

## Cytogenetic relationships between *Delphinium oxysepalum* Borb. et Pax and *D. elatum* L. from Tatra Mountains

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### INTRODUCTION

*Delphinium oxysepalum* Borb. et Pax is an endemic species from Tatra Mts. It grows in rock fissures or on screes in higher elevations from ab. 900 to ab. 2200 m.a.s.l., especially on limestone. On lower elevations (700 to 1700 m.a.s.l.) in more humid and shadowy places in Tatras grows another closely related species — *D. elatum* L. It has a large distribution in Asia and in European Russia westwards to Carpathian Mts and Alps. In Alps like in Tatra Mts. a vicarious alpine taxon occurs named by Pawłowski (1934) *D. dubium* (Rouy et Fouc.) Pawł. A third related alpine species is known from Pyrenees as *D. montanum* DC.

This group of species belonging to the s.c. section *Elatopsis* was thoroughly studied taxonomically by B. Pawłowski. This section was divided by him into two series: *Montana* and *Elata*. To the first belong the three above mentioned alpine species and to the second *D. elatum* L. and some related lowland species. It is evident that the series *Elata* originated in Asia where many related species occur and only *D. elatum* reached central and western Europe. Similar conditions exist in related genera like *Aconitum*, *Aquilegia* or *Trollius* with numerous Asiatic representatives and with only one species in Europe like *Aquilegia vulgaris* or *Trollius europaeus* L. Both *Trollius* and *Aquilegia* have formed in alpine conditions related taxa as *Trollius transsilvanicus* in Carpatians or *Aquilegia alpina* in Alps. According to our preliminary work done in genera *Aquilegia* or *Trollius* it seems very likely that the alpine taxa originated from the lowland mesophytic as a result of adaptation to local alpine conditions. The alpine and lowland taxa in these genera are cytogenetically very close with weak if any genetic barriers. The question we would like to elucidate in this paper was the relationship between the *Delphinium* species from the series *Montana* and *Elatum* of Pawłowski. According to Pawłowski the species of the series *Montana* represent old tertiary oreophytes in European flora, whereas *D. elatum* L. is a much younger migrant to Europe from Diluvial times only. He has found that the species of

the series *Montana* have many common traits with some Asiatic primitive species and are not directly related to *D. elatum*. He does not exclude the possibility that they have developed from *Elatum* group but this seems to him less probable.

To check the relationships between these two series we tried to analyse cytogenetically *D. oxysepalum* and *D. elatum* from Tatra Mts. as representatives of the two groups of taxa. Both these taxa are rather common in Tatra. In many places they meet and natural hybrids are known. Besides, from my personal observations in Tatra I had the impression that intermediate populations are more common than was believed. The plant with intermediate characters were perfectly fertile. To check the nature of these plants it was necessary to obtain artificial hybrids and to study their progenies.

#### MATERIAL AND METHODS

The plants were all grown in Warsaw in the Botanical Garden of the Warsaw University. They originated solely from natural populations from Tatra Mts.

*D. elatum* was chiefly from two sources:

No. 115 — whole plants transplanted from Tatra Mts., Bielanske Tatry „Dolina Sedmi Promenov” ab. 1300 m.a.s.l. on calcareous soil.

No. 108 — grown from seeds from Karkonoše Mts. (Sudeten Mts.) „Kotelne Jamy” in Czechoslovakia, ca 1200 m.a.s.l.

*D. oxysepalum* was also from two places:

No. 52 — „Bujaci Vrch” in Belanske Tatry ab. 1900 m.a.s.l. on calcareous soil, grown from transplanted plants.

No. 54 — upper part of the Canyon Kraków in Western Tatras ab. 1850 m.a.s.l. on calcareous soil, grown from transplanted plants.

