Hybrides of *Trollius pumilus* Don with other species of the same genus

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INTRODUCTION

Recent studies on the genus *Trollius* mainly concerned the morphology of its taxons as well as taxonomy (Schiepczinsky 1923, 1924; Handel-Mazzetti 1929; Miyabe 1943 and others). Also the number of chromosomes in individual species was examined (Langlet 1927, 1932; Zhukova 1961 and others) and studies on their karyotype were started (Kurita 1957, 1959, 1960, 1965; Doroszevska 1967). Up-to-date observations indicate that the *Trollius* species are $2n = 16$ diploids and may intercross. Hybrids of these species have been observed frequently under natural conditions, e.g. *T. altaicus* and *T. dschungaricus*, *T. europaeus* and *T. asiaticus* (Flora SSSR vol. V 1937, Malyshev 1965).

The first artificial hybridization was performed between *T. chinensis* and *T. europaeus* (Doroszevska 1965). A partly fertile progenies F_1 and F_2 were obtained from this combination. The author's long standing experiments indicate that various *Trollius* species intercross rather readily giving a more or less fertile progeny. *T. pumilus* constitutes an outstanding exception in this respect. Its hybrids with other species are mostly sterile.

This paper presents the results of hybridization of *T. pumilus* with nine other *Trollius* taxons. These taxons were selected rather accidentally, since the author had to use the *Trollius* species he managed to obtain and cultivate in the Botanical Garden at Warsaw. Of these taxons only *T. yunnanensis* is sympatric with *T. pumilus*; both these species grow in the mountains of south-western China. The geographical ranges of the other taxons are allopatric to *T. pumilus*.

The hybrids of *T. pumilus* with the other species were easily obtained but in most cases they were completely sterile. The sterility barrier between *T. pumilus* and the other taxons was pronounced. The results of crossing were univocal, only the F_1 generation of the *T. pumilus* $\times$ *T. yunnanensis* combination was partly fertile. Data concerning these hybrids will be discussed in the experimental part.

Though the author did not dispose of all *Trollius* taxons and the hybridizations performed do not give a complete picture, the results obtained are sufficiently interesting. They indicate that *T. pumilus* is a distinct taxon completely isolated from many other species. The occurrence of *T. pumilus* on areas overgrown with an ancient Tertiary flora points to its primordality. The results of hybridization confirm this supposition.

MATERIAL AND METHODS

Only a part of the plants used for hybridization was obtained from their natural habitats. In view of the difficulties in obtaining the species required from natural habitats the author had to base his studies also on the material derived from Botanical Gardens. Some species could not be obtained even so, thus only the following plants were used for hybridization with *T. pumilus*:

T. pumilus Don No. 10, 74, 76 — from the Warsaw Botanical Garden, all three plants identical

T. altaicus C. A. M. No. 20 — bred from seeds collected in the Altai Mts.

T. asiaticus L. No. 5 — brought from the environs of Ulan Bator in Mongolia

T. chinensis Bge. No. 25 — from the Warsaw Botanical Garden Nos. 66, 89 — bred from seeds obtained from the Upsala Botanical Garden

T. dschungaricus Rgl. No. 22 — bred from seeds obtained from the Moscow Main Botanical Garden of the Academy of Sciences

T. europaeus L. Nos. 47, 48, 57 — brought from the Białowieża forest in Poland

T. europaeus L. var. *transsilvanicus* (Schur) Bł. No. 23 — from the Polish Tatra

T. Ledebouri Rchb. No. 4 — from the Warsaw Botanical Garden

T. ranunculinus (Smith) Stearn No. 82 — bred from seeds obtained from the Frohnleiten Botanical Garden in Austria

T. yunnanensis (Franch.) Ulbr. No. 134 — from the Warsaw Botanical Garden

Three plants of each species: *T. pumilus*, *T. chinensis* and *T. europaeus* were used in the experiments. The individuals of the same species were very similar, thus they could be treated as a whole and the results of their hybridization were univocal. The *T. chinensis* individuals were garden forms having more sepals than normally observed in individuals derived from natural habitats. *T. altaicus* No. 20 differed from typical representatives of this species by spherical flowers similar in shape to those of *T. europaeus*, but since this plant was derived from its natural habitat it was used in the experiments.

The following hybridizations were performed:

	number of seeds obtained	number of seedlings obtained
with <i>T. altaicus</i>		
No. 150 = <i>T. pumilus</i> No. 10 × <i>T. altaicus</i> No. 20.	103	30
No. 151 = <i>T. altaicus</i> No. 20 × <i>T. pumilus</i> No. 10	17	2
with <i>T. asiaticus</i>		
No. 31 = <i>T. pumilus</i> No. 10 × <i>T. asiaticus</i> No. 5	155	78
No. 32 = <i>T. asiaticus</i> No. 5 × <i>T. pumilus</i> No. 10	478	135
with <i>T. chinensis</i>		
No. 79 = <i>T. pumilus</i> No. 74 × <i>T. chinensis</i> No. 66	72	4
No. 80 = <i>T. chinensis</i> No. 66 × <i>T. pumilus</i> No. 74	132	4
No. 111 = <i>T. pumilus</i> No. 10 × <i>T. chinensis</i> No. 25	17	11
No. 117 = <i>T. pumilus</i> No. 10 × <i>T. chinensis</i> No. 89	5	2

No. 123 = <i>T. chinensis</i> No. 89 × <i>T. pumilus</i> No. 10	41	0
with <i>T. dschungaricus</i>		
No. 148 = <i>T. pumilus</i> No. 10 × <i>T. dschungaricus</i> No. 22	57	45
No. 149 = <i>T. dschungaricus</i> No. 22 × <i>T. pumilus</i> No. 10	90	31
with <i>T. europaeus</i>		
No. 28 = <i>T. europaeus</i> No. 57 × <i>T. pumilus</i> No. 10	15	2
No. 70 = <i>T. europaeus</i> No. 48 × <i>T. pumilus</i> No. 74	72	0
No. 72 = <i>T. pumilus</i> No. 74 × <i>T. europaeus</i> No. 48	23	2
No. 77 = <i>T. pumilus</i> No. 10 × <i>T. europaeus</i> No. 48	18	3
No. 108 = <i>T. europaeus</i> No. 48 × <i>T. pumilus</i> No. 10	5	0
with <i>T. europaeus</i> var. <i>transsilvanicus</i>		
No. 73 = <i>T. europ.</i> v. <i>transsil.</i> No. 23 × <i>T. pumilus</i> 76	72	2
No. 75 = <i>T. pumilus</i> No. 76 × <i>T. europ.</i> v. <i>transsil.</i> 23	38	3
with <i>T. Ledebouri</i>		
No. 33 = <i>T. pumilus</i> No. 10 × <i>T. Ledebouri</i> No. 4	61	17
No. 34 = <i>T. Ledebouri</i> No. 4 × <i>T. pumilus</i> No. 10	69	15
with <i>T. ranunculinus</i>		
No. 81 = <i>T. pumilus</i> No. 74 × <i>T. ranunculinus</i> No. 82	19	9
No. 107 = <i>T. ranunculinus</i> No. 82 × <i>T. pumilus</i> No. 74	60	22
with <i>T. yunnanensis</i>		
No. 127 = <i>T. pumilus</i> No. 76 × <i>T. yunnanensis</i> No. 134	16	10
No. 138 = <i>T. yunnanensis</i> No. 134 × <i>T. pumilus</i> No. 76	34	10

All the plants mentioned above were cultivated in the Warsaw Botanical Garden under identical conditions on experimental plots. *T. altaicus*, *T. dschungaricus* and *T. yunnanensis* were most difficult in breeding, since in some years they hardly flowered. The young seedlings invariably flower in the second year of cultivation, thus experiments with these plants require a long period.

Observations on the morphology, fertility etc. of these plants were carried out in the period 1959—1967. These observations concern only the specimens mentioned above, bred in Warsaw, and not their species in general. Single plants, being more or less typical representatives of their species, were used for hybridization, thus they could not give a complete picture of the wide morphologic variability within the range of some taxons.

