Observations on the *Trollius chinensis × T. europaeus* hybrids

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**INTRODUCTION**

As earlier studies have shown, the genus *Trollius* has a diploid number of chromosomes equal to $2n=16$ (Langlet 1927, 1932; Sokolovskaia 1958; Skalińska, Czapik, Piotrowicz et al. 1959; Zhukova 1961). The tetraploid number of chromosomes $2n=32$ given by Langlet (1932) for *T. americanus* has not been confirmed so far. The tetraploid *T. europaeus* described by the author arose artificially, under the influence of foreign pollen placed on the stigma of a diploid *T. europaeus* plant (Doroszewska 1964). All the plants taken for the hybridizations in this work were diploid.

The greatest difficulty encountered in the work on *Trollius* hybrids was the slow development of the plants, which normally flower only in the second year after planting out the seedlings in the ground, and also their low fertility. Seed of pure species normally germinate in the second year after sowing. The hybrids behave similarly, which considerably extends the duration of experiments. Flowering time is not simultaneous in all the species, which hinders the performance of all the necessary crosses.

**MATERIAL AND METHODS**

The observations on *Trollius chinensis × T. europaeus* hybrids were conducted in the years 1958—1964. The following crosses were made:

1 — *chinensis* no. 25 × *europaeus* no. 1, made in 1958, 10 $F_1$ plants obtained
2 — *chinensis* no. 25 × *europaeus* no. 2, " " 1960, 8 $F_1$ " "
3 — *chinensis* no. 89 × *europaeus* no. 2, " " 1960, 12 $F_1$ " "
4 — *chinensis* no. 66 × *europaeus* no. 2, " " 1959, 5 $F_1$ " "

The plant *T. europaeus* no. 1 came from the Warsaw Botanic Garden and died shortly after the cross has been made. The second was brought from the Białowieża Forest. All the specimens of *Trollius chinensis*
were taken from botanical gardens, since no other material was available. No. 25 came from the Warsaw Botanic Garden and nos. 66 and 89 were grown from seed obtained from the Botanical Garden in Uppsala.

*T. chinensis* is often cultivated as an ornamental plant and it differs from the individuals coming from natural conditions in that it has a larger number of sepals in the perianth. All the *T. chinensis* individuals used in the crosses had more numerous perianth segments than is normal for the species as given in the Flora of the USSR (1937). In other respects the plants agreed with the descriptions of this species.

All the crosses were made in one direction, pollinating *T. chinensis* with *T. europaeus* pollen. All the *T. chinensis* individuals flower much later than *T. europaeus*. In spite of attempts made over several years, even trying to hasten the plants in the greenhouse, not a single successful reciprocal cross was made.

In the cross no. 1 the progeny was not uniform. In the remaining three crosses the F₁ hybrids were uniform and similar to each other. The F₁ hybrids of the 4th cross were most fertile and thus it was possible to obtain from them the F₂ generation. However the fertility of the F₁ hybrids in the 4th cross was so low that in order to obtain 200 individuals in the F₂ generation it was necessary to collect seed over three years (1961, 62 and 63). In the remaining crosses no F₂ progeny was obtained and therefore the observations are restricted to the F₁ hybrids.

All the plants were grown in experimental plots in the Warsaw Botanic Garden. Measurements of height, plant fertility, flowering etc. were made every year. Meiosis was studied on squash preparations. The material was fixed in acetic-alcohol and stained with propricarmine. Preparations of mitotic chromosomes were made according to the Dyer (1963) method, somewhat modified and adjusted to material.

**MORPHOLOGICAL OBSERVATIONS**

The general habitus of *T. chinensis* and *T. europaeus* plants is very similar. Basal leaves on their petioles form a loose clump from which the flowering shoots sprout in the spring. Basal and cauline leaves of both these species are very similar. *T. chinensis* individuals are usually somewhat taller than *T. europaeus*, but these differences are relatively small, and therefore this character was not included in the observations. A definite increase in height was evident in the F₁ progeny of all the crosses performed. During fruiting the *T. europaeus* plants were about 60—75 cm tall, *T. chinensis* 60—80 cm and occasionally 90 cm, and the F₁ hybrids were 80—100 cm or even up to 120 cm.
The significant differences between the species *T. chinensis* and *T. europaeus* concern the morphology of flowers and fruit and the time of flowering. These characters will be discussed below.