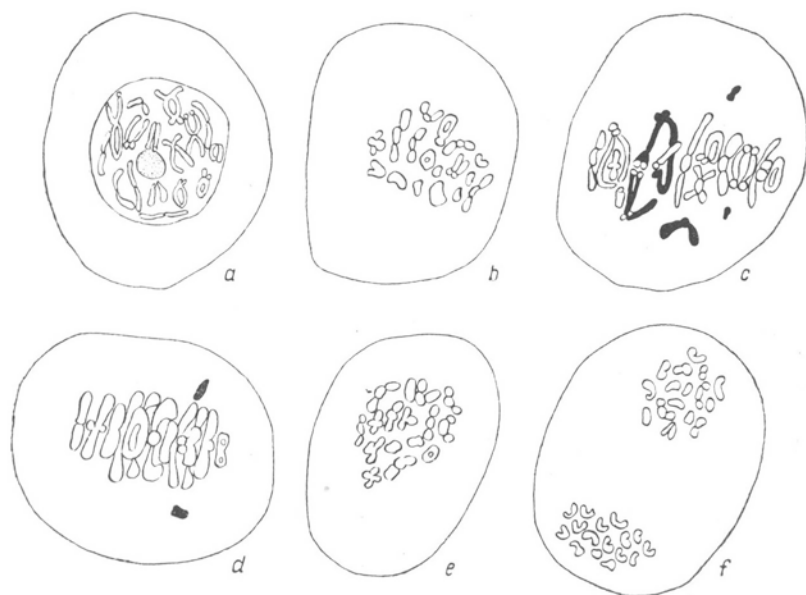
Besides many other plants from various other places in Tatra Mts. were grown through many years.

The crosses were done under cellophane bags. The emasculated flowers were pollinated the second and third day after emasculation. The cytological observations of flower buds were done on smear preparations stained with propio-carmin.

#### RESULTS

##### Number and homology of chromosomes

Both species are tetraploids with  $2n=32$  as was stated before by Skalińska (1950, 1959). No differences in chromosome numbers in many studied plants were found. The most common configuration

Fig. 1. Meiosis in *Delphinium oxysepalum* (52)

a — Diakinesis with 16II; b — metaphase I with 14II and 4I; c — metaphase I with a quadri-valent, two univalents and with one bivalent outside the plate; d — metaphase I with two univalents, e — metaphase I with 14II and 2I; f — anaphase I with 16 chromosomes in each group

at MI is 16 bivalents and the course of meiosis in the great majority of the PMC is quite normal. In few PMC's 2 to 4 univalents were observed at MI and AI. Polyvalents like tri- or quadrivalents were

Table 1

Meiosis in *D. oxysepalum*, *D. elatum* and in the hybrids.

Name of plant	MI			AI		
	Normal with 16 II	With 1-2 I	Noncon-gression of 1-2 bivalents	Normal	Lagging univalents	Bridges
<i>D. elatum</i> No. 115	65	8	3	116	6	2
<i>D. elatum</i> No. 108	124	11	-	108	12	4
<i>D. oxysepalum</i> No. 52	28	2	2	25	4	-
<i>D. oxysepalum</i> No. 54	41	3	5	130	6	4
F <sub>1</sub> 52 x 115	32	21	3	156	45	18
F <sub>1</sub> 52 x 108	25	5	2	85	14	6
F <sub>2</sub> 52 x 115 No. 1	52	6	3	48	8	-
No. 2	38	-	2	86	5	3
No. 5	35	4	-	67	5	2
No. 7	40	5	1	16	2	3

