*, pochodzących z Tatr. Enzymami różniącymi badane gatunki są: dehydrogenaza kwasu glutaminowego, dehydrogenaza izocytrynianowa, esterazy i peroksydazy. Wyodrębniono grupę roślin o fenotypach pośrednich między *A. napellus* i *A. variegatum*, wśród których znajdowały się naprawdopodobniej zarówno mieszańce $F_1$ jak i mieszańce introgresywne.

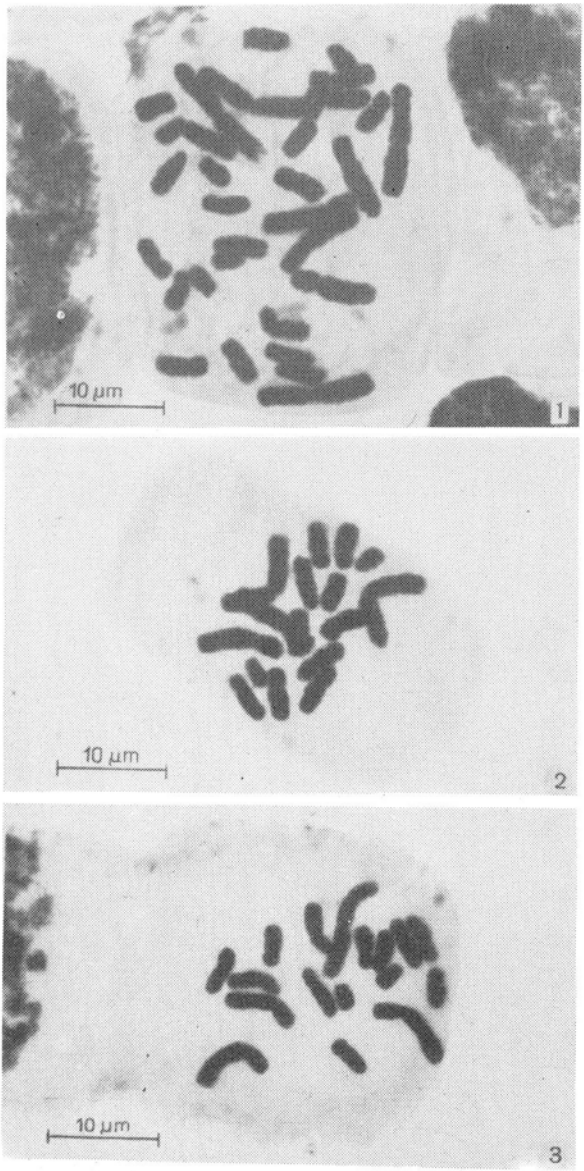



Fig. 1. *Aconitum napellus* (2n = 32)
Fig. 2. *Aconitum variegatum* (2n = 16)
Fig. 3. *Aconitum variegatum* (2n = 17)