1. Shape of the flowers

In the genus *Trollius*, the perianth is differentiated into the external perianth segments and the internal nectaries. The external perianth segments are broadly ovate, can be completely flat or to a varying degree incurved. The shape of the flower depends on the degree of that curvature. The nectaries are narrow, elongate, with a nectar secreting cavity at the base. Various species of *Trollius* differ from one another in the length and shape of the nectaries and the shape and pigmentation of the flowers.

The flowers of *T. chinensis* are almost flat, with only a few external perianth segments slightly upturned. The long nectaries form a tall erect whorl (Plate 1a). In contrast to *T. chinensis* the flowers of *T. europaeus* are globose with small nectaries (Plate 1b). This globose shape results from the external perianth segments being incurved, deeply concave and imbricate.

The flower can be completely closed. Inside the globe so formed there are hidden the styles, stamens and nectaries.

In order to describe the shape of the flowers a scale was adopted, with six grades, according to which the parents and the progenies were classified (Fig. 1). In this diagram the flowers of *T. chinensis* correspond to Fig. 1b and flowers of *T. europaeus* to Fig. 1f. In the F₁ progeny of the crosses performed all the flowers were semiglobose (Plate 1c, Fig. 1d). In the F₂ progeny of the 4th cross all the types of flower

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**Fig. 1.** A diagram representing the shape of *Trollius* flowers on a longitudinal section, demonstrating the arrangement of external perianth segments.

*a* — a flat flower, some of the segments may be curved downwards; *b* — a slightly concave flower, some of the segments are slightly curved upwards as in *T. chinensis*; *c* — a concave flower with segments more strongly curved upwards than in *T. chinensis*; *d* — a semiglobose flower as in F₁ hybrids; *e* — an almost globose flower, but widely open on the top; *f* — a globose flower, closed or almost closed as in *T. europaeus*. 
The flowers of *Trollius chinensis*, *T. europaeus* and their hybrids

a — *Trollius chinensis*; b — *Trollius europaeus*; c — F₁ hybrids; d—l — F₂ hybrids.
were found as shown in Fig. 1, except the last, globose form typical for *T. europaeus*. In the photograph (Plate I d—j) flowers of different *F₂* individuals are arranged according to size. As is evident from the numerical data, most abundantly represented in the *F₂* progeny are the individuals with flowers of the semiglobose type, characteristic for the *F₁* generation (Table 1d; Plate I, g, i, l), and individuals with concave flowers, intermediate in shape between the *F₁* and *T. chinensis* (Table 1c; Plate I f, k). Slightly concave flowers of the *chinensis* type were found in only two plants (Table 1b; Plate I j) and none of the plants had globose flowers as in *T. europaeus*. From the data presented in Table 1 it can be seen that most of the plants in the *F₂* progeny had flowers as the *F₁* generation or similar to *T. chinensis*.

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F₂</em> hybrids</td>
<td>157</td>
<td>2</td>
<td>2</td>
<td>73</td>
<td>74</td>
<td>6</td>
<td>—</td>
</tr>
<tr>
<td><em>F₁ × europaeus</em></td>
<td>11</td>
<td></td>
<td>—</td>
<td>2</td>
<td>7</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td><em>F₁ × chinensis</em></td>
<td>24</td>
<td>3</td>
<td>16</td>
<td>4</td>
<td>1</td>
<td></td>
<td>—</td>
</tr>
</tbody>
</table>

In the back-crosses, in spite of the small number of individuals obtained it can readily be seen that the differences in flower shape are dependent on the parent that was used to pollinate *F₁* hybrids.

Variation of the flower shape in the *F₂* is continuous. This has hindered the classification of the plants into different groups. There is no doubt that this character is dependent on the action of multiple factors.