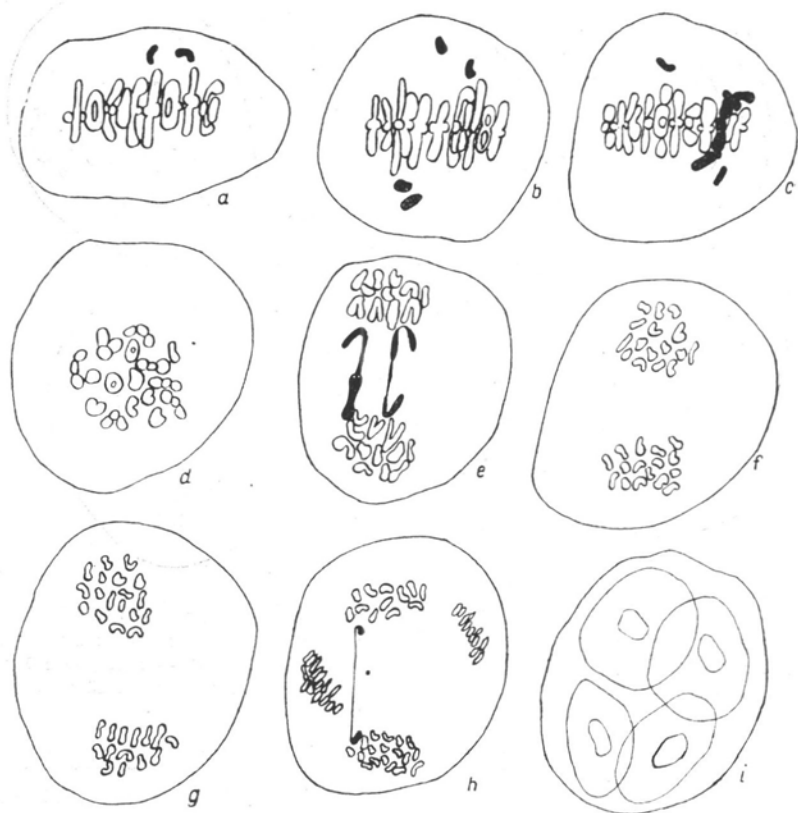


Fig. 2. Meiosis in  $F_1$  *D. oxyssepalum* (52)  $\times$  *D. elatum* (115)

a — metaphase I with two univalents; b — metaphase I with four univalents; c — metaphase I with a quadrivalent and two univalents; d — metaphase I with 16II; e — anaphase I with two stretched bivalents; f — metaphase II with 16 chromosomes in each plate; g — metaphase II with 14 and 18 chromosomes in the plates; h — anaphase II with inversion bridge; i — tetrad

also found occasionally (Fig. 1). At MI in few PMC's one or two bivalents showed noncongression and at AI rarely some bivalents were delayed in separation and the stretched chromosomes formed some kind of bridges. The second meiotic division was usually perfectly normal and the pollen fertility is high.

The artificially raised  $F_1$  hybrids show the same abnormalities as parental species, with a somewhat higher frequency (Table 1, Fig. 2). The same is true also for the few  $F_2$  segregants in which meiotic divisions were studied. The pollen fertility of the  $F_1$  and  $F_2$  hybrids was very high as it could be expected from the course of meiotic divisions. All these results show that the chromosomes of the two studied species are highly homologous.

## Morphological differences between parental species

The two species differ in many traits as it is described in a detailed way in Pawłowski's monographical study. They differ in height of floral shoots, number of flowers per shoot, kind of harriness, shape of leaves, but especially in the size and shape of floral parts chiefly sepals. The sepals of *D. oxysepalum* are distinctly elongate, 2—3 times longer than broad and are acuminate, whereas the sepals of *D. elatum* are shorter, more ovate and obtuse. The size of the *D. oxysepalum* sepals is nearly twice that of *D. elatum*.

As the length of stems, number of flowers and size and shape of sepals are the easiest to measure or count and can be easily compared they were chiefly used in the comparison of pure species, hybrids and segregants.

Table 2 shows height of shoots, number of nodes and flowers per shoot in parental species. All measurements are from the same year 1961 from uniform experimental plots. The averages from 10 to 20 plants

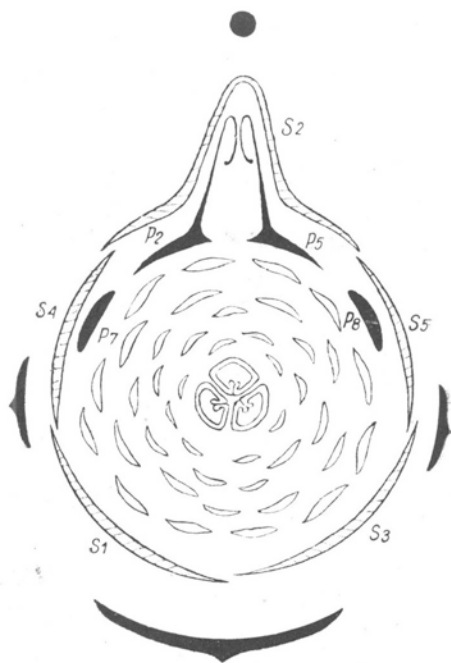


Fig. 3. A diagram of the floral structures in *Delphinium*

S1-S5 — sepals, P1-P5 — petals

are given. In this respect the differences between the two species are quite clear and are maintained in lowland conditions so that they must be genetically fixed.