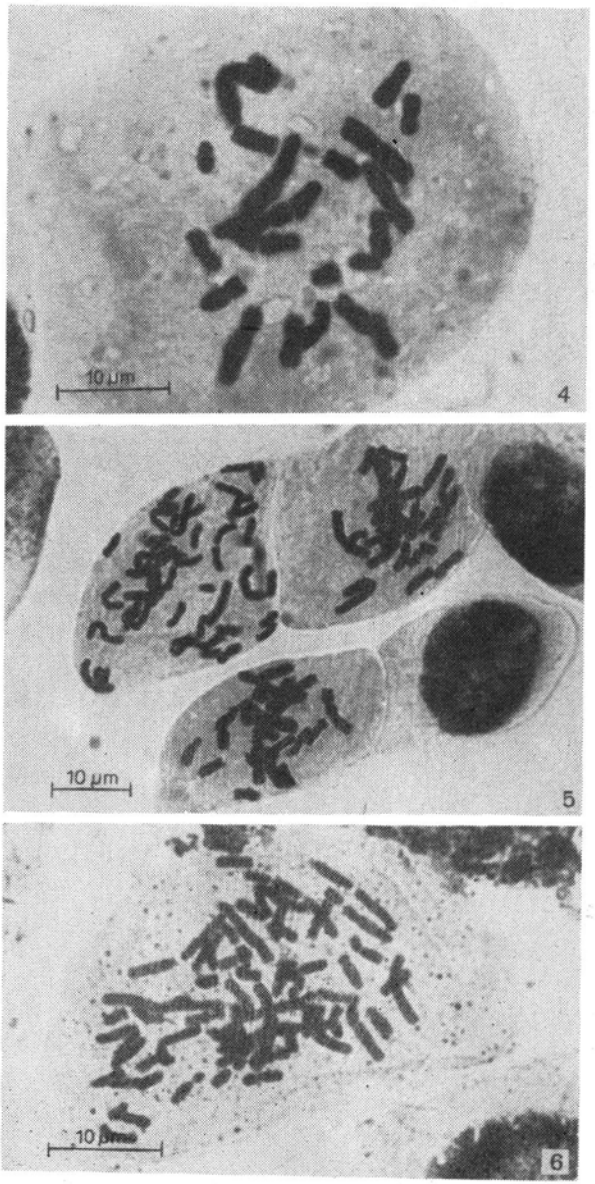
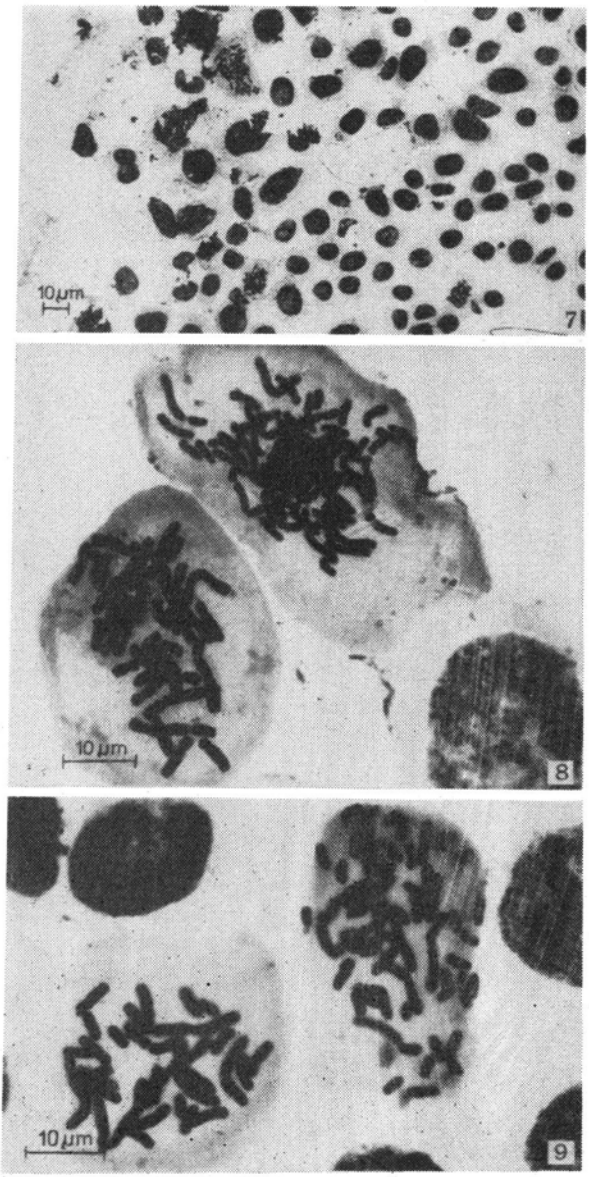



Fig. 4. Triploid hybrid (2n=24)
Fig. 5. Polysomatic cells in triploid hybrid (2n=24 and 2n=48)
Fig. 6. Polysomatic cell 2n=64 in *A. napellus* (2n=32)




Figs. 7-9. 2n = 32 and 2n = 64 in one growth apex of *A. napellus* of hybrid peroxidase phenotype ($PX^{ab}$)