The hybridization of individual *Trollius* species presented no difficulties and the only obstacle consisted sometimes in too small a number of flowers or in flowering at different periods. In most combinations the hybrids were completely sterile, thus the observations were confined to a comparison of parent plants with their F_1 progeny. Only the F_1 hybrids of the *T. pumilus* × *T. yunnanensis* combination were partly fertile, but this fertility was so low that the number of F_2 individuals was very small.

Since the results of all the hybridizations performed were in general similar they will be discussed together in the part concerning morphology; also the cytological observations will be presented together.

Measurements were performed on ten flowers and five fruit heads from each individual. Arithmetical means of these measurements are given in the tables. The seed production capacity was established by counting the mean number of well developed seeds per 1 follicle. For pure-bred species these means were calculated on the basis of 5-head samples, whereas in the case of cross-pollination all the pollinated flowers were taken into account.

The percentage of well developed pollen, i.e. the pollen fertility was established by counting 200 microspores per each plant on preparations stained with carmine-glycerine. These observations were repeated in the course of several consecutive years.

Meiosis was analyzed in the course of microsporogenesis in fresh preparations of squashed pollen sacs. The material was fixed in alcohol with acetic acid and chloroform and stained with acetocarmine.

GEOGRAPHICAL RANGE

Among the species used for hybridization only *Trollius pumilus* and *T. yunnanensis* grow on the same area in the mountains of South China. The geographical ranges of the other species spread over the territory of North Asia and Europe and are situated at various distances from that of *T. pumilus*.

T. pumilus is a small alpine plant occurring in the eastern Himalaya Mts. in Bhutan, Sikkim, eastern Tibet and South China. It grows in the alpine and subnival zones, reaching an altitude above 3000 m a. s. l., i.e. the glacier and snow line.

The range of *T. yunnanensis* includes the Yunnan and Szechwan provinces as well as South China and is mostly coextensive with that of *T. pumilus*. *T. yunnanensis* is an alpine plant and is able to contact *T. pumilus* under natural conditions. The other Asiatic *Trollius* species are allopatric to *T. pumilus*.

T. altaicus occurs in the mountains of Central Asia. Its geographical range spreads to the north east from the Alay Mts. over Tien Shan, Altai, East Sayan, West Sayan, Stanovoi Mts. to the western part of Stanovoy Khrebet. *T. altaicus* grows on alpine meadows and in lower parts of the forest zone.

T. asiaticus occurs in north-eastern Asia in Siberia between the Ural Mts. and the River Lena line. Tian Shan, southern Altai, Khangay, Khentey and Yablonovye Mts. constitute the southern border of its range. This species grows on lowlands on humid meadows, whereas in mountains it reaches up to the lower parts of the alpine zone.

T. chinensis occurs mainly in the central and lower River Amur basin, but its range reaches the shore of the Yellow Sea and the Sea of Japan. It grows on lowlands and in lower mountain zones.

The range of *T. Ledebouri* is similar to that of *T. chinensis* but shifted somewhat further to the east. It occurs mainly in the upper and central River Amur basin but in the east it reaches the Sea of Japan and the northern Yellow Sea coast. Like *T. chinensis* it grows on lowlands and in the lower mountain zones.

The geographical range of *T. dschungaricus* includes the Pamir and Atai Mts. as well as the western and central Tien Shan. It grows in the alpine as well as the conifer and deciduous forest mountain zones.

The Caucasian species *T. ranunculinus* as well as *T. europaeus* occurring in Europe and on the western Siberian lowlands are most distant from the range of *T. pumilus*. *T. europaeus* var. *transsilovanicus* occurs in the mountains of Central Europe.

Though the ranges of some of the Asiatic species are not distant from that of *T. pumilus* they are separated by dry areas such as the Takla Makan, Alashan and Gobi deserts and the Ordos and Loess uplands. These desert areas constitute an impassable barrier for *Trollius* species, since all of them belong to mesophytes and mostly grow on humid meadows.

RESULTS

I. Morphological observations

The F_1 individuals in each of the hybridizations did not differ from each other, thus the entire generation was uniform. Also reciprocal crosses between the parent plants gave the same results, thus all the hybrids obtained from two species could be treated as a whole and the data given in the tables concern the entire F_1 generation obtained. Slight differences observed in several cases will be reported in the discussion of individual features.

All the results of hybridization of *T. pumilus* obtained are in general similar. The F_1 hybrids were similar to the parental forms and exhibited an intermediate morphology. In most cases the F_1 hybrids were completely sterile and neither F_2 generations nor back-cross progeny were obtained. In view of this, the morphological observations are confined to a comparison of the F_1 hybrids with parent plants.

The results of all hybridizations are discussed together. The more important morphological features are discussed in succession, and thus *T. pumilus* is compared with other species and the hybrids obtained.

General habit of the plants

All the *Trollius* species are perennial plants with an intensively developed fasciculate root system. The overground portion consists of basal leaves on petioles and flower stems. The blades are palmatisect with a roundish outline. The flowers vary in form from completely flat to cupular or spherical.

T. pumilus is the smallest of the species studied. When flowering it is 12–30 cm high, whereas in the course of fructification occasionally the stem elongates to 40 cm. The plants of *T. yunnanensis* are but slightly higher. *T. chinensis*, the length of which frequently reached 1 m was the highest species. The other species reached 40–70 cm. The height of F_1 hybrids of all the combinations was intermediate.

The stems of *T. pumilus* are mostly without leaves and have a single flower on the top, whereas those of the other species are more or less ramified, with numerous stem leaves and their general habit is rather similar.

In all the combinations the F_1 hybrids resembled both the parental forms. In principle their general habit was intermediate but they grew exuberantly and developed more stems and flowers than their parental forms. This exuberance appeared in all the sterile hybrids. The photos of parental plants and F_1 hybrids of the *T. pumilus* $\times$ *T. europaeus* combination are given as example in Plate I.

The partly fertile F_1 hybrids of *T. pumilus* $\times$ *T. yunnanensis* developed very poorly, were small and gave but few flowers. The general habit of these plants was intermediate between that of the parental plants. The hybrid vigour involved here only leaves, which formed particularly wide leaflets.

The general habit of the few individuals of generation F_2 (30 individuals) obtained from this combination were rather similar to *T. pumilus*. All of them were small, their stems had no leaves and single flowers. The latter were somewhat darker and the leaves less incised than in *T. pumilus*. The F_2 hybrids inherited these two features from *T. yunnanensis*.

Morphology of leaves

The basal leaves of *Trollius* have petioles of varying length; the lower stem leaves have petioles, whereas the upper ones are mostly sessile. The blades are palmately, tri- or quinque-sected. Their segments are either wide, rhomboidal or incised into narrow pointed lobes.

Among the species studied *T. pumilus* has the smallest leaves. They are 3—4, at the most 5 cm, in diameter. The segments are finely incised (Plates II and III). In *T. pumilus* this feature is variable — the lobes may be wider or narrower.

Individuals of *T. altaicus*, *T. asiaticus*, *T. chinensis*, *T. dschungaricus* and *T. europaeus* had the largest leaves. The diameter of their basal leaves oscillated within the limits of 8—12 cm. The representative of *T. ranunculinus* had somewhat smaller leaves, 6—10 cm in diameter. The shape of the blades of all these species was closely similar. All of them were palmately quinque-sected, cut into pointed lobes. *T. altaicus* and *T. asiaticus* had the narrowest lobes. All the F_1 hybrids obtained with these species had invariably intermediate leaves as regards both their size and shape (Plate II).

The *T. Ledebouri* and *T. yunnanensis* individuals had distinctly different leaves. Their segments are wide and rounded, not separated into narrow lobes. The blades are rather thick and frequently skinny. The F_1 generation of the *T. pumilus* $\times$ *T. Ledebouri* combination had leaves closely similar to those of *T. Ledebouri*, but somewhat thinner (Plate III).

