An investigation on polyploidy and sex determination within the Genus *Rumex*

by

J. ŻUK

I. INTRODUCTION

The aim of this work has been to investigate how polyploidy arises and what is the mechanism of sex determination in dioecious hybrids between *Rumex thyrsiflorus* and *Rumex arifolius*. According to Löve (1957a) both these species belong to the subsection *Acetosa*, section *Acetosa*, subgenus *Acetosa*, genus *Rumex*. They are closely related with each other, so much so that A. Löve (1944) once classified them as subspecies of the collective, Linnaean species *Rumex acetosa* L. because *Rumex thyrsiflorus*, *Rumex arifolius*, and *Rumex acetosa* can inter-cross without any difficulty giving fertile progenies. Z. Świetlińska's (1960 and unpublished) investigations indicate that *R. acetosa* and *R. arifolius* are in fact the lowland and the mountain varieties of the same species, whereas *R. thyrsiflorus* appreciably differs in its morphology and ecology as well as to some extent in its genetic constitution.

Löve (1942) informs that the first to study cytologically the section *Acetosa* in the genus *Rumex* were the embryologists Fink (1899), Roth (1906), and Dudgeon (1918), but the chromosome number that they had reported was not confirmed in later investigations. Roth (1906) stated that the chromosome number of *R. acetosa* was $2n=16$, whereas Kihara and Ono (1923, 1925), Sinoto (1924, 1929), Kihara and Ono (1926), Meurman (1927), Ono (1928, 1930, 1935), Kihara and Yamamoto (1931), Yamamoto (1938), and Löve (1944) all agree that in all the investigated species from the subsection *Acetosa* the karyotype is $12a + XX$ in the female plants and $12a + X + YY$ in the male plants. According to Löve (1957) cytological investigations have covered about 20 species and subspecies of the subsection *Acetosa* and about a quarter of all the species in the genus *Rumex*.

The workers listed above also observed the course of meiosis in some

of the species of the subsection *Acetosa*: in diakinesis and in metaphase I of male individuals the autosomes formed 6 bivalents and the sex chromosomes formed one trivalent XXY. In anaphase I the X-chromosomes passed to one of the poles and the Y + Y chromosomes to the other, the result being that half of the produced gametes were $6a + X$ and half $6a + 2Y$.

Under natural conditions single tri- and tetraploid individuals occasionally appear in the *R. acetosa* populations; this was observed by many, mainly Japanese authors (Ono 1928, 1930, 1935; Ono & Shimotomai 1928; Kihara and Yamamoto 1931; Yamamoto 1938; Löve 1942; and Takenaka 1955). Nobody, however, explained what could be the mechanism of the formation of diploid gametes. Only Ono (1928) having found diploid plates in MII of *R. acetosa* PMC's supposed that they might be the source of diploid gametes and thus of triploid and tetraploid individuals.

In the F_1 generation of the cross between *R. thyrsiflorus* and *R. acetosa* Z. Świetlińska (1960) observed the abolition of the second meiotic division and the formation of diads in male individuals: in F_2 she obtained many polyploids. The abolition of the second meiotic division also occurred in the F_2 generation and in the backcrosses *R. thyrsiflorus* $\times$ F_1 and F_1 $\times$ *R. thyrsiflorus*. As a result of this phenomenon a higher order of polyploidy was obtained in F_3 . Z. Świetlińska assumed that in this case a gene of *R. thyrsiflorus* was responsible for the abolition of the second meiotic division. This mechanism is not responsible for the appearance of polyploid plants found in natural populations. The male individuals of the species from the subsection *Acetosa* have the karyotype $2n=15$ ($12a + X + 2Y$); therefore, when the second meiotic division is missing they produce gametes with 14 ($12a + 2X$) or 16 ($12a + 4Y$) chromosomes. Such gametes, when uniting with the female gametes, which have 7 ($6a + X$) chromosomes, should give either female individuals with $3n=21$ ($18a + 3X$) or male individuals with $3n=23$ ($18a + X + 4Y$), whereas actually in the natural populations mostly intersexes with the karyotype $3n=22$ ($18a + 2X + 2Y$) were found.

To examine more closely the mechanism of the formation of unreduced gametes and the appearance of polyploid plants crosses were made between *R. thyrsiflorus* and *R. arifolius* on the assumption that in the hybrids the irregularities in meiosis would be more frequent and thus easier to observe. The abolition of the second meiotic division observed by Świetlińska (1960) in the cross *R. thyrsiflorus* $\times$ *R. acetosa* was expected to occur again.

The polyploid plants in the progeny of the crosses between *R. thyrsiflorus* and *R. arifolius* allowed the study of the mechanism of sex determination in the two parental species. In *R. acetosa* the mechanism

of sex determination was fully investigated by Ono (1935) and Yamamoto (1938), who found that in this species sex depended on the ratio of autosomes to the X-chromosomes: when this ratio was $2A^*: 2X$ the female sex and when it was $2A : X$ the male sex was obtained. This means that the male factors are present in the autosomes and the female factors in the X-chromosomes. The mechanism of sex determination in *R. acetosa* is thus, similarly as in *Drosophila* of the X/A type. The species *R. arifolius* and *R. thyrsiflorus* are very closely related with *R. acetosa*; moreover, cytological investigations (Meurman 1927; Kihara 1926; Löve 1944a) have demonstrated that the sex chromosomes of these species are very similar ($\text{♀} - XX$, $\text{♂} - XYY$) and in meiosis behave in exactly the same way. No intersexes occur among the crosses of these species. On this evidence it has been generally assumed that also in *R. thyrsiflorus* and *R. arifolius* the mechanism of sex determination is the same as in *R. acetosa*, but no direct proof for this supposition has as yet been supplied.

II. MATERIAL AND METHODS

1. The origin of the experimental plants

The plants for the experiments were collected from natural stands and were grown in the garden of the Department of Genetics in Warsaw and in Skierniewice. *R. thyrsiflorus* was collected on flood dikes of the Vistula near Puławy and Nowy Dwór. Plants of *R. arifolius* were transplanted from meadows near Krościenko in the Pieniny Mts. and from the Furcówka Valley and the Długa Hala Meadow in the Gorce Mts.

2. Interspecific crosses

The following crosses were made between the particular populations of the two species:

1. *R. arifolius* Krościenko $\times$ *R. thyrsiflorus* Nowy Dwór — Cross 241.
2. *R. thyrsiflorus* Puławy $\times$ *R. arifolius* Krościenko — Cross 239.
3. *R. arifolius* Krościenko $\times$ *R. thyrsiflorus* Puławy — Cross 240.
4. *R. arifolius* Gorce $\times$ *R. thyrsiflorus* Puławy — Cross 313.

Since both *R. thyrsiflorus* and *R. arifolius* are dioecious species the procedure of crossing them was rather simple. The female individuals were isolated before the development of the stigmata; on the day before the cross was made an isolating bag was placed over a polliniferous shoot of the needed male plant; to obtain cross-pollinations the male

* $2A = 12a$ = the diploid set of autosomes.

inflorescence was cut off, placed in a test-tube with water and put under the same isolator as the female plant. The cultivation of both species was very easy. The seeds sown out at the beginning of March in a greenhouse germinated after two weeks; in the middle of the May the young plants were transplanted onto the plots the distances between the plants being 50×50 centimeters.

3. Cytological techniques

Löve and Sarkar (1956) having reported that acetocarmine and acetoorcein did not give good results in the observations of mitosis in the genus *Rumex* at first the paraffin method was used and later a procedure of making squashed preparations was developed. With the paraffin method root tips were first treated for 3—4 hours in 0.03 per cent 8-oxyquinoline according to Levan (1950) and then fixed with Navashin fixative as modified by Müntzing (1932—33). The thickness of microtome sections was 12—14 microns. The buds for the observations of meiosis were also fixed in Navashin fixative modified by Müntzing after treatment for 5—10 minutes in Carnoy's acetoalcohol; the thickness of sections was 12—16 microns. The sections were stained with crystal violet according to Newton's method.