2. A comparison of the perianth segment morphology

External perianth segments in *T. chinensis* and *T. europaeus* differ from one another in colour. Their number, size and shape are similar in both species. They are broadly ovate, spirally arranged, the wider ones towards the outside, narrowing towards the centre of the spiral. A photograph of these perianth segments has been published in the previous paper (Doroszewsk a 1964, Figs. 2, 3). The size and shape of the external perianth segments is generally the same as in *T. europaeus* and *T. chinensis*. The mean length of these segments in the parental forms and in the *F₁* hybrids from all the 4 crosses is about 24 mm.

In the *F₂* generation the size of flowers in different individuals is very variable (Plate I d—j), and thus the dimensions of the external
perianth segments also vary. The range of variation is very much greater than in the $F_1$ hybrids, and the mean is somewhat shifted towards the smaller dimensions. A similar situation is to be found in the back cross to $T. \text{chinensis}$ (Fig. 2).

The number of external perianth segments in the parental plants and in their progeny is on the average between 10 and 12 in one flower.

![Graph showing length of perianth segments](image)

Fig. 2. The length of the external perianth segments.

The nectaries of $T. \text{chinensis}$ and $T. \text{europaeus}$ differ from one another in size, shape and colour. In $T. \text{chinensis}$ they are linearly elongated, somewhat pointed at the tip (Fig. 4a). Nectaries of $T. \text{europaeus}$ are very much shorter and more rounded at the tip (Fig. 4b). In the $F_1$ generation the shape and length of nectaries are markedly intermediate (Fig. 4c). In the $F_2$ generation there is a great variability. Individuals have been found with nectaries shorter than in $T. \text{europaeus}$, however the longest nectaries found did not reach the value of the arithmetical mean for the nectaries of $T. \text{chinensis}$ (Fig. 3).

In the back-crosses the nectaries of the progeny from $F_1 \times T. \text{europaeus}$ are generally shorter than the progeny from $F_1 \times T. \text{chinensis}$, which was expected. However, even though the $F_1 \times T. \text{europaeus}$ pro-
The progeny has nectaries with dimensions intermediate between the F₁ and *T. europaeus*, the progeny from F₁ × *T. chinensis* does not have nectaries intermediate in size between the F₁ and *T. chinensis*. It resembles more an extended range of the F₁ and is intermediate between the original parental forms. Thus the dimensions of the nectaries are shifted in the F₁ × *T. chinensis* back-cross towards the smaller values, just as the F₂ generation was (Fig. 3).

The number of nectaries in *T. chinensis* is about 18 (11–30) per flower and about 10 (7–18) in *T. europaeus*. In the F₁ the number of nectaries in the flowers is similar to that in *T. chinensis*. In the F₂ generation there are considerable differences between individuals in the number of nectaries, ranging from 6 to 27 per flower.

The continuous variation in the dimensions of external perianth segments and in the nectaries indicates that we are dealing with the inheritance of quantitative traits. The number of individuals in the F₂ progeny (186) and in the back-crosses (29, 13), does not permit an estimation of the number of factors operating here.
3. Colour of the flowers

In the genus *Trollius* the flowers are generally yellow or orange. The nectaries are usually darker than the external perianth segments. In order to estimate the colours the Horticultural Colour Chart (1941) was used. The external perianth segments of *T. europaeus* are yellow in colour, Aureolin 3, while the nectaries are Saffron yellow 7. Flowers of *T. chinensis* are much darker. The external perianth segments were described as Cadmium orange 8 or Tangerin orange 9 and the nectaries as Orpiment orange 10. The F₁ hybrids have Lemon yellow 4 external perianth segments, and their nectaries are Tangerin orange 9 or Orpiment orange 10/1. In the F₂ generation only two types of nectaries appeared, Cadmium orange 8 and Orpiment orange 10. The external perianth segments were classified into five different colours. The results in the F₂ were as follows:

Plants with Orpiment orange 10 nectaries had external perianth segments of the following colours:

<table>
<thead>
<tr>
<th>Colour</th>
<th>Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemon yellow 4</td>
<td>38</td>
</tr>
<tr>
<td>Buttercup yellow 5</td>
<td>1</td>
</tr>
<tr>
<td>Indian yellow 6</td>
<td>36</td>
</tr>
<tr>
<td>Tangerin orange 9</td>
<td>2</td>
</tr>
</tbody>
</table>

\[ n = 77 \]