The leaves of *T. yunnanensis* are mostly tri-sected. The F_1 generation of *T. pumilus* $\times$ *T. yunnanensis* had leaves identical to those of *T. yunnanensis* but far more exuberant. The leaf-segments were so wide that they overlapped when the leaf

Habitus of *Trollius* plants

- a — *T. pumilus*
- b — F₁ *Trollius pumilus* × *Trollius europaeus*
- c — *T. europaeus*

Basal leaves of *Trollius* plants

a — *T. pumilus*

b — *T. asiaticus*

c — F₁ *Trollius pumilus* × *Trollius asiaticus*

Basal leaves of *Trollius* plants

a — *T. pumilus*

b — *T. Ledebouri*

c — F_1 *Trollius pumilus* $\times$ *Trollius Ledebouri*

Plate IV

Flowers of *Trollius* plants

- a* — *T. chinensis*
- b* — F_1 *Trollius chinensis* $\times$ *Trollius pumilus*
- c, f* — *T. pumilus*
- d* — *T. asiaticus*
- e* — F_1 *Trollius asiaticus* $\times$ *Trollius pumilus*

was laid flat. Leaves closely similar to those of *T. yunnanensis* but less exuberant than in F_1 prevailed in the F_2 generation.

A comparison of the results of individual combinations indicates that the F_1 hybrids of the combinations of *T. pumilus* and species having blades cut into lobes invariably had leaves of an intermediate morphology. This was observed in the hybrids of *T. altaicus*, *T. asiaticus*, *T. chinensis*, *T. dschungaricus*, *T. europaeus* and *T. ranunculinus* (Plate II).

On the other hand, in cases when the plant used for hybridization with *T. pumilus* had unlobed leaf-segments, this feature distinctly dominated in the F_1 generation (Plate III). The hybrids of *T. Ledebouri* and *T. yunnanensis* illustrate this phenomenon. The leaf width of the F_1 generation obtained from *T. pumilus* $\times$ *T. yunnanensis* exceeded that of the latter species. In general these hybrids were weak and grew worse than the parental plants. The hybrids exuberance was manifested here only in the form of widened leaf-segments of the blade. This is particularly noteworthy, since a taxon closely similar to *T. yunnanensis* having wide overlapping leaf-segments exists in nature. It is *T. anemonifolius* Stapf. It occurs in the Yunnan Province on the area of occurrence of *T. yunnanensis* and has been established by Stapf (1928) as a separate species owing mainly to its characteristic leaf morphology. The leaves of *T. anemonifolius* Stapf and those of the F_1 hybrids of *T. pumilus* $\times$ *T. yunnanensis* have an identical shape.

Morphology of flowers

The *Trollius* species flower in spring, mostly in May and June. The blooming season of a single individual generally lasts 1—2 weeks in dependence on the number of flowers and temperature of the environment. Observations on all the representatives of individual species were carried out in the course of several years. Considering the annual oscillations, and taking into account a larger number of plants observed, it may be established that the blooming season of a single species lasts about a month.

T. altaicus, *T. dschungaricus* and *T. Ledebouri* exhibited the earliest, and *T. chinensis* and *T. yunnanensis* the latest anthesis. The latter species had to be forced in a greenhouse to allow its hybridization with *T. pumilus*. The F_1 hybrids generally flower in the same period as the parental plants.

The flowers of the genus *Trollius* are radial with numerous carpels, stamina, nectaria petals and externally arranged sepals. These elements are arranged in a spiral.

Colour. The flowers of *Trollius* are yellow or orange with a varying intensity of these colours. The nectaria are generally darker than the sepals. The stamina and styles are generally also yellow. The colours were established according to the scale of the "Horticultural Colour Chart" (1941).

The flowers of *T. pumilus* are light yellow, their sepals — Canary yellow 2, nectaria Saffron yellow 7 (Table 1). The sepals of *T. europaeus*, *T. europaeus* var. *trans-*

silvanicus and *T. ranunculinus* are somewhat darker, i.e. Aureolin 3, whereas their nectaria are Saffron yellow 7 as those of *T. pumilus*. *T. altaicus*, *T. dschungaricus*, *T. chinensis* and *T. asiaticus* have orange flowers each of them darker than in the former species. The nectaria and sepals of *T. asiaticus* have the same intensive orange colour denoted as Orpiment orange 10 (Table 1).

Table 1
Colour of flower according "Horticultural Colour Chart" 1941

<i>Trollius</i>	<i>pumilus</i>	<i>altaicus</i>	<i>asiaticus</i>	<i>chinensis</i>	<i>dschungaricus</i>	<i>europaeus</i>	<i>europaeus</i> var. <i>transsilvanicus</i>	<i>Ledebouri</i>	<i>ranunculinus</i>	<i>yunnanensis</i>
Sepala										
P	2	7	10	8,9	7,8	3	3	9,1	3	4
F ₁	—	4	5	4	4	3	3	5	3,4	3,4
Nectaria										
P	7	9,1	10	10	9	7	7	9,1	7	8,9
F ₁	—	10	10	10	9	7	7	10	7—9	8,9

The F₁ hybrids of almost all the combinations had sepals of an intermediate colour as compared with that of the parental plants. In the case of closely similar colours, e.g. colours Nos 2 and 3, as in the *T. pumilus* × *T. europaeus* combination the F₁ generation had sepals of a colour approximating No. 3. Similarly, in the *T. pumilus* × *T. yunnanensis* combination the colour of sepals of the F₁ hybrids approximated that of the second parental species. It is a colour intermediate between Nos 3 and 4 (Table 1). In the combination No. 107 *T. pumilus* × *T. ranunculinus* the F₁ hybrids had sepals of No. 4/1 colour, i.e. somewhat darker than those of *T. ranunculinus*. Thus in this combination the colour intensity of the hybrid's sepals exceeded that of the sepals of both parental forms.

All the F₁ hybrids had nectaria of the same colour as those of the parental plant possessing darker nectaria. Combination No. 107 was the only exception in this respect. The nectaria of its hybrid were Tangerine orange 9, while in both parental forms they were Saffron yellow 7. Further observations proved that the individual *T. ranunculinus* used in this combination was not a homozygote as regards the colour of its flowers, since its inbred progeny had flowers of various yellow and orange tints.

In the not numerous F₂ progeny of *T. pumilus* × *T. yunnanensis*, individual specimens had sepals of Nos. 2, 3, 4 and nectaria of Nos. 7, 8, 9 colours. These were either those of the parental plants or intermediate colours.

The styles of *Trollius* flowers are mostly darker or lighter yellow. Only *T. altaicus* has purple Oxblood red 0.0823/3 styles, whereas those of *T. yunnanensis* are white. The *T. pumilus* × *T. altaicus* hybrids invariably had light-purple styles, whereas

all the F_1 and F_2 hybrids of the *T. pumilus* $\times$ *T. yunnanensis* combination had light yellow styles.

Scent. In *Trollius* species flowers have either a pleasant but very weak, practically imperceptible scent or none. It is interesting that a strong pleasant scent appeared in most of the sterile hybrids. In principle the *Trollius* species are entomophilous. The strong scent in hybrids may be interpreted as an additional factor attracting insects. In this case, however, this factor does not fulfil its role, since these hybrids are completely sterile and pollination of their flowers with pollen of fertile plants gives no result. In general the production of a strong scent and numerous flowers increase the chance of procreation, but in this case it seems only a symptom of "nature's lavishness".

Sepals. The number of sepals varies within the limits of 5—20 per 1 flower. In *T. pumilus* and *T. yunnanensis* it is 5, occasionally 6, whereas in other species this number is higher. The largest number of sepals was observed in the flowers of *T. altaicus*, *T. europaeus* and *T. Ledebouri* (Table 2).

Table 2
Number and length of the sepals

<i>Trollius</i>	<i>pumilus</i>	<i>altaicus</i>	<i>asiaticus</i>	<i>chinensis</i>	<i>dzungaricus</i>	<i>europaeus</i>	<i>Ledebouri</i>	<i>ranunculinus</i>	<i>yunnanensis</i>
Number of sepals per one flower									
P	5	14,0	10,5	12,0	9,0	13,3	15,1	9,0	5,0
F_1	—	7,0	5,4	7,0	8,2	8,0	7,0	7,1	5,0
Mean length of sepals in mm									
P	18,0	26,2	23,9	22,1	19,1	24,3	21,5	21,6	24,2
F_1	—	22,0	19,0	21,3	18,0	23,1	22,0	23,0	16,5

The size of sepals is more or less equal in all the species, their length being on the average about 22 mm. Only *T. pumilus* has distinctly shorter sepals and owing to this its flowers are the smallest.