Root tips were usually fixed in the morning, between 7 and 10 a. m., and the buds in the afternoon: in this way the greatest number of divisions was obtained on the slides.

The procedure for squash preparations was as follows: root tips treated for 3—4 hours in 0.03 per cent solution of 8-oxyquinoline and washed for 5—10 minutes in distilled water were fixed for 2—48 hours in Carnoy fixative (3 parts absolute alcohol: one part glacial acetic acid), then placed for about 15 minutes in 2 per cent solution of orcein in 50 per cent acetic acid mixed with hydrochloric acid (9 parts 2 per cent orcein solution in 50 per cent acetic acid: one part of 1-normal hydrochloric acid) and squashed after about 15 minutes.

Other procedures were also tried out: root tips were treated with para-dichlorobenzene and Carnoy fixative with chloroform (absolute alcohol, glacial acetic acid, and chloroform mixed in various proportions) or Carnoy fixative with glacial acetic acid and absolute alcohol mixed in the following proportions: 1 : 1, 1 : 2, 1 : 4 and 1 : 5. These procedures did not however yield better results.

Observations of chromosomes were made with a Zeiss immersion objective 100 X and a PK eye-piece 15 X. The drawings were made using an Abbe apparatus and a Zeiss 100 X objective with a 15 X eye-piece. For microphotography Isopan FF 10 Din. films and an Exacta-Varex camera with 16 X, 40 X, and 100 X objectives and a Projectiv 4 : 1 eye-piece were used.

III. MORPHOLOGICAL TRAITS OF THE HYBRIDS

1. Diploid hybrids

Cross 241

The F_1 progeny was obtained from the cross ♀ *R. arifolius* Krościenko $\times$ ♂ *R. thyrsiflorus* Nowy Dwór. The seeds for the F_1 generation were set very abundantly and had a high germination rate. The F_1 progeny consisted of 83 individuals. The whole population was uniform, morphologically intermediate between the parental species, and manifested no well marked heterosis: e. g. the height of the stems in *R. arifolius* averaged 73 centimeters, in *R. thyrsiflorus* 93 centimeters and in F_1 plants 86 centimeters. The seeds for F_2 were set abundantly and had a high germination rate. The total number of F_2 individuals planted on the experimental plot was 185. The F_2 generation was less uniform than F_1 : some of the plants closely resembled *R. thyrsiflorus* and others *R. arifolius*, but the most numerous were the intermediate ones. The average height of stems was 90 centimeters, which was slightly more than in F_1 . To obtain the F_3 progeny the following crosses of F_2 plants were made:

- ♀ 3 $\times$ ♂ 11 giving 7 seeds,
- ♀ 48 $\times$ ♂ 11 giving 170 seeds,
- ♀ 138 $\times$ ♂ 1 giving 200 seeds, and
- ♀ 68 $\times$ ♂ 1 giving 74 seeds.

The F_3 generation morphologically resembled the F_2 population with a somewhat more pronounced segregation of morphological traits. A few individuals had thick, fragile leaves: when the chromosome number of these plants was counted it was found that they were polyploids. The cross between two tetraploid F_3 plants, ♀ 28 ($4X + 24a$) and ♂ 30 ($2X + 4Y + 24a$), produced no seeds whatever. An *R. thyrsiflorus* Nowy Dwór plant was crossed with an F_2 individual producing unreduced gametes: the setting of seeds was very abundant, but of the several hundred seeds sown out only 77 plants were obtained.

Cross 239

The F_1 generation was obtained from the cross ♀ *R. thyrsiflorus* Puławy $\times$ ♂ *R. arifolius* Krościenko. The seeds were set abundantly and their germination rate was 68 per cent. The number of individuals planted on the experimental plot was 23. The F_1 population was uniform and morphologically approached *R. thyrsiflorus*. The filial generation manifested a marked heterosis as compared to the parental species. The average height of stems of female and male individuals of *R. thyrsiflorus*

from Puławy was respectively 137 and 83 centimeters, of *R. arifolius* from Krościenko 118 and 70 centimeters, and of the F_1 generation 161 and 107 centimeters. The setting of seeds for F_2 was good and the germination rate was 50 per cent, which was somewhat lower than for the F_1 generation. The number of individuals planted on the plot was 407. Morphologically the F_2 population was not uniform being composed of plants resembling either *R. thyrsiflorus* or *R. arifolius* and of intermediate ones. Heterosis was less marked than in the F_1 generation the average height being 136 centimeters in female plants and 79 centimeters in male plants. The F_3 generation morphologically resembled the F_2 progeny. Two backcrosses were made:

♀ $F_1 \times \sigma R. arifolius$, and

♀ *R. thyrsiflorus* $\times \sigma F_1$.

The germination rate in the first of these backcrosses was 50 per cent; 180 plants were planted on the plot. Morphologically this population was rather uniform resembling *R. arifolius*. The average height of stems of female and male individuals was 155 and 82 centimeters respectively. The germination rate of seeds from the other backcross was much higher (80 per cent); 210 plants were planted. This population morphologically resembled *R. thyrsiflorus*, but it was not very uniform. The average height of stems of female and male individuals was 152 and 102 centimeters. The setting of seeds was very abundant.

Cross 240

To obtain the F_1 generation the cross was made ♀ *R. arifolius* Krościenko $\times \sigma R. thyrsiflorus$ Puławy. The setting of seeds for the F_1 progeny was poor; the number of individuals planted on the plot was 206. Morphologically the F_1 population was not uniform consisting of plants resembling either *R. thyrsiflorus* or *R. arifolius* and of intermediate ones. The fertility of both female and male individuals was appreciably reduced.

Cross 313

The cross was ♀ *R. arifolius* Gorce $\times \sigma R. thyrsiflorus$ Nowy Dwór. The number of plants obtained was 104. The whole F_1 population was morphologically uniform. The leaves were small, dark green; the stems were less tall than in the parental species. Pollen fertility was 85 per cent. Among the 104 plants there were only three male individuals.

The F_2 population was composed of 215 plants, which morphologically resembled the F_1 generation: the plants were small and had few leaves. There were only six male plants. Cytological examinations having revealed no polyploids among the progeny of this cross it was disregarded in the further investigations.

2. Polyploid Hybrids

General Description

Numerous polyploid plants were obtained from the interspecific crosses described above. Some of the polyploids being partly fertile it was possible to obtain their progeny.

The differences of morphological traits between the triploids or tetraploids and the diploid plants were mainly of a quantitative and not a qualitative nature. Generally speaking the polyploid plants were stronger and more vigorous than the diploid ones; their leaves were much larger, thicker, and more fragile; the floral shoots were usually thicker and taller. In male individuals with karyotypes $30 = 2X + 4Y + 24a$, $24 = X + 3Y + 20a$ and $23 = X + 4Y + 18a$ the flower buds and the anthers were appreciably larger than in the diploids. In the intersexes with karyotypes $3X + 2Y + 24a$ or $2X + 18a$ the flower buds and the anthers were small and very often dried and dropped off before producing any pollen. When comparing the progeny of the tetraploid No. I ($29 = 3X + 2Y + 24a$) from the backcross *R. thyrsiflorus* Puławy $\times$ F_1 239 with the progeny of the tetraploid No. II ($29 = 3X + 2Y + 24a$) from the F_1 240 population it was found that the progeny of the first tetraploid was morphologically very similar to *R. thyrsiflorus*, with narrow leaves and a compact and very much ramified inflorescence. The progeny of the second tetraploid had wider leaves and a less compact inflorescence. This was natural since in the former instance the chromosomes of *R. thyrsiflorus* ($3X + 18a$) were prevailing, whereas in the latter the karyotype was composed of chromosomes from the two parental species in equal numbers.