Plants with Cadmium Orange 8 nectaries had external perianth segments of the following colours:

<table>
<thead>
<tr>
<th>Colour</th>
<th>Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aureolin 3</td>
<td>102</td>
</tr>
<tr>
<td>Lemon yellow 4</td>
<td>1</td>
</tr>
<tr>
<td>Indian yellow 6</td>
<td>11</td>
</tr>
</tbody>
</table>

\[ n = 114 \]

From the data obtained in the F₂ it appears that the pigmentation of nectaries and external perianth segments is inherited independently. In the F₁ the nectaries are coloured as the *T. chinensis* parent or slightly more bright. In the F₂ out of 191 plants 77 had nectaries like *T. chinensis* and 144 had an intermediate colour between *T. chinensis* and *T. europaeus* and brighter than the F₁. This ratio is 2:3.

The pigmentation of the external perianth segments alone segregated in the F₂ in the following manner:

<table>
<thead>
<tr>
<th>Colour</th>
<th>Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aureolin 3</td>
<td>102</td>
</tr>
<tr>
<td>Lemon yellow 4</td>
<td>39</td>
</tr>
<tr>
<td>Buttercup yellow 5</td>
<td>1</td>
</tr>
<tr>
<td>Indian yellow 6</td>
<td>47</td>
</tr>
<tr>
<td>Tangerin orange 9</td>
<td>2</td>
</tr>
</tbody>
</table>

\[ n = 191 \]

All the F₁ hybrids had the external perianth segments not much darker than *T. europaeus* (1 degree on the colour scale). In the F₂ gene-
ration about half of the progeny have segments coloured as *T. europaeus*, about 1/4 as the F₁, the remainder is intermediate between F₁ and *T. chinensis*, and only 2 plants have a pigmentation as *T. chinensis*. The apparent numerical ratio of 2:1:1 is suggestive of a simple inheritance, however the colour of the perianth in *Trollius* is inherited in a much more complicated fashion. The intensity of the colour is dependent on the action of multiple cumulative factors. Often the qualification of colours has presented difficulties. The results obtained indicate that the pigmentation of the flowers in the F₂ hybrids is dependent on the segregation of factors conditioning the intensity of colour as well as on the action of modifier and inhibitor genes. The results obtained are insufficient to permit a clarification of this complicated mechanism. It will be necessary to return to this problem when more material is obtained for comparison from other crosses.

4. Anthesis

In the Polish lowland, the time of flowering of *T. europaeus* is in May. *T. chinensis* will flower in June and in the beginning of July, however single individuals can come to bloom towards the end of May. The F₁ hybrids have flowered in the latter part of May and in the first half of June. The F₂ hybrids flowered in the latter part of May, in June and in the first part of July. None of these plants flowered as early as *T. europaeus*.

5. Follicles

In the genus *Trollius* fruits are follicles bunched together into heads. Both in *T. europaeus* and in *T. chinensis* there can be many follicles per head. There can be over 50 or even over 60 follicles per head. As an average, there are about 30 follicles per one head in the parental forms as well as in their progeny.

The follicles of *T. chinensis* are greater than those of *T. europaeus* and they have long beaks at their tips (Fig. 5a). The follicles of *T. europaeus* are small with much shorter beaks (Fig. 5b). In the F₁ progeny the dimensions of the follicles are intermediate between the parental forms (Figs. 5c; 6). In the F₂ the length of the follicle is very variable. Individuals have been found with follicles much shorter than in *T. europaeus*, but none had follicles as long as *T. chinensis* (Fig. 6).

The beaks on the follicles of *T. chinensis* and *T. europaeus* differ substantially in length (Fig. 5). In *T. chinensis* the beaks vary in length from 1.2 to 3.8 mm and in *T. europaeus* from 0.1 to 1.3 mm. In the F₁ and F₂ hybrids the beaks are intermediate in length between the two parental forms (Fig. 7).

The variation in the dimensions of follicles and beaks is continuous both in the parental forms and in the hybrids.
Fig. 5. The follicles.

a - T. chinensis; b - T. europaeus;
c - F₁ hybrids.