In F_1 hybrids both the number and size of sepals is intermediate as compared with those of the parental plants (Table 2). Only in *T. pumilus* $\times$ *T. Ledebouri* and *T. pumilus* $\times$ *T. ranunculinus* hybrids the sepals of the F_1 generation were larger than in the parental plants, and therefore their flowers were larger than those of *T. Ledebouri* and *T. ranunculinus*, respectively. On the other hand the flowers of the F_1 generation of *T. pumilus* $\times$ *T. yunnanensis* hybrids were smaller than those of *T. pumilus*.

The sepals of all the *Trollius* species exhibit a similar, oval-ovate shape. The outmost sepals are the widest and become narrower towards the centre. Owing

to this only the length of sepals was taken into account in measurement, whereas their width was not considered.

The sepals may be completely flat, as in *T. pumilus*, or curved upward to a various degree and bent into a spoon-shape. This feature is most pronounced in *T. europaeus*, the flowers of which are spherical.

Nectaria. The number of nectaria in the flowers of *Trollius* species varies. The largest number was observed in *T. asiaticus* and *T. chinensis*, whereas the other species had mostly 10–15 nectaria per 1 flower. The F_1 hybrids exhibited a similar number (Table 3). The differences in shape and size of the nectaria were more significant, since their morphology varies and is characteristic of the given species (Table 3).

Table 3
Number and length of the nectaria

<i>Trollius</i>	<i>pumilus</i>	<i>altaicus</i>	<i>asiaticus</i>	<i>chinensis</i>	<i>dschungaricus</i>	<i>europaeus</i>	<i>Ledebouri</i>	<i>ranunculinus</i>	<i>yunnanensis</i>
Number of nectaria per one flower									
P	11,6	12,0	26,0	19,9	12,0	9,9	12,5	15,0	11,5
F_1	—	11,0	18,5	13,3	10,0	8,0	13,4	13,4	10,4
Mean length of nectaria in mm									
P	5,1	10,0	20,8	24,6	9,5	11,6	15,4	12,0	9,9
F_1	—	8,3	12,5	12,7	6,1	6,9	10,5	10,3	5,9

Among the species studied *T. pumilus* has the smallest nectaria. The latter widen at the tip into an oval swollen lobe. This lobe is convex, vividly orange and intensely shiny, thus it serves as a decoy. The nectarostigma is situated directly above the short unguis. It is spherical, swollen at the bottom.

T. yunnanensis has similar nectaria, only their lobe is larger and more elongated. Similarly as in *T. pumilus* it is swollen and shiny. In *T. yunnanensis* the entrance to the nectarostigma is spout-shaped, whereas the nectarostigma is intensively swollen. The nectaria of F_1 of *T. pumilus* $\times$ *T. yunnanensis* hybrids have an intermediate form, but their nectarostigma is swollen as in *T. yunnanensis* (Fig. 1).

The nectaria of *T. asiaticus*, *T. chinensis* and *T. Ledebouri* are completely different. They are long, ensiform, of a uniform orange colour. Their lobe is thin and not shiny. The nectarostigma is pocket-shaped and not swollen. The nectaria of these three species differ from each other only by their length. The nectaria of their hybrids with *T. pumilus* are intermediate in shape and size. Their lobes are somewhat swollen and shiny, whereas the nectarostigma is somewhat swollen and of a shallow pocket shape (Fig. 1).

The nectaria of *T. altaicus*, *T. dschungaricus* and *T. ranunculinus* are of moderate

Fig. 1. Nectaria of *Trollius* plantsa - *T. pumilus*b - *T. altaicus*c - F_1 *T. pumilus* $\times$ *T. altaicus*d - F_1 *T. dschugaricus* $\times$ *T. pumilus*e - *T. dschungaricus*f - *T. asiaticus*g - F_1 *T. asiaticus* $\times$ *T. pumilus*h - F_1 *T. chinensis* $\times$ *T. pumilus*i - *T. chinensis*j - *T. ranunculinus*k - F_1 *T. ranunculinus* $\times$ *T. pumilus*l - F_1 *T. Ledebouri* $\times$ *T. pumilus*m - *T. Ledebouri*n - *T. europaeus*o - F_1 *T. europaeus* $\times$ *T. pumilus*p - F_1 *T. yunnanensis* $\times$ *T. pumilus*r - *T. yunnanensis*

—

length and in general are similar to each other. Their lobes are ensiform, rounded at the tip, somewhat swollen and shiny but less than those of *T. pumilus*. Their width varies. The nectarostigmata are not swollen of a shallow pocket shape. All the hybrids of these species and *T. pumilus* have nectaria of an intermediate form. Their lobes are ovaly elongated, swollen and shiny as in *T. pumilus*. Also the shape of their nectarostigma is similar to that in *T. pumilus* (Fig. 1).

T. europaeus has nectaria of a type similar to *T. pumilus*. They are only thinner, more elongated and not so shiny. Their nectarostigma is identical as that of *T. pumilus*. The nectaria of the variety *transsylvanicus* do not differ from those of *T. europaeus*. The *T. pumilus* $\times$ *T. europaeus* hybrids have nectaria of an intermediate morphology (Fig. 1).

Shape of flowers. When the sepals are completely flat they form flat flowers such as those of *T. pumilus*. On the other hand, sepals curved upward and spoon-shaped give the flower the form of a shallow or deep bowl or even sphere in dependence on the degree of curving.

The short nectaria may be covered by stamina and almost invisible, whereas long nectaria may protrude upward from the flowers giving a particularly ornamental form as in *T. chinensis*, which has the longest nectaria of all the *Trollius* species (Plate IV, a). The flowers of *T. pumilus* are quite flat and their small nectaria do not affect the flower shape (Plate IV, c, f).

The flowers of *T. yunnanensis* have the shape of shallow bowls, whereas the F_1 and F_2 hybrids of this species had flat flowers like *T. pumilus*.

The flowers of *T. chinensis* are also cupular but their nectaria protrude high upward. The F_1 hybrids had either flat or shallow cupular flowers. Though their nectaria were considerably shorter than those of *T. chinensis*, they protruded from the flowers (Plate IV, a, b).

The flowers of *T. dschungaricus* and *T. ranunculinus* are also of shallow cupular shape, but their nectaria are considerably shorter than those of *T. chinensis*. Their F_1 hybrids invariably had flowers of an intermediate morphology.

T. asiaticus and *T. Ledebouri* have flowers in the shape of somewhat deeper bowls, some of them hemispherical. Their hybrids with *T. pumilus* developed flowers in the form of shallow or deeper bowls. The nectaria of *T. asiaticus* are so long that they protrude beyond the flower, whereas those of *T. Ledebouri* are somewhat shorter and hidden within the flower (Plate IV, d, e).

The flowers of *T. europaeus* and *T. altaicus* are spherical. Their sepals are so strongly bent that they overlap forming a closed or almost closed sphere. Their hybrids with *T. pumilus* had either hemispherical or cupular flowers.

The results of all the combinations were similar, i.e. the flowers of F_1 hybrids had intermediate shapes. No new forms, exceeding those of the parental plants were obtained. An entire scale of variability of this feature, from flat to spherical flowers, can be established on the basis of the shapes of flowers of the pure species and their hybrids.

Morphology of fruits. All the *Trollius* species develop dry fruits in the form of follicles ended with beaks. The follicles form heads. Both the length and number

of follicles and beaks varies. The beaks develop from the style which dry together with the ovary and transform into a fruit.

In all the sterile hybrids obtained the follicles were small and underdeveloped, and owing to this the spiral arrangement of the styles became pronounced. Most follicles of the partly fertile F_1 hybrids of the *T. pumilus* $\times$ *T. yunnanensis* combination were normal.

In all the F_1 hybrids the number of follicles was either more or less the same or intermediate as compared with that of the parental plants. The length of follicles was not taken into account since the underdeveloped follicles of hybrids were considerably smaller than those of fertile plants. On the other hand the length of beaks was considered since they were normally developed even on the underdeveloped follicles. The length of beaks is an important taxonomic feature in the genus *Trollius*. The beaks of hybrids generally had the same length as those of the parental plants, only in some cases they were bent outward. In the *T. pumilus* $\times$ *T. europaeus* and *T. pumilus* $\times$ *T. Ledebouri* hybrids the beaks were longer than those of the parental plants (Table 4).