The rate of growth of the polyploids was much slower: the polyploid seeds germinated later and the plants that grew out of them flowered at a later date than in the case of diploid plants. In the triploids the guard-cells and the epidermal cells were much larger than in the diploids; the sizes of these cells in the tetraploids were essentially the same as in the triploids.

The size of pollen grains of the polyploids was not measured: the pollen produced by the tetraploids and the triploids being sterile and degenerate in nearly 100 per cent the results of such measurements would be meaningless (Phot. 35, 36).

No pollen whatever was produced by the male individuals with the karyotypes $24 = X + 3Y + 20a$, $23 = X + 4Y + 18a$, and $20 = X + 2Y + 17a$.

The polyploid plants obtained in this investigation were, according to Stebbins's (1947, 1958) classification, segmental allopolyploids, because they contained the partly homologous chromosomes of both *R. thyrsiflorus* and *R. arifolius*.

The Weight of Seeds

The weight of seeds of *R. thyrsiflorus*, *R. arifolius*, the F_1 and F_2 crosses, and of the tetraploids No. I and II is shown in Table 1.

Table 1
Weight of seeds of *R. thyrsiflorus*, *R. arifolius*, F_1 , F_2
hybrids and tetraploid plants I and II

Plant	Weight of 100 seeds in mg.
<i>R. thyrsiflorus</i> Nowy Dwór	48
<i>R. thyrsiflorus</i> Puławy	47
<i>R. arifolius</i> Tatry	90
<i>R. arifolius</i> Gorce	85
F_1 (<i>R. thyrsiflorus</i> $\times$ <i>R. arifolius</i>)	75
F_2 (<i>R. thyrsiflorus</i> $\times$ <i>R. arifolius</i>)	60
I ($29 = 3X + 2Y + 24a$)	150
II ($29 = 3X + 2Y + 24a$)	110

As is to be seen the seeds of the tetraploid plants were heavier than of the diploids, whether pure species or crosses. The seeds of *R. arifolius* were twice as heavy as the seeds of *R. thyrsiflorus*. The seeds of the two parental species and of the tetraploid individual No. I are shown in Phot. 37.

The Fertility of the Triploids and Tetraploids

The pollen fertility rate of the triploids was very low. In the majority of the triploid plants all the pollen was sterile the fertility rate of 1.8 per cent being quite exceptional. Pollen sterility was associated with triploidy itself as well as with the disturbed balance of the ratio of the X-chromosomes to the autosomes. It is noteworthy that no pollen was produced by the polyploid individuals I/31 with $23 = X + 4Y + 18a$ and II/7 with $24 = X + 3Y + 20a$: though, both these individuals had large flower buds and large anthers they did not form pollen grains. The anthers did not burst but after some time they withered and dropped off. Similarly, the triploid intersexes either produced no fertile seeds or at the most produced only a very few such seeds. The fertility of the female triploids was somewhat higher.

The rate of pollen fertility of the tetraploids with the chromosome set $4n = 29 (3X + 2Y + 24a)$ equalled 0. In individual with the chromosome set $4n = 30 (2X + 4Y + 24a)$ pollen fertility was 40 per cent. The tetraploid intersexes with the karyotype $4n = 29 (3X + 2Y + 24a)$ and the tetraploid female plants with the karyotype $4n = 28 (4X + 24a)$ did

not set seeds or their seed formation rate amounted to only 10 per cent of the rate of the diploid species. The germination rate of the seeds collected from tetraploid plants ranged 50 to 65 per cent.

IV. CYTOLOGICAL EXAMINATIONS

1. Parental Plants

a) Karyotypes

Counts of the chromosome number were made for numerous plants derived from the natural stands. The chromosome number was established in

26 plants of *R. thyrsiflorus* Puławy

21 plants of *R. thyrsiflorus* Nowy Dwór

2 plants of *R. thyrsiflorus* Ząbki

17 plants of *R. arifolius* Krościenko

7 plants of *R. arifolius* Gorce.

All the examined plants but one were diploids with the karyotypes $14 = 2X + 12a$ or $15 = X + 2Y + 12a$; the one exception was a *R. thyrsiflorus* plant from Puławy, which was a triploid with the chromosome set $21 = 3X + 18a$ (Fig. 5).

The plates on which the chromosomes could be counted not always were suitable for determining the karyotype of the given plant and, therefore, not in all plants the morphology of chromosomes was accurately established. It was found that in all the examined plants the sex chromosomes X and Y always had the centromere in the medial position. The autosomes were of four types: those of type j had the arms of distinctly different lengths, those of type t had a subterminal centromere and a trabant, those of type i had a subterminal centromere, and those of type v were short with the centromere in the medial position (Fig. 14).

The variability of the composition of autosome sets in the examined species was appreciable: karyotypes were found in which the chromosomes were all of type i, or of type i + v, or i + j etc. The karyotypes established for the particular populations are listed below:

R. thyrsiflorus Puławy — $8i + 2j + 2v$ (Fig. 4, 5, 9, 11, Phot. 3).

R. thyrsiflorus Nowy Dwór — $9i + j + 2v$ (Fig. 1).

R. thyrsiflorus Nowy Dwór — $10i + j + v$ (Fig. 2, Phot. 2).

R. thyrsiflorus Nowy Dwór — $9i + j + v + t$ (Fig. 10).

R. thyrsiflorus Nowy Dwór — $8i + j + 2v + t$ (Phot. 1).

R. thyrsiflorus Ząbki — $6i + 2j + 2v + 2t$ (Fig. 6).

R. arifolius Krościenko — $12i$ (fig. 12, 13).

R. arifolius Gorce — $9i + j + 2t$ (Fig. 7).

R. arifolius Gorce — $11i + t$ (Fig. 8).

R. arifolius Gorce — $11i + j$ (Phot. 4, 5).

Fig. 1—13. Metaphasal plates from root tips

1 — *R. thyrsiflorus* Nowy Dwór ♀ ($2X + j + 2v + 9i$); 2 — *R. thyrsiflorus* Nowy Dwór ♂ ($X + 2Y + j + v + 10i$); 3 — *R. thyrsiflorus* Pulawy ($3X + 2v + 16i$); 4 — *R. thyrsiflorus* Pulawy ♀ ($2X + 2j + 2v + 8i$); 5 — *R. thyrsiflorus* Pulawy ♂ ($X + 2Y + 2j + 2v + 8i$); 6 — *R. thyrsiflorus* Ząbki ♀ ($2X + 2j + 2t + 2v + 6i$); 7 — *R. arifolius* Gorce ♂ ($2X + j + 2t + 9i$); 8 — *R. arifolius* Gorce ♂ ($X + 2Y + t + 11i$); 9 — *R. thyrsiflorus* Pulawy ♀ ($2X + 2j + 2v + 8i$); 10 — *R. thyrsiflorus* Nowy Dwór ♀ ($2X + j + v + t + 9i$); 11 — *R. thyrsiflorus* Pulawy ♂ ($X + 2Y + 2j + 2v + 8i$); 12 — *R. arifolius* Krościenko ♀ ($2X + 12i$); 13 — *R. arifolius* Krościenko ♂ ($X + 2Y + 12i$). Fig. 1—8: smears; Fig. 9—13: paraffin preparations. Magnific.: 1575 ×

Table 2

Absolute length of chromosomes of *R. thyrsiflorus* and *R. arifolius*
(in microns)