The flower of *Delphinium* has special structure which is diagrammed on Fig. 3. Lateral external sepals S1 and S3, internal-ones S4 and S5 as

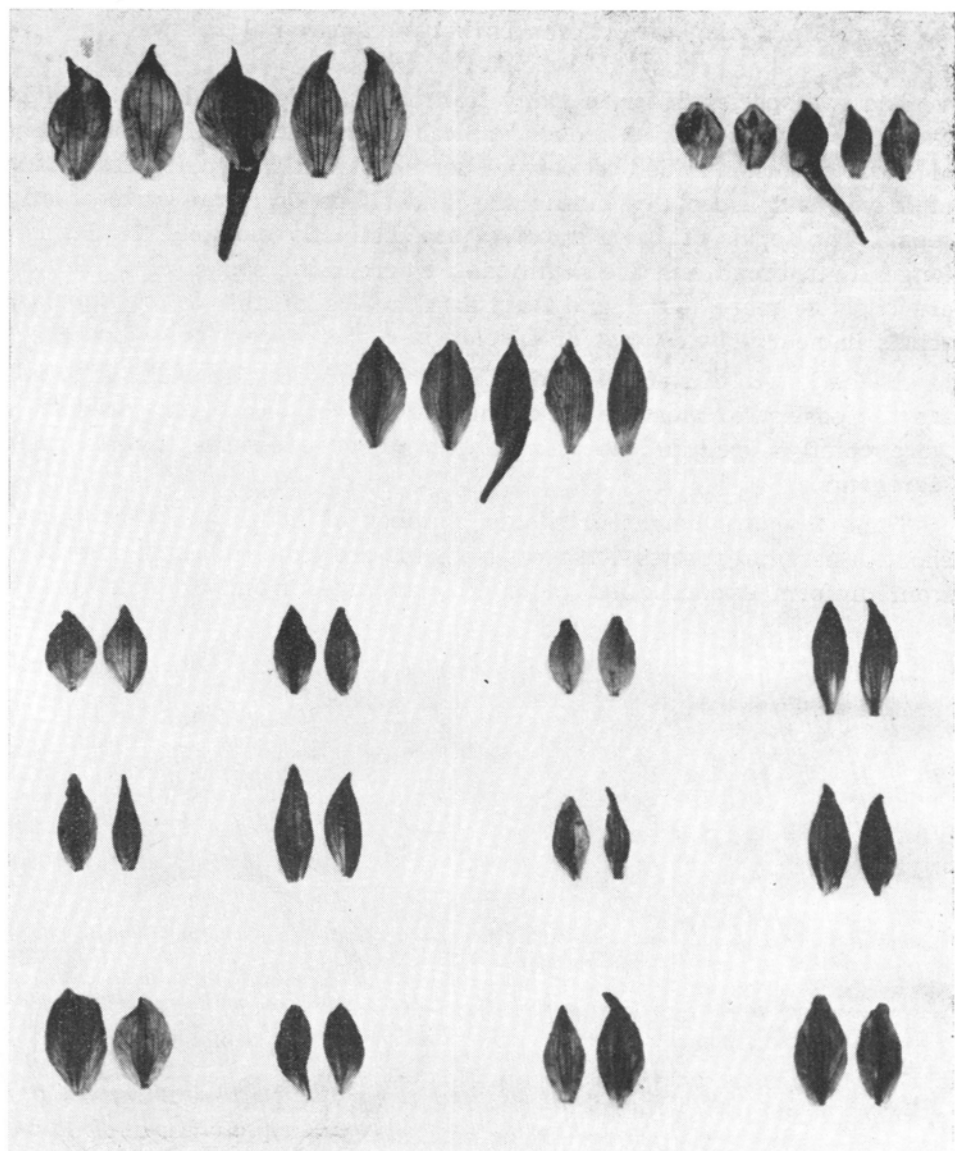


Fig. 4. The inheritance of sepals size and shape

Upper left: *D. oxysepalum* (52); upper right: *D. elatum* (115), lower row  $F_1$  *D. oxysepalum* (52)  $\times$  *D. elatum* (115). The three lower rows S4 and S5 sepals from 12  $F_2$  plants of the same cross

well as the spurred-one S2 were measured. The data for lateral sepals are averaged as the two symmetrical sepals are very similar. Other floral parts differ also but they are more difficult to measure and do not show such conspicuous differences.

Table 3 gives the dimensions and shape indexes of the sepals of the two parental species. The averages from ten flowers from each plant are given.