Table 4

Mean length of the beaks at the follicles in mm

<i>Trollius</i>	<i>pumilus</i>	<i>altaicus</i>	<i>asiaticus</i>	<i>chinensis</i>	<i>dschungaricus</i>	<i>europaeus</i>	<i>europaeus</i> var. <i>transsilvanicus</i>	<i>Ledebouri</i>	<i>ranunculinus</i>	<i>yunnanensis</i>
P	1,4	2,0	2,6	2,1	2,0	0,7	3,5	2,0	2,0	2,4
F_1	—	2,0	2,5	2,1	2,0	3,0	4,0	2,5	2,0	1,9

The length of the beaks in fertile *Trollius* plants depends on the activity of numerous polygens. This was proved by the results of the *T. chinensis* $\times$ *T. europaeus* combination (Doroszewska 1965 and other observations of the author). The character of this feature is more complicated in the sterile hybrids of *T. pumilus*, where the long styles and beaks develop on underdeveloped follicles. It seems that the factors inhibiting the normal development of ovaries do not affect the development of the style and the stigma. The beaks develop normally notwithstanding the lack development of the ovary. It seems improbable that their length would be solely the result of hybrid exuberance. Similarly as in fertile plants, also in hybrids the length of beaks depends on the number of polygens transferred, but, doubtless, also additional factors are responsible for such features as e.g. intensive bending of the beaks or their length.

II. Fertility

The number of seeds produced by self- and crosspollinated plants as well as the germination capacity of the seeds and the amount of normally developed pollen were taken into account in the studies on fertility of plants (Table 5). Generally

Table 5
Comparison of fertility of parental plants and hybrids

<i>Trollius</i>	Mean number of seeds per 1 follice			Germinating seeds, %		Good pollen, %	
	after auto-gamy P	after pollination with <i>T. pumilus</i>	after auto-gamy F ₁	P	F ₁	P	F ₁
<i>pumilus</i>	4.0	—	—	30.0—50.0	—	90.0—100.0	—
<i>altaicus</i>	4.3	1.5	0.0	20.0—30.0	25.0	90.0— 96.0	1.5— 9.0
<i>asiaticus</i>	4.0	6.6	0.0	32.3	33.6	90.0— 97.0	4.5—10.0
<i>chinensis</i>	3.3	2.6	0.0	50.0	7.8	90.0—100.0	1.0— 5.0
<i>dschungaricus</i>	3.3	3.3	0.0	5.7	45.0	95.5	1.0— 6.0
<i>europaeus</i>	1.1	2.2	0.0	1.0	4.5	90.0—100.0	1.0— 6.7
<i>europaeus</i> var. <i>transsilvanicus</i>	1.8	3.2	0.0	3.0	4.5	95.0— 99.0	2.0— 3.0
<i>Ledebouri</i>	2.5	2.5	0.0	25.0	24.6	90.0— 97.0	0.0— 2.2
<i>ranunculinus</i>	2.4	2.4	0.0	10.0	30.0	70.0— 90.0	0.5— 9.5
<i>yunnanensis</i>	2.5	1.6	1.0	50.0	39.0	81.0— 97.0	40.0—80.0

two or more hybridizations were performed in each combination (see "Material and methods"). The mean data of all hybridizations between the given two species are summarized in the Tables. Detailed data of the *T. pumilus* × *T. chinensis* combination are given as an example. The following numbers of seeds and seedlings were obtained from cross-pollination of *T. pumilus* × *T. chinensis*:

A

No. of combination	number of seeds	number of follices	mean number of seeds per 1 follice	number of seedlings
79	72	22	3.2	4
80	132	53	2.5	4
111	17	9	1.8	11
117	5	5	1.0	2
123	41	11	3.7	—
Total	267	100	2.6	21

A total of 267 seeds in 100 follices, i.e. a mean of 2.6 seeds per 1 follice, were obtained. The largest number of seeds per 1 follice occurred in the reciprocal combinations Nos. 79 and 123. The summed results of combinations Nos. 79, 111 and 117, in which *T. pumilus* was the material plant, was 94 seeds in 36 follices. Thus, here, similarly as before, the mean is 2.6 seeds per 1 follice. These data indicate that the direction of hybridization exercised no effect, and the general mean 2.6 may represent all the combinations. The situation was analogous with other species.

The seeds of hybrids germinated weakly and only single F₁ individuals were

obtained from individual combinations. A total of 21 seedlings were obtained, i.e. only 7.8 per cent of the seeds exhibited germinating power. The relatively highest number of seedlings were obtained from combination No. 111, but they were so weak that most of them perished. In No. 123, the mean number of seemingly good seeds was the highest, but none of them germinated. This may be attributed to the fact that in the course of collecting the author took into account only the seeds which were well and normally developed at first sight, whereas underdeveloped or greatly shrivelled seeds were omitted in calculations. In all combinations the follicles contained such underdeveloped seeds. This indicates that after fertilization a part of the zygotes perishes in earlier or later developmental stages. Also a part of the collected seeds, being at first sight normally developed, may have been already dead or incapable of further development, though their death occurred relatively late. The mortality of seedlings is as if a further stage of the same phenomenon.

In all the cases in which a relatively high number of seeds was obtained, the percentage of germinating seeds was low, and when many seeds germinated most seedlings perished soon.

This phenomenon is well visible in the *T. pumilus* $\times$ *T. asiaticus* combination from which the following results were obtained:

B

No. of combination	number of seeds	number of follicles	mean number of seeds per 1 follicle	seedlings	
				number	percentage
31	155	30	5.1	78	50.0
32	478	66	7.2	135	30.0
Total	633	96	6.6	213	33.6

A particularly high number of seeds was obtained from this combination. The mean number of seeds per 1 follicle considerably exceeded that observed in self-pollination of the parental plants (Table 5). In other combinations the cross-pollination gave the same or somewhat smaller numbers of seeds than those obtained in self-pollination of pure species (Table 5). The percentage of germinating seeds varied widely. These data, however, could hardly be considered, since in pure species the germinating power of seeds was far from uniform. For instance only 1 per cent of seeds of *T. europaeus* germinated. The seeds of *T. pumilus*, *T. chinensis* and *T. yunnanensis* germinating in about 50 per cent belonged to the best. The seeds of hybrids of *T. asiaticus*, *T. dschungaricus*, *T. Ledebouri*, *T. ranunculinus* and *T. yunnanensis* germinated just as well as those of the parental plants. In the other hybrids the percentage of germinating seeds was considerably decreased (Table 5).

Most of the F_1 hybrids were completely sterile and gave no seeds both from free and back-pollination. Their follicles were underdeveloped.

The F_1 hybrids of the *T. pumilus* $\times$ *T. yunnanensis* combination were the only, partially fertile ones. In self pollination they gave on the average 1 seed per 1 follicle. A total of 238 F_2 seeds were collected, out of which 93 seeds germinated. A part of the latter perished and 72 were planted. Of this number only 30 plants came into