Plant	Sex	X ₁	X ₂	Y ₁	Y ₂	1	2	3	4	5	6	7	8	9	10	11	12
<i>R. thyrsiflorus</i> (Puławy) smears with 8-oxyquinoline pretreatment	♀	3.6 + 3.9 = 7.5	3.6 + 3.6 = 7.2			(j) 1.8 + 3.6 = 5.4	(j) 1.6 + 3.2 = 4.8	(i) 0.9 + 3.4 = 4.3	(i) 0.9 + 3.2 = 4.1	(i) 0.9 + 3.0 = 3.9	(i) 0.9 + 2.9 = 3.8	(i) 0.7 + 2.9 = 3.6	(i) 0.7 + 2.7 = 3.4	(i) 0.9 + 2.3 = 3.2	(i) 0.9 + 2.3 = 3.2	(v) 1.6 + 1.6 = 3.2	(v) 1.3 + 1.9 = 3.2
	♂	3.9 + 4.3 = 8.2		2.5 + 3.2 = 5.7	2.5 + 2.9 = 5.4	(j) 1.8 + 3.9 = 5.7	(j) 1.8 + 3.6 = 5.4	(i) 1.1 + 3.9 = 5.0	(i) 1.1 + 3.9 = 5.0	(i) 0.7 + 3.9 = 4.6	(i) 0.7 + 3.9 = 4.6	(i) 0.7 + 3.4 = 4.1	(i) 0.7 + 3.2 = 3.9	(i) 0.7 + 3.2 = 3.9	(i) 0.7 + 3.2 = 3.9	(v) 1.8 + 2.0 = 3.8	(v) 1.6 + 2.0 = 3.6
<i>R. thyrsiflorus</i> (Puławy) paraffin preparation with 8-oxyquinoline pretreatment	♀	2.5 + 2.9 = 5.4	2.5 + 2.7 = 5.2			(j) 0.9 + 2.5 = 3.4	(j) 0.9 + 2.5 = 3.4	(i) 0.5 + 2.9 = 3.4	(i) 0.5 + 2.7 = 3.2	(i) 0.5 + 2.5 = 3.0	(i) 0.5 + 2.3 = 2.8	(i) 0.5 + 2.1 = 2.6	(i) 0.5 + 2.0 = 2.5	(i) 0.5 + 1.9 = 2.4	(i) 1.1 + 1.2 = 2.3	(i) 0.5 + 1.7 = 2.2	(v) 1.1 + 1.1 = 2.2
	♂	2.7 + 2.7 = 5.4		1.6 + 2.3 = 3.9	1.8 + 2.0 = 3.8	(j) 0.9 + 2.9 = 3.8	(j) 0.9 + 2.7 = 3.6	(i) 0.5 + 2.5 = 3.0	(i) 0.5 + 2.5 = 3.0	(i) 0.5 + 2.3 = 2.8	(i) 0.5 + 2.1 = 2.6	(i) 0.5 + 2.0 = 2.5	(i) 0.5 + 2.0 = 2.5	(i) 0.5 + 1.8 = 2.3	(i) 0.5 + 1.8 = 2.3	(v) 1.1 + 1.1 = 2.2	(v) 0.9 + 1.3 = 2.2
<i>R. thyrsiflorus</i> (Nowy Dwór) smears with 8-oxyquinoline pretreatment	♀	2.9 + 3.9 = 6.8	3.2 + 3.6 = 6.8			(j) 1.8 + 3.6 = 5.4	(i) 0.9 + 3.0 = 3.9	(i) 0.5 + 3.4 = 3.9	(i) 0.9 + 2.9 = 3.8	(i) 0.7 + 3.0 = 3.7	(i) 0.5 + 3.2 = 3.7	(i) 0.5 + 3.0 = 3.5	(i) 0.5 + 3.0 = 3.5	(i) 0.5 + 3.0 = 3.5	(i) 0.5 + 2.7 = 3.2	(v) 1.4 + 1.6 = 3.0	(v) 1.1 + 1.8 = 2.9
	♂	3.6 + 3.9 = 7.5		3.2 + 3.2 = 6.4	2.5 + 2.9 = 5.4	(j) 2.1 + 3.6 = 5.7	(i) 0.9 + 3.6 = 4.5	(i) 0.9 + 3.6 = 4.5	(i) 0.7 + 3.6 = 4.3	(i) 0.7 + 3.6 = 4.3	(i) 0.5 + 3.2 = 3.7	(i) 0.5 + 3.0 = 3.5	(i) 0.5 + 2.9 = 3.4	(i) 0.5 + 2.9 = 3.4	(i) 0.5 + 2.9 = 3.4	(i) 0.5 + 2.9 = 3.4	(v) 1.6 + 1.6 = 3.2
<i>R. arifolius</i> (Krościenko) paraffin preparation	♀	2.9 + 3.4 = 6.3	2.9 + 3.2 = 6.1			(i) 0.5 + 4.1 = 4.6	(i) 0.5 + 3.9 = 4.4	(i) 0.5 + 3.6 = 4.4	(i) 0.5 + 3.4 = 3.9	(i) 0.5 + 3.4 = 3.9	(i) 0.5 + 3.2 = 3.7	(i) 0.5 + 2.3 = 3.7	(i) 0.5 + 3.2 = 3.7	(i) 0.5 + 2.9 = 3.4	(i) 0.5 + 2.7 = 3.4	(i) 0.5 + 2.1 = 2.6	(i) 0.5 + 2.0 = 2.5
	♂	2.9 + 2.9 = 5.8		2.3 + 2.5 = 4.8	2.3 + 2.3 = 4.6	(i) 0.5 + 3.2 = 3.7	(i) 0.5 + 3.2 = 3.7	(i) 0.5 + 3.2 = 3.7	(i) 0.5 + 3.2 = 3.7	(i) 0.5 + 3.2 = 3.7	(i) 0.5 + 3.2 = 3.7	(i) 0.5 + 2.7 = 3.2	(i) 0.5 + 2.3 = 2.8	(i) 0.5 + 2.3 = 2.8	(i) 0.5 + 2.3 = 2.8	(i) 0.5 + 2.1 = 2.6	(i) 0.5 + 2.1 = 2.6
<i>R. arifolius</i> (Turbacz) smears with 8-oxyquinoline pretreatment	♀	2.9 + 3.2 = 6.1	2.9 + 2.9 = 5.8			(j) 1.3 + 3.2 = 4.5	(i) 0.7 + 2.7 = 3.4	(i) 0.7 + 2.5 = 3.2	(i) 0.7 + 2.5 = 3.2	(i) 0.7 + 2.5 = 3.2	(i) 0.7 + 2.3 = 3.0	(i) 0.7 + 3.2 = 3.0	(i) 0.7 + 2.1 = 2.8	(i) 0.7 + 2.1 = 2.8	(i) 0.7 + 2.1 = 2.8	(i) 0.5 + 1.8 = 2.3	(i) 0.5 + 1.6 = 2.1
	♂	2.5 + 2.9 = 5.4		2.1 + 2.1 = 4.2	2.0 + 2.0 = 4.0	(i) 0.5 + 2.7 = 3.2	(i) 0.5 + 2.7 = 3.2	(i) 0.5 + 2.5 = 3.0	(i) 0.7 + 2.1 = 2.8	(i) 0.5 + 2.3 = 2.8	(i) 0.5 + 2.3 = 2.8	(i) 0.5 + 2.3 = 2.8	(i) 0.5 + 2.3 = 2.8	(i) 0.5 + 2.1 = 2.6	(i) 0.5 + 1.8 = 2.3	(i) 0.5 + 1.8 = 2.3	(i) 0.5 + 1.6 = 2.1

Table 3

Relative length of chromosomes of *R. thyrsiflorus* and *R. arifolius* calculated as percentage of the total length of chromosome set