We see that the sepals of *D. oxysepalum* are much bigger, distinctly more elongated and acuminate (Fig. 4).

The pollen and seed fertility of both parental species is very high ranging from 80 to 95%. The germination of seeds was always difficult and only 50—60% of seeds do germinate.

### The hybrids

The compatibility of the two taxa is nearly perfect and the seed setting after cross-pollination is normal. The seeds like in pure species are difficult to germinate. The  $F_1$  hybrids are easy to grow and are very vigorous. The height of shoots and the number of nodes and flowers per shoot are like in *D. elatum* (Table 2). The dimensions and shape of the sepals are more intermediate (Table 3). The  $F_1$  hybrids are highly fertile. For instance in an  $F_1$  generation *D. elatum* 115  $\times$  *D. oxysepalum* 42 consisting of 43 plants, 40 had pollen fertility ranging from 70 to 95% and only 3 plants had pollen fertility between 50 and 65%. The same was true for other  $F_1$  hybrid populations. The seed setting was not exactly measured but seems to be on the level of parental species. The only striking difference was a very low (ab. 1%) germination of the seeds. From many hundreds of seeds sown only few  $F_2$  plants were obtained in two succeeding years. This was true both for seeds from selfed  $F_1$  plants as from intercrossed  $F_1$  plants.

I have obtained two small  $F_2$  generations. One from the cross *D. elatum* 115  $\times$  *D. oxysepalum* 52 consisting of 17 plants and the second from the cross *D. elatum* 108  $\times$  *D. oxysepalum* 52 with 12 plants. The numbers are too small to observe any regular segregation, but a distinct segregation of many parental traits was obvious. Some plants were up to 1. m. high with very numerous flowers like in *D. elatum*, others were

Table 2

Height of floral shoots, number of nodes and flowers per shoot.

Name of plant	Height of shoots in cm.	Number of nodes per shoot	Number of flowers per shoot
<i>D. elatum</i> No. 115	92.4	33.2	41.5
<i>D. elatum</i> No. 108	90.3	21.3	38.8
<i>D. oxysepalum</i> No. 52	12.1	4.5	18.2
<i>D. oxysepalum</i> No. 54	14.3	6.2	15.4
$F_1$ 115 $\times$ 52	101.1	28.4	35.6
$F_1$ 52 $\times$ 115	98.4	27.7	30.4
$F_1$ 108 $\times$ 52	80.1	18.1	30.4
$F_1$ 52 $\times$ 108	82.2	17.3	32.2





T a b l e 4

Floral dimensions in F<sub>2</sub> hybrids D.elatum /115/ x D.oxysepalum /52/

Number of plant	Sepals S1 a. S3			Sepals S4 a. S5			Sepal S2			
	Length	Breadth	Length: Breadth	Length	Breadth	Length: Breadth	Length	Breadth	Length of blade: Length of spur	Length: Breadth
1	21.5	9.1	2.36	20.9	12.1	1.72	22.3	14.7	1.11	1.51
2	20.4	9.6	2.12	19.0	12.0	1.58	18.9	12.7	1.10	1.49
3	20.4	8.4	2.73	22.5	10.3	2.18	23.7	11.9	1.46	1.99
4	20.4	9.2	2.21	18.9	10.4	1.81	19.9	12.6	1.58	1.59
5	17.4	7.2	2.41	16.8	9.5	1.76	19.6	11.9	1.03	1.64
6	17.7	8.0	2.21	17.2	11.8	1.45	18.4	12.5	1.06	1.45
7	17.3	6.1	2.82	17.8	7.8	2.41	18.4	10.6	1.26	1.73
8	17.5	5.8	3.01	19.0	5.6	3.39	17.0	7.6	1.37	2.13
9	19.2	5.8	3.31	20.5	6.4	3.20	20.5	9.5	1.00	2.15
10	18.9	5.5	3.43	18.1	5.6	3.23	20.1	8.2	1.02	2.45
11	24.2	10.8	2.24	23.2	12.2	1.90	25.2	14.7	1.13	1.70
12	18.5	6.6	2.80	18.4	7.0	2.62	17.6	10.4	1.04	1.69
13	17.2	7.1	2.40	17.6	8.2	2.14	15.8	10.6	1.01	1.49
14	17.3	6.8	2.54	17.4	7.9	2.20	17.1	9.4	0.89	1.83
15	17.5	8.1	2.15	17.2	8.8	1.95	16.6	9.7	0.97	1.91
16	20.9	8.9	2.34	20.5	9.4	2.18	21.6	10.8	1.05	2.00
17	21.9	9.8	2.23	21.4	10.4	2.05	21.7	10.4	1.04	2.08

not more than 55 cm. high and with only 6 to 10 flowers per shoot like in *D. oxysepalum*. Also in the size and shape of the sepals a variation was evident (Table 4). The majority of plants had a normal pollen fertility from 80 to 95%; only few plants showed lowered pollen fertility from ab. 60 to 70%.