Table 6

Course of meiosis in F₁ hybrids of *T. pumilis* with other species

A. Conjugation of chromosomes in metaphase I																		
	<i>altaicus</i> number of PMC		<i>asiaticus</i> number of PMC		<i>chinensis</i> number of PMC		<i>dschungaricus</i> number of PMC		<i>europaeus</i> number of PMC		<i>europaeus</i> <i>v. transsiv.</i> of PMC		<i>Ledebouri</i> number of PMC		<i>ranunculinus</i> number of PMC		<i>yunnanensis</i> number of PMC	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Metaphase I	100		1100		400		200		439		200		600		500		500	
8 _{II}	65.0		83.6		78.5		53.5		81.6		56.5		93.0		88.6		95.6	
7 _{II} + 2 _I	20.0		13.3		17.5		29.0		15.5		24.0		6.6		10.0		4.2	
6 _{II} + 4 _I	12.0		2.8		3.0		17.5		2.5		15.5		0.2		1.4		0.2	
5 _{II} + 6 _I	3.0		0.3		0.7		5.5		0.4		3.0		—		—		—	
4 _{II} + 8 _I	—		—		0.2		2.0		—		1.0		—		—		—	
3 _{II} + 10 _I	—		—		0.1		1.0		—		—		—		—		—	
2 _{II} + 12 _I	—		—		—		1.5		—		—		—		—		—	
	100.0		100.0		100.0		100.0		100.0		100.0		100.0		100.0		100.0	
B. Further course of meiosis — total number of cells examined (n) and percentage of disturbances																		
Anaphase I	100	30.0	579	14.7	251	24.7	35		460	28.1	200	29.1	201	12.0	100	10.0	248	3.2
Telophase I	100	24.0	785	6.7	250	19.2	137	52.0	600	22.1	200	25.5	443	3.5	441	7.4	353	5.1
Metaphase II	100	32.0	701	14.2	205	28.8	200	51.5	516	27.5	200	27.0	493	9.7	500	13.8	450	3.3
Anaphase II	100	34.0	522	16.1	138	22.6	40	—	276	34.4	200	35.0	132	9.1	146	15.8	141	12.4
Telophase II	100	25.0	924	6.9	314	15.4	200	58.5	455	26.9	200	20.5	453	7.1	500	7.4	394	7.1
Tetrads	200	28.0	2200	8.8	800	18.0	400	55.5	800	21.5	600	17.4	1023	12.6	1000	10.0	1000	6.7

flower, whereas the rest perished before the flowering period. The F_2 hybrids grew very weakly.

Pollen fertility. Most of the parental plants produced normal amounts of well developed pollen, but in some of them, as for instance *T. ranunculinus* and *T. dschungaricus* the percentage of normal pollen was somewhat decreased (Table 5).

All the sterile F_1 hybrids had a very low percentage of good pollen. It oscillated within the limits of 0.0 to 10.5 per cent, moreover it is not sure whether this normally developed pollen had any fertilization capacity. Only the partly fertile hybrids of the *T. pumilus* $\times$ *T. yunnanensis* combination produced 40—80 per cent of well developed pollen. The F_2 hybrids of this combination had 41.2—90.0 per cent of good pollen (Table 5).

III. Cytological observations

The observations concerned the course of meiosis in pollen mother cells (PMC). In the parental plants the course of meiosis was quite regular, the conjugation of chromosomes in the metaphase was complete, each cell containing 8_{II} ; at the most 2 per 100 cells contained $7_{II}+2_I$. The course of further division stages was also regular and disturbances amounted to only a few per cent. Owing to this, these plants produced 98.0—100.0 per cent of normally developed tetrads and the fertility of their pollen oscillated within the limits of 90.0—100.0 per cent (Table 6).

The meiotic division in hybrids is not as regular as that in the parental plants and a number of characteristic disturbances occur. Mostly the same irregularities occurred in all the hybrids only they were more or less frequent. Data concerning the course of meiosis in PMC of F_1 hybrids of individual combinations are tabulated for comparative purposes. The percentual numbers of PMC regularly dividing in successive meiotic stages are given. The author endeavoured to count 100, and at the tetrad stage 200 cells per each plant and to follow the course of meiosis of five F_1 individuals in each combination, but in some cases this was impossible (Table 6).

The irregularities observed in consecutive stages of meiosis are described below. In metaphase I 2—12 univalents were observed per 1 cell. The most successful conjugation of chromosomes was observed in the F_1 individuals of the *T. pumilus* $\times$ *T. yunnanensis* and *T. pumilus* $\times$ *T. Ledebouri* combinations in which 95.6 and 93.0 per cent of cells with 8_{II} were observed and the number of univalents did not exceed 4 per 1 cell.

The lowest conjugation occurred in the *T. pumilus* $\times$ *T. dschungaricus* hybrids in which only 53.5 per cent of F_1 PMC contained exclusively bivalents, whereas the number of univalents rose to 12 per 1 cell. This was the lowest conjugation of chromosomes observed in *Trollius* hybrids (Table 6).

In the course of division some chromosomes did not migrate to the poles but remained on the division plate. In meiotic division I the author observed belated bivalents, lagging univalents or fragments of chromosomes and chromatine bridges.

The 1—2 micronuclei per 1 cell observed in telophase I were the result of these disturbances. Some of the AI bridges did not break and could remain intact through-

hout meiotic division II. In metaphase II they linked two metaphasal plates. When these bridges were not broken in anaphase II, they could be observed in telophase II. Chromatine bridges could develop also in meiotic division II. In consequence, chromatine bridges formed in meiotic division I and II could be observed in the course of anaphase II and telophase II. Frequently the cells containing bridges showed also lagging chromosomes or micronuclei.

Some of the cells in telophase contained nuclei which did not undergo division; in this stage they remained joined by a constricted part. In consequence, instead of two, a single restitution nucleus was formed. Such restitution nuclei were found in division I and II of the PMC. Owing to these disturbances normal tetrads were accompanied by diads, triads and even monads. Also pentads, hexads, septads and tetrads with nuclei of different dimensions were observed. This was due to an uneven division of chromosomes.

In general the percentage of disturbances in a given hybrid is more or less equal in successive division stages. The most regular meiosis was observed in the F_1 hybrids of the *T. pumilus* $\times$ *T. yunnanensis* combination, in which 95.6 per cent of cells in metaphase I contained exclusively bivalents and 93.4 per cent of tetrads were normally developed. The percentage of good pollen in these hybrids was 40.0—80.0 (Table 2). The most irregular division was observed in the *T. pumilus* $\times$ *T. dschungaricus* F_1 hybrids, in which only about one half of the PMC in metaphase I contained exclusively bivalents and only 44.5 per cent of tetrads were normally developed. In the other hybrids the percentage of disturbances was lower, and in consequence these hybrids produced above 80 per cent of regular tetrads (Table 6).

As mentioned above, among the hybrids obtained only those of the *T. pumilus* $\times$ *T. yunnanensis* combination were partly fertile, whereas the others were completely sterile. In these sterile hybrids the meiotic divisions were regular in most PMC and in metaphase I cells with 8 bivalents prevailed. This indicates that all the chromosomes of the hybrid's cells had a conjugation capacity, thus they should be at least partly homologous. Despite, however, such a successful conjugation of chromosomes in metaphase I, numerous disturbances occur in further meiotic stages and the number of normally developed tetrads is lower than that in the parental plants. This proves that, despite normal conjugation, the homology of chromosomes of *T. pumilus* with those of the other species is rather low, and to a considerable degree they are not homologous.

Not only the conjugation of chromosomes in metaphase I, but also the number of disturbances in further divisions should be taken into account in studies on the *Trollius* hybrids. These data considered together point to a lower or higher homology of chromosomes and may give evidence as regards the relationship between the parental plants.

The observations performed point to a normal course of the meiotic divisions in most PMC of the sterile hybrids. The number of regularly developed tetrads in F_1 hybrids oscillates within the limits of 44.5—91.0 per cent. On the other hand, the number of normal microspores produced by these plants is extremely small and amounts to 0.0—10.0 per cent (Table 6). This difference is striking. It indicates

that, despite the normal regular development of most tetrads, they perish in a further stage of development and do not form good pollen. This, doubtless, is due not only to chromosome disturbances in the course of meiosis, but also to some additional agent. It may be supposed that the sterility is of a genic nature. The influence of the additional agent becomes finally manifest after the development of the tetrads. Maybe, the same factor is also responsible for the degeneration and under-development of ovules in these sterile hybrids. Probably this genic incompatibility is the main cause of the existence of a complete sterility barrier between *T. pumilus* and the other species. In the *T. pumilus* $\times$ *T. yunnanensis* combination this factor is active only to a certain extent, thus the F_1 hybrids are partly fertile.

T. pumilus exhibits a considerably closer relationship with *T. yunnanensis* than with the other species. Not only are the chromosomes of these two species homologous to a high degree, but also their progeny is partly fertile.