Plant	Sex	X ₁	X ₂	Y ₁	Y ₂	1	2	3	4	5	6	7	8	9	10	11	12
<i>R. thyrsiflorus</i> (Puławy) smears	♀	5.88 + 6.47 = 12.35	5.88 + 5.88 = 11.76			(j) 2.94 + 5.88 = 7.94	(j) 2.65 + 5.29 = 7.94	(i) 1.47 + 5.59 = 7.06	(i) 1.47 + 5.29 = 6.76	(i) 1.47 + 5.00 = 6.47	(i) 1.47 + 4.71 = 6.18	(i) 1.18 + 4.71 = 5.89	(i) 1.18 + 4.41 = 5.59	(i) 1.47 + 3.82 = 5.29	(i) 1.47 + 3.82 = 5.29	(v) 2.65 + 2.65 = 5.30	(v) 2.06 + 3.24 = 5.30
	♂	5.39 + 5.88 = 11.27		3.43 + 4.41 = 7.84	3.43 + 3.92 = 7.35	(j) 2.45 + 5.88 = 8.33	(j) 2.45 + 4.90 = 7.35	(i) 1.41 + 5.39 = 6.80	(i) 1.41 + 5.39 = 6.80	(i) 0.98 + 5.39 = 6.37	(i) 0.98 + 5.39 = 6.37	(i) 0.98 + 4.66 = 5.64	(i) 0.98 + 4.41 = 5.39	(i) 0.98 + 4.41 = 5.39	(i) 0.98 + 4.41 = 5.39	(v) 2.45 + 2.70 = 5.15	(v) 2.21 + 2.70 = 4.91
<i>R. thyrsiflorus</i> (Puławy) paraffin preparation with 8-oxyquinoline pretreatment	♀	5.69 + 6.50 = 12.19	5.69 + 6.10 = 11.79			(j) 2.03 + 5.69 = 7.72	(j) 2.03 + 5.69 = 7.72	(i) 1.22 + 6.50 = 7.72	(i) 1.22 + 6.10 = 7.32	(i) 1.22 + 5.69 = 6.91	(i) 1.22 + 5.28 = 6.50	(i) 1.22 + 4.87 = 6.09	(i) 1.22 + 4.47 = 5.69	(i) 1.22 + 4.07 = 5.29	(i) 2.44 + 2.85 = 5.29	(i) 1.22 + 3.66 = 4.88	(v) 2.44 + 2.44 = 4.88
	♂	5.83 + 5.83 = 11.66		3.50 + 5.06 = 8.56	3.89 + 4.28 = 8.17	(j) 1.95 + 6.23 = 8.18	(j) 1.95 + 5.83 = 7.78	(i) 1.17 + 5.43 = 6.60	(i) 1.17 + 5.43 = 6.60	(i) 1.17 + 5.06 = 6.23	(i) 1.17 + 4.67 = 5.84	(i) 1.17 + 4.28 = 5.45	(i) 1.17 + 4.28 = 5.45	(i) 1.17 + 3.89 = 5.06	(i) 1.17 + 3.89 = 5.06	(v) 2.33 + 2.33 = 4.66	(v) 1.95 + 2.72 = 4.67
<i>R. thyrsiflorus</i> (Nowy Dwór) smears	♀	4.94 + 6.79 = 11.73	5.56 + 6.17 = 11.73			(j) 3.07 + 6.17 = 9.24	(i) 1.54 + 5.25 = 6.79	(i) 0.93 + 5.86 = 6.79	(i) 1.54 + 4.94 = 6.48	(i) 1.23 + 5.25 = 6.48	(i) 0.93 + 5.56 = 6.49	(i) 0.93 + 5.25 = 6.18	(i) 0.93 + 5.25 = 6.18	(i) 0.93 + 5.25 = 6.18	(i) 0.93 + 4.63 = 5.56	(v) 2.47 + 2.78 = 5.25	(v) 1.85 + 3.09 = 4.94
	♂	5.27 + 5.80 = 11.07		4.75 + 4.75 = 9.50	3.69 + 4.22 = 7.91	(j) 3.17 + 5.27 = 8.44	(i) 1.32 + 5.27 = 6.59	(i) 1.32 + 5.27 = 6.59	(i) 1.06 + 5.27 = 6.33	(i) 1.06 + 5.27 = 6.33	(i) 1.06 + 4.75 = 5.81	(i) 1.06 + 4.49 = 5.55	(i) 1.06 + 4.22 = 5.28	(i) 1.06 + 4.22 = 5.28	(i) 1.06 + 4.22 = 5.28	(i) 1.06 + 4.22 = 5.28	(v) 2.37 + 2.37 = 4.74
<i>R. arifolius</i> (Krościenko) paraffin preparation	♀	5.08 + 6.03 = 11.11	5.08 + 5.71 = 10.79			(i) 0.95 + 7.30 = 8.25	(i) 0.95 + 6.98 = 7.93	(i) 0.95 + 6.34 = 7.29	(i) 0.95 + 6.03 = 6.98	(i) 0.95 + 6.03 = 6.98	(i) 0.95 + 5.71 = 6.66	(i) 0.95 + 5.71 = 6.66	(i) 0.95 + 5.71 = 6.66	(i) 0.95 + 5.08 = 6.03	(i) 0.95 + 4.76 = 5.71	(i) 0.95 + 3.81 = 4.76	(i) 0.95 + 3.49 = 4.44
	♂	5.21 + 5.21 = 10.42		4.23 + 4.56 = 8.79	4.23 + 4.23 = 8.46	(i) 0.98 + 5.86 = 6.84	(i) 0.98 + 5.86 = 6.84	(i) 0.98 + 5.86 = 6.84	(i) 0.98 + 5.86 = 6.84	(i) 0.98 + 5.86 = 6.84	(i) 0.98 + 5.86 = 6.84	(i) 0.98 + 4.89 = 5.87	(i) 0.98 + 4.23 = 5.21	(i) 0.98 + 4.23 = 5.21	(i) 0.98 + 4.23 = 5.21	(i) 0.98 + 3.91 = 4.89	(i) 0.98 + 3.91 = 4.89
<i>R. arifolius</i> (Turbacz) smears	♀	5.90 + 6.64 = 12.54	5.90 + 5.90 = 11.80			(j) 2.58 + 6.64 = 9.22	(i) 1.48 + 5.53 = 7.01	(i) 1.48 + 5.17 = 6.65	(i) 1.48 + 5.17 = 6.65	(i) 1.48 + 5.17 = 6.65	(i) 1.48 + 4.80 = 6.28	(i) 1.48 + 4.80 = 6.28	(i) 1.48 + 4.43 = 5.91	(i) 1.48 + 4.43 = 5.91	(i) 1.48 + 4.43 = 5.91	(i) 1.11 + 3.69 = 4.80	(i) 1.11 + 3.32 = 4.43
	♂	5.34 + 6.11 = 11.45		4.58 + 4.58 = 9.16	4.20 + 4.20 = 8.40	(i) 1.15 + 5.73 = 6.88	(i) 1.15 + 5.73 = 6.88	(i) 1.15 + 5.34 = 6.49	(i) 1.53 + 4.58 = 6.11	(i) 1.15 + 4.98 = 6.13	(i) 1.15 + 4.96 = 6.11	(i) 1.15 + 4.96 = 6.11	(i) 1.15 + 4.96 = 6.11	(i) 1.15 + 4.58 = 5.73	(i) 1.15 + 3.82 = 4.97	(i) 1.15 + 3.82 = 4.97	(i) 1.15 + 3.44 = 4.59

The frequency of the particular karyotypes in the various populations was not established since the available data were insufficient for this purpose. The above list seems to indicate, however, that the karyotypes with the chromosomes of type j and v are more frequent in *R. thyrsiflorus* than in *R. arifolius*. Such morphological variation of autosome was described by many authors also for *R. acetosa* and is probably due to reciprocal translocations between different autosomes.

Chromosome lengths were measured in a few chosen plates with the best visibility where as far as possible all the chromosomes were lying in the same plane. The results of the measurements are listed in Table 2 and illustrated in Fig. 14.

The measurements made in this way were exposed to different errors: for instance, as is to be seen in Table 2, in the case of two plants of *R. thyrsiflorus* from Puławy the chromosomes of one ($X = 7.2; 7.5$) were much longer than of the other ($X = 5.2; 5.4$). It does not seem possible that natural variation could be responsible for so large a difference. In this case it was quite certainly caused by the different procedures followed in fixing and preparing slides; in the former case the procedure was acetoalcohol and smears, in the latter Navashin fixative and paraffin.