### Plants from natural populations

According to Pawłowski (1934) the natural hybrids between these two species are not very common. He lists the known localities and describes some forms which are different but in general intermediate between parental species. He has found that the hybrids have lowered pollen fertility (from 15 to 34,2% of undeveloped pollen). Pawłowski has also observed that sometimes in Tatra Mts. there are populations which to some extent are intermediate and could be the result of ancient hybridizations.

From my personal observations in Tatra Mts. and also from the breeding experiments from seeds taken from natural populations it seems to me that in some places the populations are truly intermediate between *D. elatum* and *D. oxysepalum*. In those places plants occur that have intermediate traits. The progenies raised from seeds of such plants maintain their characters or segregate. *D. oxysepalum* in Bielanske Tatry or in Czerwone Wierchy range where it occurs abundantly above 1800 m. a.s.l. is a typical alpine *D. oxysepalum* with short shoots, few, large flowers with elongate and acuminate sepals. But the populations which are below this belt, like for instance in the valley Dolina Sedmi Promenov in Bielanske Tatry or in Miętusia valley or Kraków Canyon in Czerwone Wierchy on altitudes between 1000 and 1600 m. a.s.l., show sometimes untypical characters combinations. The seed progenies from these plants maintained in Warsaw their characteristic intermediate traits (Table 5). Among about one hundred of plants studied in Warsaw many were very similar to the artificially raised  $F_1$  or  $F_2$  hybrids and perfectly fertile. The variation concerned the size and shape of sepals but also the height of floral shoots and the number of flowers per shoot. Obvious segregation in shape of leaves and in the colour of the flowers was many times observed. For many of these plants it was impossible to classify them to any pure species.

### DISCUSSION

*D. oxysepalum* and *D. elatum* in Tatra Mts. are both tetraploid and their chromosomes are highly homologous. Many specific traits are genetically fixed and maintained in low land conditions. In  $F_1$  hybrids they show dominant or intermediate expression and in  $F_2$  a segregation

T a b l e 5

Floral dimensions of seed progenies from plants of natural population in Lietusia valley.  
/From each plant two the most different progeny plants are given/.

Number of plant	Sepals S1 a. S3			Sepals S4 a. S5			Sepal S2			Length: Breadth
	Length	Breadth	Length: Breadth	Length	Breadth	Length: Breadth	Length	Breadth	Length: Breadth	
20.1a	14.1	8.3	1.70	13.8	10.0	1.38	12.7	9.9	19.6	1.28
20.1b	16.8	8.3	2.02	15.8	9.9	1.59	14.2	8.0	17.0	1.77
20.2a	17.5	7.0	2.50	16.5	8.0	2.06	16.4	10.0	19.0	1.64
20.2b	22.6	11.5	1.96	22.5	12.1	1.89	20.8	13.5	18.1	1.54
20.3a	23.3	10.4	2.82	28.4	11.4	2.49	25.3	13.1	23.6	1.93
20.3b	23.1	9.2	2.51	21.7	9.7	2.24	18.3	11.7	21.7	1.56
20.4a	26.0	11.0	2.36	25.1	13.1	1.92	22.0	13.0	18.0	1.69
20.4b	20.5	7.0	2.93	19.2	8.5	2.26	19.3	9.0	16.0	2.14
20.5a	22.5	7.5	3.00	23.0	9.5	2.42	20.5	10.1	17.5	2.05
20.5b	24.8	9.5	2.61	23.5	10.9	2.15	19.8	10.2	21.8	1.94
20.6a	19.9	7.0	2.84	19.2	8.5	2.26	14.5	9.7	17.7	1.49
20.6b	27.6	11.6	2.38	26.3	13.4	1.89	22.7	14.7	20.4	1.54
20.7a	21.5	10.6	2.03	21.2	11.8	1.79	17.2	13.0	15.4	1.32
20.7b	25.0	14.5	1.72	24.5	14.5	1.69	18.5	15.5	20.6	1.19
20.8a	21.8	9.6	2.27	20.4	11.2	1.82	19.4	12.5	12.5	1.55
20.8b	26.9	12.2	2.20	25.4	14.3	1.77	21.5	16.0	19.7	1.34
20.9a	20.5	7.5	2.73	19.5	8.0	2.44	16.5	9.5	20.0	1.74
20.9b	22.5	10.0	2.25	21.4	11.3	2.44	19.5	13.5	16.0	1.44
20.10a	23.1	9.7	2.38	22.5	11.8	1.91	19.0	13.2	15.8	1.44
20.10b	21.4	9.7	2.21	20.6	11.4	2.28	17.8	12.8	16.6	1.39