DISCUSSION

Comparison of the morphology of hybrids and that of the parental plants indicates that almost invariably the F_1 individuals are similar to the parental forms and exhibit intermediate characteristic. Most of the latter belong to polygenic features depending on a number of quantitative factors. This concerns such morphological features as e.g. the height of the plants, dimensions of sepals, shape and colour of flowers, etc. These results are consistent with observations on *Trollius* hybrids. In the *T. europeus* $\times$ *T. chinensis* hybrid the F_1 generation exhibited an intermediate morphology, whereas in the F_2 generation segregation occurred, owing to which continuous variability sequences of individual features were obtained (Doroszewska 1965, Figs. 1—4 and 7). In other hybrids, such as for instance *T. altaicus* $\times$ *T. europaeus* and *T. chinensis* $\times$ *T. ranunculinus*, the results were similar (unpublished data of the author).

A high morphological variability is observed also in nature within some *Trollius* species. This continuous natural variability renders difficult the classification of this genus. Some of the taxons cannot be distinctly separated and are insufficiently differentiated; they are frequently interlinked by continuous sequences of varying features. This morphological variability is attributable to the polygenic character of these features. This indicates that the differentiation of a number of morphological features in the genus *Trollius* occurred gradually in the form of minute mutations. These small effects cumulated and formed new evolutionary lines. Similarly, the spurs of various length and shape in *Aquilegia* (Prażmo 1965) and flower tubes of various length in *Mirabilis* (Kruszewska 1961) developed under the influence of polygenes.

In the genus *Trollius* we deal mostly with features of a quantitative character, such as for instance the flower shape from flat in *T. pumilus* to spherical in *T. europaeus* or the length of nectaria in *T. asiaticus* and *T. chinensis* (Fig. 1), etc.

The colour of nectaria is one of the few dominating features hitherto observed

in the genus *Trollius*. In the F_1 hybrids the nectaria invariably exhibit the colour of nectaria of the parental plant exhibiting darker nectaria. The colour of nectaria is inherited independently of the colour of sepals.

The sepals are generally lighter than the nectaria. In all but one combinations the sepals of F_1 hybrids had an intermediate colour (Table 1). Only the *T. pumilus* $\times$ *T. ranunculinus* F_1 hybrids had both sepals and nectaria darker than those of parental plants. Further breeding, however, proved that the *T. ranunculinus* individual was not a homozygote and its progeny developed flowers of various colours.

The shape of the leaf blade with rounded, non-incised segments is another dominating feature. Such leaves are characteristic of *T. Ledebouri* and *T. yunnanensis*. The F_1 hybrids of these species and *T. pumilus* invariably had leaves with rounded, non-incised segments (Plate III).

The hybrids of species with incised blades such as *T. altaicus*, *T. asiaticus*, *T. chinensis*, *T. europaeus*, *T. dschungaricus* and *T. ranunculinus* invariably had intermediate leaves (Plate II).

Hybrid vigour was observed in all the sterile F_1 individuals, which reached the flowering stage. Generally this phenomenon was manifested by an increased size and number of individual organs. The F_1 hybrids of the *T. europaeus* $\times$ *T. chinensis* combination exhibited a similar exuberance (Doroszevska 1965).

In the *T. pumilus* $\times$ *T. yunnanensis* hybrids this exuberance was manifested only by a widening of the leaf-segments. In the F_1 generation they were so wide that they overlapped. Similar leaves, with wide, overlapping segments have been observed in *T. anemonifolius* Stapf. The latter taxon is closely similar to *T. yunnanensis* and occurs also in the mountains of the Yunnan Province in China. It is possible that *T. anemonifolius* developed by hybridization of *T. yunnanensis* with another species. Probably it was not *T. pumilus*, but rather another taxon more similar to *T. yunnanensis*. The flowers of *T. anemonifolius* Stapf are fertile and almost identical to these of *T. yunnanensis*, whereas the flowers of the *T. pumilus* $\times$ *T. yunnanensis* hybrids are rather similar to those of *T. pumilus* and sterile to a high degree.

The hybridization of *T. europaeus* $\times$ *T. chinensis* proved that the length of the beaks and shape of fruits belong to polygenic features (Doroszevska 1965, Fig. 7). In the *T. pumilus* hybrids the results were somewhat different. In all the sterile hybrids the follicles were underdeveloped, whereas their beaks were normal and long, only sometimes somewhat more curved. These sterile follicles invariably had beaks as long as those of the parental plant with longer beaks, or even longer (Table 4). Only in the partly fertile *T. pumilus* $\times$ *T. yunnanensis* hybrids the follicles of generation F_1 were normally developed with beaks of intermediate length, whereas in the F_2 generation this feature underwent segregation. It seems that the beaks of sterile hybrids develop otherwise than those of fertile plants. Their length cannot be the result of hybrid exuberance only and probably some additional factors were responsible for this.

All the species taken for experiments exhibited a capacity of hybridization with *T. pumilus*. Cross-pollination resulted in the production of considerable numbers of seeds, frequently higher than in self-pollination. The F_1 seeds obtained germi-

nated nonuniformly, but the differences in their germination power could not be taken into account, since the germination of seeds of pure species was also nonuniform (Table 5). Further breeding proved that the higher was the percentage of germinating F_1 seeds the more seedlings perished. Not only mortality of gametes but also that of zygotes occurs in the *Trollius* hybrids. Their follicles contained a considerable number of underdeveloped seeds, which died at an earlier or later development stage. This low viability of the hybrids should be attributed to the incompatibility of genes, which inhibited the normal development of the organism in F_1 individuals.

The *Trollius* species are as a rule allogamous plants and exhibit a number of adaptations to cross-pollination. In some plants even a partial self-sterility is observed. This may explain the larger number of seeds obtained from cross-pollination than from autogamy. On the other hand, most of these numerous seeds perished either at the seed stage or after germination. Only few F_1 individuals survived to the flowering stage. All but one F_1 hybrids proved sterile and gave no seeds even in back-crosses with pollen of one of their parental plants.

Observations on microsporogenesis indicate that the course of meiosis in *Trollius* hybrids is generally identical to that in the parental plants, i.e. in most cells it is regular. Disturbances of the same type occur both in parental plants and hybrids, only in the latter they are considerably more frequent. Similar disturbances were observed in the meiosis of F_1 individuals of the *T. europaeus* $\times$ *T. chinensis* combination (Doroszevska 1965). Intact chromatine bridges, lagging chromosomes and micronuclei also occurred in the PMC of this hybrid. On the other hand, neither triads, diads nor monads were found here. Maybe, polyploid microspores develop very rarely.

Among the 400 tetrads counted in the *T. pumilus* $\times$ *T. dschungaricus* F_1 hybrid 10.7 per cent proved to be triads, and 10.2 per cent of diads were found. Among the 800 tetrads counted in the *T. pumilus* $\times$ *T. europaeus* F_1 hybrid 1.5 per cent were found to be triads, 2.5 per cent diads and 0.7 per cent monads. Examination of the pollen morphology revealed some pollen grains twice as large as the normal ones. These grains, doubtless, developed by the disintegration of triads or diads. This indicates that the production of unreduced gametes is possible in the genus *Trollius*. On the other hand, the frequency of this phenomenon is very low and in practice probably it plays no role. Many hybrids of some *Trollius* species occur under natural conditions, but hitherto no natural polyploids were found in the genus *Trollius*.

Despite the chromosome disturbances occurring in the course of meiosis, the sterile F_1 hybrids produce a relatively high number of tetrads (44.5—91.2 per cent). On the other hand the amount of pollen produced by these hybrids is minimal. In individual hybrids the author found 0.0—10.5 per cent of normal microspores staining in carmine-glycerine. It is rather doubtful whether this pollen is effective in fertilization despite its normal appearance. Back-pollination of parental plants with this pollen gave no results.

It may be supposed that the sterility of pollen in these hybrids was in the first

place due to the action of genes and not only to the disturbances in chromosome conjugation in the course of meiosis. This genic incompatibility is probably responsible not only for the mortality of pollen, but also for the underdevelopment of ovules and follicles. It constitutes the main barrier isolating *T. pumilus* from the other species. The results of *T. europaeus* $\times$ *T. chinensis* hybridization proved that the fertility of pollen of the F_2 progeny depends on hereditary factors (Doroszevska 1965). This indicates that in *Trollius* species the fertility of pollen may be regulated by genes. This fact confirms the hypothesis that not only the disturbances in meiotic divisions reduce the pollen fertility, but also a genic sterility occurs in the *T. pumilus* hybrids.