But there was still another source of errors which could have caused the observed differences: as is well known the length of chromosomes depends on the stage of the nucleic division. For instance in prometaphase the chromosomes are somewhat longer than in metaphase.

In view of these errors it was thought more adequate to compare the relative lengths of chromosomes expressed in percentages, since then the results would be more comparable. The relative chromosome lengths were computed from the obtained results and are listed in Table 3. For the computations the total length of all the chromosomes in a plate was accepted to be 100 and the lengths of the particular chromosomes were then calculated as the percentage of the total length.

The thus calculated data indicate that the largest were in all instances the X-chromosomes. In *R. thyrsiflorus* their length ranged 11.7 to 12.35 per cent of the total length of chromosomes and in *R. arifolius* 10.42 to 12.54 per cent. The length of the Y-chromosomes ranged 7.35 to 9.50 per cent in *R. thyrsiflorus* and 8.40 to 9.11 per cent in *R. arifolius*. As is to be seen in both species the Y-chromosomes were appreciably smaller than the X-chromosomes. The sizes of j-chromosomes were of the same order as of the Y-chromosomes their length ranging 7.35 to 9.24 per cent in *R. thyrsiflorus*. In *R. arifolius* only one j-chromosome was measured (9.22 per cent). The autosomes of type i, their number in the particular karyotypes varied from 12 to 6, greatly differed in size: the largest ones were only slightly smaller than or almost of the same size as the chromosomes of type j, whereas others were the smallest

chromosomes in the plate. Their lengths ranged 7.72 to 4.88 per cent in *R. thyrsiflorus* and 8.25 to 4.43 per cent in *R. arifolius*. The size of the chromosomes of type v was of the same order as of the smallest chromosomes of type i ranging 5.30 to 4.66 per cent in *R. thyrsiflorus*.

Fig. 14. Chromosome sets of *R. thyrsiflorus* and *R. arifolius*

a — *R. thyrsiflorus* Pulawy ♀; b — *R. thyrsiflorus* Pulawy ♂; c — *R. thyrsiflorus* Pulawy ♀; d — *R. thyrsiflorus* Pulawy ♂; e — *R. thyrsiflorus* Nowy Dwór ♀; f — *R. thyrsiflorus* Nowy Dwór ♂; g — *R. arifolius* Krościenko ♀; h — *R. arifolius* Krościenko ♂; i — *R. arifolius* Gorze ♀; j — *R. arifolius* Gorze ♂. Fig. a, b, e, f, i, j from smear preparations; Fig. g, h from paraffin preparations; Fig. c, d from paraffin preparations with 8-oxyquinoline pretreatment

No v-chromosomes were found in the metaphase plates of *R. arifolius* in which the chromosome lengths could be measured.

The chromosomes of type X, Y, and v were found to have the two arms usually of slightly different lengths. Often the difference between the two arms was so marked that it could be easily observed.

Among the chromosomes of type v some had arms exactly equal in length, whereas others had arms either slightly or considerably differing in length. These chromosomes morphologically closely resembled the smallest chromosomes of type i.

All this shows that in the populations of *R. thyrsiflorus* and *R. arifolius* the variations of karyotypes and of the size of chromosomes were considerable.

b) The Course of Meiosis in Male Plants

The course of meiosis was investigated in plants of *R. thyrsiflorus* from Puławy and Nowy Dwór and of *R. arifolius* from Krościenko. No instances of major irregularities in meiosis were observed. In diakinesis the chromosomes formed six bivalents and one trivalent composed of $Y_1 + X + Y_2$. At anaphase I the X-chromosome moved to one pole and the $Y_1 + Y_2$ one moved to the other. After the second meiotic divisions cytokinesis took place and regular tetrads were formed. Of the four microspores thus formed two had the karyotype $7 = X + 6a$ and determined the female sex whereas two had the karyotype $8 = 2Y + 6a$ determining the male sex. Chromatin bridges caused by difficulties in the parting of chromosomes at anaphase I were sporadically found in both *R. thyrsiflorus* and *R. arifolius*. Among the 100 PMC's of *R. arifolius* from Krościenko examined at anaphase I chromatin bridges were found in 10. The observations showed that the course of meiosis in both *R. thyrsiflorus* and *R. arifolius* was the same as in *R. acetosa* and the other species of the subsection *Acetosa*. The results obtained in this investigation are concordant with the results obtained earlier for *R. arifolius* (Kihara 1926) and *R. thyrsiflorus* (Meurman 1927).

2. Hybrids

a) Cross 241 (*R. arifolius* Krościenko $\times$ *R. thyrsiflorus* Nowy Dwór)

In F_1 the chromosome number was counted for 13 individuals and the following chromosome sets were found:

$$\left. \begin{array}{l} \text{♀ } 2X \\ \text{♂ } X + 2Y \end{array} \right\} + j + v + 10i \text{ (Fig. 15, 16).}$$

Meiosis was investigated in 12 male plants: in all instances the course of meiosis was regular and no major disturbances were observed.

15

16

17

18

19

20

21

22

23

Fig. 15—23. Root tips metaphases of hybrids between *R. arifolius* Krościenko and *R. thyrsiflorus* Nowy Dwór (cross 241)

15 — $F_1 \varnothing$ ($14 = 2X + j + v + 10i$); 16 — $F_1 \sigma^7$ ($15 = X + 2Y + j + v + 10i$); 17 — $F_2 \varnothing$ ($14 = 2X + j + 2v + 9i$);
 18 — $F_2 \varnothing$ ($14 = 2X + j + v + 10i$); 19 — $F_3 \varnothing$ ($14 = 2X + j + 11i$); 20 — $F_3 \varnothing$ ($14 = 2X + j + v + 10i$); 21 —
 — F_3 ($28 = 4X + 24a$); 22 — F_3 ($21 = 3X + 18a$); 23 — F_3 ($22 = 2X + 2Y + 18a$); Paraffin preparations. Magnific.:
 1575 $\times$

In the F_2 generation the chromosome number was counted in 12 individuals. All the examined plants proved to be diploids. Since in F_1 the karyotypes were asymmetrical each containing one j -chromosome and one v -chromosome in F_2 the karyotypes were segregated into those containing two, one, or no j -chromosomes as well as those containing two, one, or no v -chromosomes.

The karyotype $2X + j + 2v + 9i$ is shown in Fig. 17,

the karyotype $2X + j + v + 10i$ in Fig. 18, and

the karyotype $2X + j + 11i$ in Fig. 19.

The course of meiosis was examined in 8 male plants of the F_2 generation. Irregularities in meiosis leading to the formation of diads instead of tetrads were observed in five individuals (No. 1, 11, 12, 15, and 18); in three individuals (No. 17, 42, and 55) meiosis was regular.

Meiosis in the individuals No. 1, 11, 12, 15, and 18.

The conjugation of chromosomes at diakinesis was regular with six bivalents and one trivalent visible (Fig. 24). Similarly, no irregularities were observed at metaphase I (Fig. 25). At anaphase I the chromosomes parted forming two groups the composition of which was respectively $X + 6a$ and $2Y + 6a$ (Fig. 26—29). In a small proportion of pollen mother cells bridges with acentric fragments (Fig. 27) and lagging bivalents (Fig. 28) could be seen. In the second meiotic division the irregularities were much greater. Cytokinesis occurred immediately after the end of the first anaphase leading to the formation of diads (Fig. 30, 31, 33, 43, 50, 51, and Phot. 7, 9). One of the diad cells contained $X + 6a$ chromosomes and the other $2Y + 6a$ chromosomes. At this stage the chromosomes ceased to be visible and part of the diads disintegrated (Fig. 33).