Fig. 6. The length of the follicles in mm.

Fig. 7. The length of the beaks at the follicles in mm.

Fig. 8. Pollen fertility of the F₂ generation (n=180).
THE FERTILITY

Seed set

In many species of *Trollius* it is possible to observe great differences in the number of fully formed seeds produced in different years. Local conditions have a great influence. *T. europaeus* is particularly sensitive, and in some years it almost gives no seeds at all, even after self-pollination.

| Table 2 |
| Seed set |
|---------------------------------|-----------------|-----------------|-----------------|
| | Number of seeds | Number of follicles | Average number of seeds per one follicle |
| After cross-pollination | | | |
| cross 2 (*chin. 25 × europ. 2*) | 23 | 13 | 1.8 |
| cross 3 (*chin. 89 × europ. 2*) | 65 | 28 | 2.1 |
| *T. chinensis × europaeus* | cross 4 (*chin. 66 × europ. 2*) | 431 | 83 | 5.4 |
| total | 539 | 124 | 4.3 |
| After self-pollination | | | |
| *F₁* hybrids of first cross | 88 | 283 | 0.3 |
| *F₁* hybrids of second cross | 64 | 118 | 0.6 |
| *F₁* hybrids of third cross | 223 | 298 | 0.7 |
| *F₁* hybrids of fourth cross | 642 | 660 | 0.8 |

In the follicles of *T. chinensis* there are 9—12 ovules out of which about 50% develop into seeds. The number of seed produced in this species under conditions of free pollination is on the average about 4.7 per follicle. When self-pollinated under controlled conditions the number is reduced to 3.3 seeds per follicle. In *T. europaeus* the follicles are shorter and contain about 5—7 ovules. Under conditions of free pollination there are about 2.5 well formed seeds per follicle, and under self-pollination 1.1.

Since the counting of ovules in the follicles is very laborious, the seed set was estimated as number well formed seeds per follicle rather than per number of ovules. When pollinating the flowers of *T. chinensis* with *T. europaeus* pollen seeds were readily obtained. There were on the average 4.3 seeds per follicle (Table 2). This is more than was obtained as a result of self-pollination of *T. chinensis*, where only 3.3 seeds per follicle were obtained. From the obtained seeds about 11% germinated within two years. The *F₁* hybrids were subjected to self-pollination and seeds were collected (Table 2). About 1/3 of the follicles in the *F₁* hybrids can be underdeveloped which markedly lowers their seed set.
As can be seen from the figures given in Table 2 the F₁ hybrids when self-pollinated gave small numbers of seeds. Out of the 642 seeds collected from the F₁ individuals only 210 germinated. A part of the plants died during cultivation, thus for the purpose of measurements and calculations only about 200 plants of the F₂ generation remained.

The fertility of the F₂ hybrids was estimated in conditions of free pollination, taking one floral head per plant. The following results were obtained:

\[
\begin{array}{c|c|c}
\hline
\text{seeds} & \text{plants} \\
0 & 4 \\
0.1—1.0 & 29 \\
1.1—2.0 & 77 \\
2.1—3.0 & 32 \\
3.1—4.0 & 6 \\
4.1—5.0 & 1 \\
5.1—6.0 & 1 \\
\hline
\text{average value per follicle} & \text{\textit{n} = 150} \\
\hline
\end{array}
\]

Among the F₂ hybrids there were most plants producing 1.1—2.0 seeds per follicle, in fact about half of the F₂ plants did so. This seed set is comparable to the 1.8 seeds per follicle observed in the selfed F₁ plants.

Seeds of various Trollius species generally germinate very weakly. The seeds of \textit{T. chinensis} germinate relatively readily, and this even in the first year after sowing, however the seeds of \textit{T. europaeus} germinate with great difficulty. For example out of the 268 seeds obtained from self-pollination of \textit{T. europaeus}, scarcely 10% germinated. Over the same period of time 50% of \textit{T. chinensis} seeds germinate. Since the seeds of the parental forms have a weak and uneven germinability, this factor hinders the scoring of the hybrid progeny with respect to seed germination.