Comparison of the course of meiosis in individual combinations indicates that in some hybrids it is more, and in others less, regular. On the other hand, this observation gives insufficient grounds for advancing conclusions as regards the degree of relationship between individual species and *T. pumilus*. Doubtless, however, the most regular meiosis observed in the *T. pumilus* $\times$ *T. yunnanensis* F_1 hybrids points to a higher homology of chromosomes of these species, i.e. also to their closer relationship. On the other hand, the most numerous disturbances were observed in the meiosis of the *T. pumilus* $\times$ *T. dschungaricus* hybrids, though it does not seem that these taxons are the most distant ones. These problems should be solved on the basis of results obtained from a more ample comparative material. Moreover a number of both morphological and other features as well as mutual relations between individual species should be considered in these studies.

A distinct sterility barrier developed between *T. pumilus* and the other species. Since the hybrids are completely sterile, a gene exchange between these taxa is impossible even under artificial breeding conditions. Only between *T. pumilus* and *T. yunnanensis* this barrier is not complete and these species can produce partly fertile hybrids. These species grow in the mountains of South China and their geographical ranges indicate that they belong to the oldest representatives of the genus *Trollius*. Also cytological observations point to their close relationship. Owing to this, the meiosis is relatively regular in the PMC of the F_1 generation and the morphology of their chromosomes is similar. The karyotypes of *T. pumilus* and *T. yunnanensis* are similar to each other and distinctly differ from those of the other species studied. The karyotypes of *T. pumilus* and *T. yunnanensis* are characterized by the occurrence of a large satellite at the third pair of chromosomes (Doroszevska 1967, Plate II).

The following consistent results should be stressed:

1. Comparison of the morphology of *T. pumilus* and *T. yunnanensis* indicates that they are well distinguishable separate taxa. Though a number of closely similar taxonomic units have been described within the range of *T. pumilus* and *T. yunnanensis*, the difference between these groups is distinct. In the case of other *Trollius* species these differences are not always as distinct, e.g. *T. Ledebouri* is linked with *T. asiaticus* by intermediate forms.

2. The geographical ranges of *T. pumilus* and *T. yunnanensis* point to the relict character of both these taxa.

3. The hybridizations performed indicate that *T. pumilus* is completely isolated from the other species, but it has preserved the capacity of producing a partly fertile progeny with *T. yunnanensis*.

Finally, the cytological data confirm the close relationship between *T. pumilus* and *T. yunnanensis*, since their chromosomes are homologous to a high degree. Despite the distinct morphological differences these species are closely related. They have also the same, flat type of flowers with few sepals. If we assume that *T. pumilus* and *T. yunnanensis* are older than the other species we may claim that their structure is more primeval. This allows to establish the lines of differentiation of some features leading to the development of younger phylogenetic taxonomic units. Thus the establishment of the direction of changes is particularly important in the study of the evolutionary development of the genus *Trollius*. This problem will be the subject of further publications of the author.

SUMMARY

1. The reciprocal crosses were performed between *T. pumilus* and the following taxa: *T. altaicus*, *T. asiaticus*, *T. dschungaricus*, *T. chinensis*, *T. europaeus*, *T. europaeus* var. *transsylvanicus*, *T. Ledebouri*, *T. rammunculinus* and *T. yunnanensis*. F_1 individuals were obtained from all these combinations. No difficulties occurred in the hybridization of these species.

2. All but one F_1 hybrids were completely sterile, their follicles were underdeveloped and only several per cent of their pollen was normally developed. Only the F_1 progeny of the *T. pumilus* $\times$ *T. yunnanensis* was partly fertile, but its fertility was very low.

3. All the F_1 hybrids exhibited an intermediate morphology as compared with that of the parental plants. Only the following two features were dominant: the darker colour of nectaria and the shape of blades with wide rounded leaf-segments characteristic of *T. Ledebouri* and *T. yunnanensis*.

4. Most of the morphological features in the genus *Trollius* depend on the cumulative action of a number of polygenes. Owing to this, both in artificial hybrids and under natural conditions continuous variability sequences of individual features may be observed. This continuous variability constitutes the main difficulty in the taxonomic classification of the genus *Trollius*.

5. In most PMC of the F_1 hybrids the course of meiosis is normal, i.e. all the chromosomes in these cells conjugate in metaphase I and form 8 bivalents. All the chromosomes of *T. pumilus* are capable of conjugating with the chromosomes of the species selected for hybridization. The following disturbances were observed in the meiotic divisions: chromatine bridges occurred in divisions I and II, univalents or bivalents remained in the cytoplasm and were not included into the telophasal nuclei, additional micronuclei were formed and triads, diads and even monads developed.

6. Chromosome disturbances mostly occurred in 20–30 per cent of PMC, and in the *T. pumilus* $\times$ *T. dschungaricus* hybrids were most frequent, occurring in 55 per cent of PMC. The amount of good pollen produced by the sterile hybrids did not exceed 10 per cent. This indicates that the sterility of pollen is due not only to chromosome disturbances, but also to the action of genes, which are responsible for the degeneration of pollen.

7. *T. pumilus* is separated from the other species by a complete sterility barrier; only between *T. pumilus* and *T. yunnanensis* it is not complete. The latter species produce a partly fertile progeny. The course of meiosis in the F_1 hybrids of *T. pumilus* $\times$ *T. yunnanensis* is relatively regular and the karyotypes of these two taxa are closely similar. Both these species occur in the mountains of South China. Their geographical ranges as well as cytological observations and experimental data point to a close relationship between these species.

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Mieszkańce T. pumilus Don z innymi gatunkami w obrębie rodzaju

Streszczenie

Trollius pumilus Don skrzyżowano z następującymi gatunkami: *T. altaicus* CAM, *T. asiaticus* L., *T. chinensis* Bge., *T. dschungaricus* Rgl., *T. europaeus* L., *T. europaeus* L. var. *transsilvanicus* (Schur) BL., *T. Ledebourii* Rchb., *T. ranunculinus* (Smith) Stearn i *T. yunnanensis* Ulbr. We wszystkich kombinacjach wykonano krzyżówki obustronne i otrzymano mieszańce F₁. Większość mieszańców pokolenia F₁ okazała się całkowicie bezpłodna, mieszki ich były niedorozwinięte, a ilość normalnie wykształconego pyłku wynosiła u tych mieszańców zaledwie parę procent.

We wszystkich krzyżówkach morfologia mieszańców była pośrednia w porównaniu z roślinami rodzicielskimi. Dotyczy to takich cech jak wysokość roślin, kształt kwiatów, wymiary listków miodnikowych, barwa okwiatu itp. Cechy te uwarunkowane są działaniem licznych poligenów. Jedynie dwie cechy wykazały dominowanie: ciemniejsze zabarwienie listków miodnikowych i kształt blaszki liściowej o szerokich zaokrąglonych odcinkach, nie pociętych na wąskie łaty charakterystycznych dla *T. yunnanensis*.

Wszystkie rośliny wzięte do doświadczeń były diploidami o $2n = 16$. W mejozie mieszańców wystąpiły liczne zaburzenia, ale większość KMP przechodziła regularnie podziały, w MI. przeważały komórki z 8 bivalentami. Wynika z tego, że wszystkie chromosomy *T. pumilus* są zdolne do koniugacji z chromosomami pozostałych gatunków, a więc są one częściowo homologiczne. Sterylność pyłku mieszańców F₁ spowodowana jest nie tylko zaburzeniami w mejozie, ale także działaniem genów, które wywołują degenerację pyłku po uformowaniu tetrad.

T. pumilus jest oddzielony od pozostałych gatunków całkowitą barierą sterylności. Jedynie między *T. yunnanensis* a *T. pumilus* bieriera ta nie wytworzyła się całkowicie, ich F₁ jest częściowo płodne. Z obserwacji cytologicznych wynika, że chromosomy *T. pumilus* i *T. yunnanensis* są w dużej mierze homologiczne, także kariotypy tych dwu gatunków są do siebie podobne. *T. pumilus* i *T. yunnanensis* wykazują bliskie pokrewieństwo i należą do grupy najstarszych żyjących obecnie przedstawicieli *Trollius*.