After this, as it were, resting stage the chromosomes again became visible behaving in a way somewhat similar to the usual course of the second prophase and metaphase. The chromosomes, however, did not assume the arrangement usual for the second metaphase and their distribution was entirely haphazard (Fig. 45, Phot. 9, 10, 11). On the slides with the somewhat later stages the fact that each chromosome was composed of two chromatids joined at the centromere was clearly visible (Fig. 52, 53). After this the centromeres divided, but the spindle of the second division was not formed and the chromosomes continued to lie in pairs without parting (Fig. 54). The result of this behaviour was the formation of unreduced gametes with the following chromosome sets: 14 ($2X + 12a$) and 16 ($4X + 12a$).

The just described mechanism of the doubling of the chromosome number was not synchronous with the separation of the diad cells. In many instances the parting of chromosomes was observed when the diads were still connected (Fig. 51). Another possibility was that in one

Fig. 24—34. Meiosis in PMCs from the plant $F_2/12$ of the cross No. 241

24 — diakinesis; 25 — M I; 26, 27, 28, 29 — A I; 30, 31 — diads; 32 — irregular tetrad; 33 — disintegrating diad; 34 — microspore after the disintegration of diad

Fig. 35—39. Metaphase plates from pollen mitosis in $F_2/12$ plant of the cross No. 241

35 — ($16 = 2X + 2Y + 2v + 10i$); 36 — ($14 = 2X + 2j + 2v + 8i$); 37 — ($16 = 2X + 2Y + 2j + 10i$); 38 — ($18 = 2X + 2Y + 2v + 12i$); 39 — ($16 = 4Y + 2j + 10i$); Paraffin preparations. Magnific.: $1575 \times$

Fig. 40—49. Meiosis in PMCs from the plant $F_2/11$ of the cross No. 241

40 — diakinesis; 41 — T I; 42 — interkinesis, formation of the phragmoplast; 43 — diad; 44 — microspore after the disintegration of diad; 45 — "M II" in microspore; 46 — irregular tetrad; 47 — triad; 48 — pentad; 49 — triad

Fig. 50—55. Meiosis in PMCs from the plant $F_2/15$ of the cross No. 241

50 — diad; 51 — "M II" in a diad; 52, 53, 54, 55 — "second meiotic division" in microspore. Paraffin preparations. Magnific.: 1575 $\times$

of the cells of the diad the chromosomes divided into two groups a new cell wall being formed between them, whereas in the other cell the chromosomes divided but remained in one common nucleus: the result then was the formation of a triad (Fig. 46, 47, 49, Phot. 8). Tetrads could also be formed if the chromosomes divided in both nuclei of a diad; however, such tetrads differed from the regular ones in that all the four microspores then lay in one plane (Fig. 48).

Mention has already been made that the abolition of the second meiotic division in PMCs led to the formation of unreduced gametes. To confirm further this observation also the mitotic divisions in pollen were examined in the individual No. 12 (Fig. 35—39, Phot. 12). In the 15 metaphase plates that were analysed the following chromosome numbers were found:

14 chromosomes in 5 plates,
16 chromosomes in 8 plates,
18 chromosomes in 2 plates.

Thus, in all instances the microspores had an unreduced number of chromosomes. The arrangement of chromosomes in pairs, seen best in Fig. 35, as well as their even numbers confirmed the above described pattern of the abolition of the reduction of the chromosome number. Not one plate with the haploid chromosome number was found, which means that in plant No. 12 the abolition of the second meiotic division and the formation of diads was the usual course of meiosis. On the other hand, the analysis of the course of microsporogenesis in the other individuals showed that the degree of these meiotic irregularities could be different. The irregularities could differ even in the various buds of the same plant: besides regular diads also tetrads could be formed. In order to gain some information about the magnitude of the disturbances occurring in the course of the second meiotic division the numbers of diads, triads, tetrads, and pentads were counted in several plants (Table 4).

Table 4

Disturbances in the microsporogenesis of F₁ and F₂ hybrids from the cross 241

Plant	Numbers of PMS (in parenthesis percentage values)				
	diads	triads	tetrads	pentads	total
F ₁ /38	3 (1,65)	1 (0,55)	177 (97,8)	—	181
F ₂ /42	—	2 (0,96)	206 (99,04)	—	208
F ₂ /1	35 (30,47)	31 (27,43)	45 (39,82)	2 (1,7)	113
F ₂ /11	50 (37,04)	65 (48,15)	20 (14,81)	—	135
F ₂ /12	141 (90,07)	14 (9,03)	—	—	155

As is to be seen nearly all the microspores produced during meiosis of the individual F₂/12 were diads and only a small proportion triads.

Irregularities of a similar magnitude were also observed in plants $F_2/15$ and $F_2/48$. On the other hand, in plants $F_2/11$ and $F_2/1$ diads, triads, and tetrads were formed in almost equal numbers showing that the course of meiosis in some pollen mother cells was regular, whereas in others the second meiotic division was abolished.

In the plants $F_2/42$ and $F_1/38$ the course of meiosis was regular (Table 4); the diads found in these individuals could have been produced by some other kind of meiotic irregularities or they were regular tetrads cut in half.

The general conclusion to be made from what has just been said seems to be that the observed disturbances in the course of meiosis usually consisted in the abolition of the second meiotic division and that they were probably caused by factors of a genetic nature.

If we assume that in the F_2 generation the female plants can also produce haploid as well as unreduced gametes, then the proportion of polyploid plants to be expected in the F_3 progeny should be high. The pattern according to which such plants would be formed is shown in Table 5.

To check this assumption the somatic chromosome number was counted in 41 plants of the F_3 generation. The following results were obtained:

	Karyotypes	Number of plants
$F_3 (3 \times 11)$	21 (3X + 18a)	1 (Fig. 22)
	14 (2X + 12a)	2
	15 (X + 2Y + 12a)	1
$F_3 (48 \times 11)$	14 (2X + 12a)	7
	15 (X + 2Y + 12a)	3
	28 (4X + 24a)	2 (Fig. 21, Phot. 26)
	22 (2X + 2Y + 18a)	1 (Fig. 23)
$F_3 (138 \times 1)$	14 (2X + 12a)	9 (Fig. 20)
	15 (X + 2Y + 12a)	9
	28 (4X + 24a)	2
$F_3 (68 \times 1)$	14 (2X + 12a)	3
	30 (2X + 4Y + 24a)	1
	Total	41

	Karyotypes	Number of plants
1)	14 (2X + 12a)	21
2)	15 (X + 2Y + 12a)	13
3)	21 (3X + 18a)	1
4)	22 (2X + 2Y + 18a)	1
5)	28 (4X + 24a)	4
6)	30 (2X + 4Y + 24a)	1
	Total	41

The analysis of the chromosome sets shows that out of the predicted seven different karyotypes (Table 5) only the one with the formula $23 = X + 4Y + 18a$ was not found in the F_3 generation.

This result confirmed the assumption that meiotic irregularities leading to the production of unreduced gametes occurred also in the female plants of the F_2 generation. Nothing is known about the nature of these irregularities since no cytological observations of the macrosporogenesis of these plants were made.

Table 5

Expected types of polyploids after partial failure of the second meiotic division

♀ \ ♂	Normal meiosis		Failure of the second division	
	X + 6a	2Y + 6a	2X + 12a	4Y + 12a
Normal meiosis X + 6a	2X + 12a 2n = 14	X + Y + 12a 2n = 15	3X + 18a 2n = 21	X + 4Y + 18a 2n = 23
Failure of the second division 2X + 12a	3X + 18a 3n = 21	2X + 2Y + 18a 3n = 22	4X + 24a 4n = 28	2X + 4Y + 24a 4n = 30

The occurrence of polyploid plants in the F_3 progeny was lower than the predictions made from the frequency of disturbances recorded in the male plants of the F_2 generation. On the other hand, among the F_3 polyploids the number of tetraploids was higher than the number of triploids, though Table 5 indicates that the reverse proportions were to be expected. This can only be explained by assuming that the union between gametes according to the pattern $\text{♀}n \times \text{♂}n$ or $\text{♀}2n \times \text{♂}2n$ was more frequent than according to the pattern $\text{♀}2n \times \text{♂}n$ or $\text{♀}n \times \text{♂}2n$. There must have been some obstacles hindering the union between the haploid and the diploid gametes.