Pollen fertility

Pollen fertility was estimated from preparations stained in 1:1 aceto-carmine with glycerine. 200 pollen grains per slide were counted. In \textit{T. europaeus} pollen fertility averaged 95.7% for plant no. 1 and 95.5% for plant no. 2. The parental \textit{T. chinensis} plants had the following mean fertility: no. 25 — 87.7%, no. 66 — 94.0%, no. 89 — 92.0%. In the F₁ hybrids various mean values of pollen fertility were found for the different crosses: cross number 1 — 70%, no. 2 — 39.3%, no. 3 — 39.2% and no. 4 — 76.0%.

The pollen fertility of the F₂ hybrids is presented on the graph in Fig. 8. In this generation the majority of the individuals had a relatively
high pollen fertility, ranging from 80—90%. Generally speaking the fertility of the F2 generation is greater than that of the F1, and in many individuals it equals the values for parental forms.

MICROSPOROGENESIS

The meiotic division in the parental plants *T. chinensis* and *T. europaeus* is perfectly normal and similar in both species. In metaphase I all the chromosomes conjugate giving 8 bivalents per cell. When univalents were found there were two per cell, thus only one pair of chromosomes did not conjugate. Such cells with univalents were found at the rate of 1—3 per 100 PMC observed (Table 3). With the same sort of frequency univalents were found in the I division, remaining in the cytoplasmic zone and not included into the telophase nuclei.

<table>
<thead>
<tr>
<th>T. europaeus no. 2</th>
<th>n</th>
<th>8n</th>
<th>7n+2l</th>
<th>6n+4l</th>
<th>6n+l4v</th>
<th>5n+l4l</th>
<th>n</th>
<th>tetrads</th>
<th>regular</th>
<th>pentads</th>
<th>hexads</th>
<th>difference in size of 4 nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>98</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>200</td>
<td>198</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>T. chinensis no. 25</td>
<td>100</td>
<td>97</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>200</td>
<td>197</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. chinensis no. 66</td>
<td>100</td>
<td>99</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>200</td>
<td>199</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 hybrids cross 3</td>
<td>100</td>
<td>74</td>
<td>16</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>200</td>
<td>164</td>
<td>26</td>
<td>2</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>F1 hybrids cross 4</td>
<td>100</td>
<td>86</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td></td>
<td>200</td>
<td>160</td>
<td>24</td>
<td>4</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

In the F1 hybrids the conjugation of chromosomes was relatively regular. In metaphase I most of the cells had 8 bivalents. This implies that all the chromosomes of *T. chinensis* and *T. europaeus* are able of conjugating with each other (Plate IIa). At the same time in these hybrids aberrations occurred during meiosis. In metaphase I about 25% of the cells had univalents. In one cell there could be as many as 2, 4 or 6 univalents. One tetravalent was also found (Table 3). In anaphase I of the F1 hybrids chromatin bridges often occurred with chromosome fragments in the cytoplasm. Out of the 200 cells investigated 159
Microsporogenesis in the F1 hybrids *Trollius chinensis* × *T. europaeus*

a — 8 bivalents in metaphase I; b — anaphase I with the bridge and fragment; c — telophase I with the small nucleus and bridge ↓; d, e — metaphase II with bridges and chromosome fragments; f — three groups of chromosomes in metaphase II; g, h — telophase II with the bridges between nuclei; i — pentad.
had a regular anaphase, in 36 there were bridges with or without fragments, and in 6 cells there were no bridges but there remained on the equatorial plate of the spindle some lagging chromosomes or their fragments. Such lagging chromosomes can from small nuclei already in telophase I (Plate IIc). Chromatin bridges found in F₁ hybrids are typical examples of heterozygosity for paracentric inversions in these plants. Chromatin bridges also occurred in telophase I, in interkinesis and in the prophase and metaphase of the second division (Plate IIc, d, e). In anaphase II, out of 200 investigated cells 164 were regular, in 20 chromatin bridges were found with fragments, in 14 bridges without fragments, and in 2 cells there were no bridges but there were lagging chromosomes in the cytoplasm. In telophase II numerous bridges and fragments were also found. (Plate IIg, h). A part of the bridges found in the second division were formed during the second anaphase, however a part originated in anaphase I, failed to break, and were preserved until the second division.