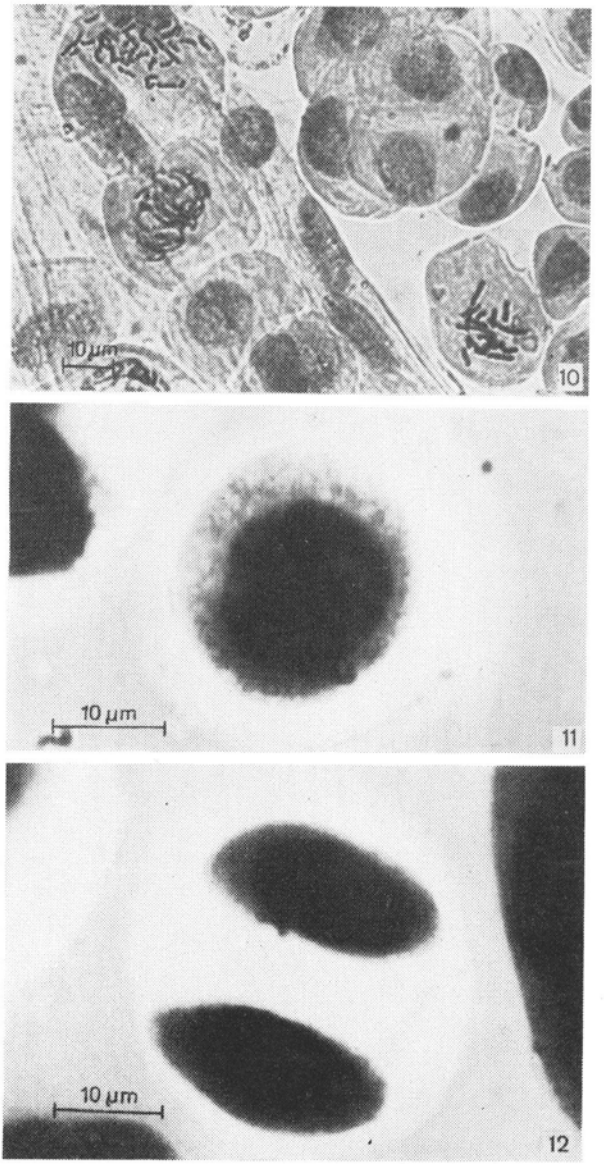



Fig. 10. Somatic reduction (2n = 32 and 2n = 16) in *Aconitum napellus* with phenotype $PX^{ab}$
Fig. 11. Monad
Fig. 12. Diad

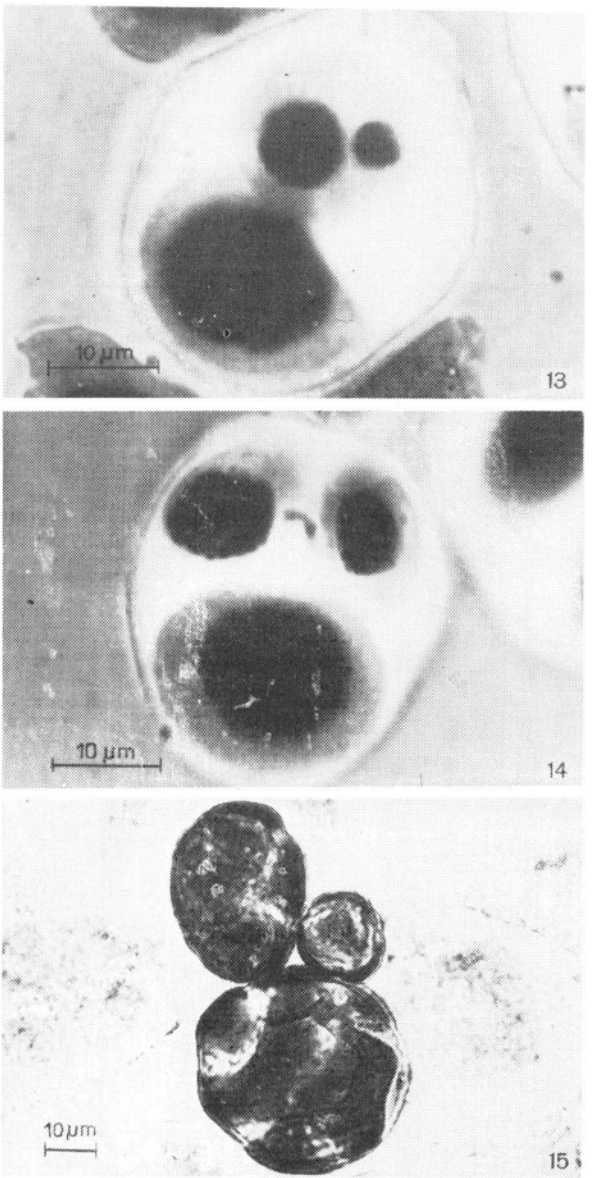



Fig. 13. Diad with micronucleus
Fig. 14. Triad
Fig. 15. Pollen grains of various sizes