was observed. The genetic barriers between these two taxa are very weak. The gene flow between two taxa is possible and very probable. The existence of populations with a mixture of specimens with different interspecific character combinations seems to indicate a probable introgression.

The cytogenetic data point to a rather very close relationship between the two taxa. It seems to me very probable that *D. oxysepalum* originated from *D. elatum* through selection of biotypes adapted to alpine conditions. The same process took place in Alps and maybe also in Pyrenees. The alpine species in all these three mountain ranges could arise independently. Their close resemblance could be explained by the fact that they originated from the common ancestors of *D. elatum* group and by convergence caused by similar alpine conditions. The same process took place probably in related genera *Trollius* and *Aquilegia* as was pointed out before. The genera *Trollius*, *Aquilegia* and *Delphinium* are all Asiatic in origin and have only few mesophytic lowland representatives in Europe and all have formed closely related alpine taxa.

In California according to Epling a. Lewis (1952) the mesophytic *Delphinium* species are relics of the old arcto-tertiary flora. From those primitive species in California numerous new species developed adapted to xeric conditions when desert and mediterranean type floras were formed. Very conspicuous changes in the morphology and physiology took place without nearly any changes in the number and structure of chromosomes. Epling a. Lewis (1959) have shown that numerous Californian desert *Delphinium* species are kept separate in nature solely by ecological or geographical barriers. The species are interfertile but hybrid swarms in nature are infrequent. In some instances pairs of species form hybrid colonies from which new species could arise only through segregation and selection of different gene combinations. *D. gypsophilum* is of such an origin from the hybrids between *D. hesperium* and *D. recurvatum*.

Both Europe and California are on the limits of the distribution of the genus *Delphinium* which has its centre in Asia. Of course, the evolutionary changes in these two parts with so different geological history were completely different but the general trend was the same: the primitive species are mesophytic.

It is quite possible that the intermediate character of some populations in Tatra Mts. is not only the result of introgression between two distinct species but may be also the result of incomplete selection in habitats with „intermediate” edaphic and climatic conditions. To this conclusion point also some facts gathered by Pawłowski from his extensive taxonomic studies based on material from the whole *Elatopsis* section. One can easily observe that nearly all traits characteristic for the species of the series *Montana* are to be found in the species from

the series *Elata*. For instance Pawłowski has observed that in *D. elatum* the number of nervatures in sepals S1-S2 varies from 3 to 12 with an average of 7 and the curve of variation is normal, whereas in *D. oxysepalum* the number varies from 3 to 9 and the curve of variation is unilateral with the maximum at 3. This seems to indicate rather that *D. oxysepalum* developed from *D. elatum* through sharp selection towards the reduction of the number of nervatures per sepal.

#### CONCLUSIONS

1. *D. oxysepalum* Borb. et Pax an alpine endemite from Tatra Mts. is closely related to an Eurasiatic species *D. elatum* L.

2. Both species have the same tetraploid chromosome number  $2n=32$  and their chromosomes are nearly completely homologous.

3. The species are intercompatible and the  $F_1$  and  $F_2$  hybrids are highly fertile. They are separated mainly by ecological and geographical barriers.

4. In Tatra Mts. populations of plants intermediate between parental species were found. Plants grown from seeds from such populations show distinct variation in many specific traits.

5. It is assumed that *D. oxysepalum* is a taxon derived from *D. elatum* and adapted to alpine conditions.

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