The tetrads of the F₁ hybrids also demonstrated some deviations from normality. Out of 200 investigated tetrads about 160 were regularly formed, while the remainder had additional pollen grains forming pentads or hexads (Plate III, Table 3).

DISCUSSION

The two diploid Trollius species taken for the crosses have morphological characters distinctly differentiating them and have geographic ranges that do not meet. Trollius chinensis occurs in the Far East and T. europaeus grows in Europe and extends as far east as the western extremities of Western Siberia (Krylov 1930—31). Both these species will readily cross, and F₁ hybrids are easily produced. All the crosses were made in only one direction, however it can be assumed that the reciprocal crosses T. europaeus × chinensis would also give satisfactory results. When pollinating T. chinensis with T. europaeus pollen on the average 4.3 seeds were obtained per pollicle. This is more than when self-pollinating T. chinensis (3.3 seeds per follicle).

Trollius is to a large extent a cross-pollinating plant. It pistils develop earlier than the stamens, thus the flowers are protogynous. Nectaries, located on the inner perianth segments, and the bright yellow colours, attract insects. Trollius flowers are visited by bumble bees, honey bees, flies and small beetles (Hegi 1912). In spite of these adaptations to cross pollination, selfed progeny can be obtained, by pollinating with the plant’s own pollen.

In all the crosses performed by the author between different Trollius species it was possible to obtain F₁ hybrids. Natural hybrids between some species can be found in the regions where the species occur together
(Flora transbaicalica 1941, Flora of the USSR 1937). It is possible that all species of *Trollius* can cross with each other.

In the cross *T. chinensis* × *T. europaeus* F₁ hybrids were partially fertile, but their fertility was considerably lowered in comparison with the parental plants. In the F₁ hybrids about 1/3 of the follicles were undeveloped, and in the normal follicles there were many malformed seeds. On the average 0.3 to 0.8 good seeds were obtained per follicle from self-pollination. Under these conditions *T. chinensis* gave on the average 3.3 and *T. europaeus* 1.1 seeds per follicle.

The fertility of the F₂ hybrids was estimated under conditions of free pollination. Completely sterile individuals were found, as well as individuals giving various numbers of seeds per follicle, including some that equalled open pollinated *T. chinensis* in their fertility. From the numerical data it appears that in the F₂ the ability to form seeds is dependent to a large extent on hereditary factors.

In two of the *T. chinensis* × *europaeus* crosses pollen fertility in the F₁ was about 70% whereas in the remaining two crosses it was about 39%. One of the reasons for the formation of abortive pollen in these hybrids could be found in the aberrations occurring in the meiotic divisions. The numerous bridges and fragments found in the anaphase indicate that these plants are heterozygous for paracentric inversions. The occurrence of such inversions causes the formation of chromatids with duplications and deficiencies during meiosis. Microspores, which receive chromosomes with duplications or deficiencies are usually inviable. In the studies on paracentric inversions in the maize *Morgan* (1950) and *Rhoades* and *Dempsey* (1953) after an accurate analysis of chromatid bridges have estimated the number of abortive pollen grains resulting from these aberrations. In maize the bridges formed during the first division get broken after anaphase I, but the bridges found in anaphase II originate only during the second division. This is dependent on the position where crossing over took place during meiosis (Burnham 1963; McClintock’s scheme, page 38). In F₁ *Trollius* hybrids part of the chromatid bridges formed during the first division do not break but persist into the second division. Analysing the bridges in anaphase II it was often difficult to decide whether they were formed during the second division or whether they are left-overs from the first division. Thus it is not possible to make such calculations in *Trollius* hybrids as were made for maize by *Morgan* (1950) and *Rhoades* and *Dempsey* (1953). A similar preservation of chromatin bridges formed in anaphase I into the second meiotic division has been observed by *Gajewski* (1948) in *Paeonia* and *Zuk* (1963) in *Rumex* hybrids. Also, in zoological material a similar phenomenon was observed by *Carson* (1946) in the eggs of *Sciara impatiens*. 
It can be concluded that the lowering of pollen fertility in *Trollius* hybrids is caused by the occurrence of chromatin bridges in meiosis, however it may also be a result of the influence of other hereditary factors, which is indicated by the segregation of pollen fertility in the F₂ generation.

As is seen from the observations performed, most of the morphological characters studied differ quantitatively, and are controlled by the action of many genetic factors. The F₁ generation is always intermediate with respect to the parents, however in the F₂ generation transgression occurs sometimes and it is possible to observe a completely continuous range of variation. The range of variability of individual characters within the F₂ generation can be shifted more towards the values for *T. chinensis* or for *T. europaeus*. This can be the result of the fact that genes of one species can modify or repress the action of genes from the other species. Consequently the expression of a given character is a result of segregation among multiple factors and the influence upon them of modifier and inhibitor genes. A similar phenomenon was observed by Clausen and Hisey (1958) in Potentilla.

In *Trollius* hybrids it is an interesting fact that for some characters genes of *T. chinensis* were repressed and for others genes of *T. europaeus*. Thus in the F₂ generation 74 individuals had semiglobose flowers, as the F₁, 73 plants had concave flowers, intermediate between F₁ and *T. chinensis*, 2 individuals had slightly concave flowers as in *T. chinensis*, only 6 plants had almost globose flowers, and globose flowers as in *T. europaeus* did not occur at all (Table 1). The pigmentation of nectaries in the F₂ segregated in such a fashion that 2/5 of the plants had nectaries coloured as *T. chinensis*, while the remainder had nectaries of a colour intermediate between F₁ and *T. europaeus*. In both the examples given above traits of *T. chinensis* are expressed much more strongly, and the action of *T. europaeus* genes is, as if, inhibited. No plants were found which would have a globose flower shape or a pigmentation of the nectaries as in *T. europaeus*.

The situation is reversed in the inheritance of such characters as pigmentation and dimensions of external perianth segments, as well as the size of the follicles. In the F₂ generation out of 102 plants, about 1/2 had external perianth segments coloured as *T. europaeus*, 39 coloured as the F₁, 48 individuals with a colour intermediate between F₁ and *T. chinensis* and 2 plants with the same colour as *T. chinensis*. Thus in the F₂ the colour of *T. chinensis* was expressed to a very much lesser extent.

From the measurements of the external perianth segments in the F₂ it can be seen that the transgression was in the direction of smaller dimensions, very substantially exceeding the values for *T. europaeus* (Fig. 2). In the opposite direction the transgression is very much less.
The length of the nectaries (Fig. 3 and 4) and of the follicles (Fig. 6) gives a similar picture. The range of variation within the F₂ is shifted towards small dimensions, more akin to T. europaeus. Similar results were obtained by Kruszewska (1961) on Mirabilis. In the F₂ generation of the hybrids Mirabilis jalapa × M. logiflora all the plants had floral tubes and styles much shorter than M. longiflora. The author has explained this result by the action of inhibitor genes.

SUMMARY

1. F₁ hybrids obtained from the cross T. chinensis × T. europaeus were partially fertile and morphologically intermediate between the parental forms. The F₂ generation had a much higher fertility then the F₁. In F₂ there is a segregation of factors determining seed set.

2. Morphological characters differentiating these two species: colour and shape of flowers, dimensions of perianth segments, follicles and beaks are all controlled by cumulative multiple-factors, and demonstrate in the F₂ a continuous variation.

3. In the PMC of F₁ hybrids a very regular chromosome pairing was observed, most of the cells contained 8 bivalents. This indicates that chromosomes of T. chinensis are to some extent homologous with the chromosomes of T. europaeus.

4. In the meiotic divisions of F₁ hybrids numerous chromatin bridges were observed in the anaphase. They could be one of the reasons for the lowering of pollen fertility in the F₁ hybrids.

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REFERENCES

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W podziałach meiotycznych KMP mieszańców F₁ stwierdzono regularną koniugację chromosomów. Większość komórek w metafazie 1 zawierała po 8 biwalentów. Wynika z tego, że chromosomy obu tych gatunków są częściowo homologiczne. Liczne mostki chromatynowe występujące w anafazie mieszańców F₁ są jedną z przyczyn obniżenia płodności pyłku tych roślin.