Meiosis was examined in one male individual from the progeny F_3 (3×11), in one male individual from the progeny F_3 (48×11) and in eight male individuals from the progeny F_3 (138×1). In the first two of these plants no irregularities of meiosis were noted. Similarly, no meiotic irregularities were found in six of the plants of the progeny F_3 (138×1), whereas in the other two there was a small proportion of diads. These diads, however, were the result not of the absence of the second meiotic division but of some other kind of irregularities, such as the irregular parting of chromosomes at anaphase I, the formation of restitution nuclei (Fig. 56—67), or the joining of groups at anaphase II. Irregularities of this kind were evidenced by the chromosome numbers found in the mitotic divisions of pollen; these chromosome numbers were:

n = 7 in 3 microspores	n = 12 in 1 microspore
n = 8 in 3 microspores	n = 14 in 1 microspore
n = 9 in 1 microspore	n = 17 in 1 microspore.

Altogether meiosis was examined in ten diploid male plants from the F_3 generation and in none of them was the second meiotic division abolished, though this meiotic irregularity was observed in the parental plants from the F_2 generation. Apparently, the capacity to produce unreduced gametes as a result of the abolition of the second meiotic division was not inherited by the progeny.

The male plants, in which the absence of the second meiotic division was observed, had a distinctly reduced fertility. This is to be seen in Table 6 listing the pollen fertility of the F_2 male plants with a regular

Fig. 56—67. Meiosis in PMCs from the plant $F_2/36$ of the cross 241

56 — diakinesis; 57 — A I; 58 — T I; 59, 60 — chromosome bridges at M II; 61 — chromosome bridges at A II; 62, 63 — anaphase of a restitution nucleus; 64 — desorganisation of meiotic division; 65 — diad resulting from the failure of chromosome separation at second meiotic division; 66 — tetrad; 67 — diad from the division of a restitution nucleus.

Paraffin preparations. Magnific.: 1575 $\times$

course of meiosis as well as of those which were characterized by the absence of the second meiotic division. The pollen fertility of the parental plants *R. thyrsiflorus* and *R. arifolius* and of the F_1 , F_2 and F_3 hybrids is also tabulated for comparison.

Table 6
Pollen fertility of *R. arifolius*, *R. thyrsiflorus* and F_1 , F_2 , F_3 hybrids

Plant	Pollen fertility in %										N	M $\pm$ m
	10	20	30	40	50	60	70	80	90	100		
<i>R. arifolius</i>								2	1		3	83,3 $\pm$ 4,1
<i>R. thyrsiflorus</i>									1	1	2	95,0 $\pm$ 5,0
F_1 normal meiosis							2	3	1		6	78,3 $\pm$ 3,1
normal meiosis		1	1			2	1	9	5		19	74,2 $\pm$ 4,5
F_2 failure of the second division	1		2		2						5	34,0 $\pm$ 7,5
F_3 normal meiosis				1	1	2			3		7	68,6 $\pm$ 7,9

Moreover, measurements were made of the size of pollen grains from four diploid male plants, which were found to produce unreduced gametes, and, for comparison, from two diploid plants with a normal course of meiosis (Table 7).

Table 7

Pollen diameters in plants with normal meiosis and with failure of the second meiotic division

Pollen diameter in μ	12,5	15	17,5	20	22,5	25	27,5	30	32,5	35	37,5
Plant											
F ₂ 241/42 tetrads		3	13	24	10						
F ₂ 241/17 tetrads		5	24	17	3	1					
F ₂ 241/12 diads and tetrads			12	48	17	10	10	3			
F ₂ 241/11 diads and tetrads					7	22	36	19	11	3	1
F ₂ 241/15 diads			2	9	24	13	2				
F ₂ 241/12 diads					2	18	26	4			

As is to be seen the distribution series for the plants producing unreduced gametes is shifted to the right: in these plants pollen grains were larger. The reason of this shift in the distribution series was that besides the haploid pollen grains these plants also produced diploid ones of a larger diameter. In the plant F_2 241/11 some of the pollen grains were enormous reaching in size twice the diameter of the normal grains. Such enormous grains were probably the product of monads and had as many as 30 chromosomes; they presumably were unviable.

b) Cross 239 ($\text{♀ } R. \text{ thyrsiflorus}$ Puławy $\times \text{♂ } R. \text{ arifolius}$ Krościenko)

In F_1 mitosis was examined in 16 plants. The chromosome number was diploid (Fig. 68, 69). The chromosome sets were as follows:

$$\left. \begin{array}{l} \text{♀ } 2X \\ \text{♂ } X + 2Y \end{array} \right\} + j + v + 10i.$$

The course of meiosis was examined in three male plants. In all of these plants irregularities of meiosis leading to the formation of restitution nuclei and diads were found in some pollen mother cells. These disturbances being the most frequent in plant No. 17 the detailed examination of the course of meiosis in this individual made it possible to analyse the whole process of the formation of diploid gametes.

As is to be seen in Fig. 77 six bivalents and one trivalent were formed at diakinesis. Univalents were very seldom found. Metaphase I was normal. At anaphase I the parting of chromosomes was hindered chromosomes lying outside the spindle and chromatin bridges being rather frequent (Fig. 78, 79, 80). Sometimes the bivalents did not part or their parting was not completed and restitution nuclei were formed, usually with the characteristic constriction in the middle (Fig. 81, 82, Phot. 13, 14).

At metaphase of the second meiotic division of the restitution nucleus all of the 15 chromosomes ($X + 2Y + 12a$) were arranged in one plane forming a metaphase plate (Fig. 83, Phot. 18). The chromosomes then parted (Fig. 84, Phot. 15) giving rise to a diad (Fig. 85, Phot. 16, 17); each of the two gametes thus formed contained $15 = X + 2Y + 12a$ chromosomes.

The division of the restitution nuclei either preceded (Fig. 36), followed (Fig. 87, 88), or was synchronous (Fig. 89) with the stage of division of the neighbouring pollen mother cells.

When in the first meiotic division not one but two nuclei had been formed and this had been followed by the second division, then chromatin bridges were very frequent at metaphase II (those left over from the first division) and at anaphase II (those left over from the first division and the newly formed ones) (Fig. 90—95). When at anaphase II the chromosomes failed to part irregularities of this kind also led to the formation of diads (Fig. 94). Some enormous pollen grains were produced and they probably contained thirty instead of seven or eight chromosomes (Fig. 100). Sometimes owing to the presence of the chromatin bridges the pollen grains did not separate (Fig. 97—99).

In order to obtain some data on the frequency of these irregularities the number of diads and tetrads was counted in three F_1 plants and for comparison in one *R. thyrsiflorus* plant (Table 8).

As is to be seen diads were also formed in the pure species, but their frequency was much lower than in the F_1 cross. Among the F_1 popula-

68

69

70

71

72

73

74

75

76

Fig. 68—72. Root tips metaphases of F_1 and F_2 hybrids between *R. thyrsiflorus* Puławy and *R. arifolius* Krościenko (cross No. 239)

68 — F_1 ($14 = 2X + j + v + 10i$); 69 — F_1 ($15 = X + 2Y + j + v + 10i$); 70 — F_2 ($14 = 2X + 2v + 10i$); 71 — F_2 ($14 = 2X + j + 2v + 9i$); 72 — F_2 ($15 = X + 2Y + 2v + 10i$).

Fig. 73. Root tip metaphase of the backcross *R. thyrsiflorus* $\times F_1$ from the cross 239 ($14 = 2X + j + 3v + 8i$)

Fig. 74, 75. Root tips metaphases of the backcross F_1 (cross 239) $\times R. arifolius$

74 — ($14 = 2X + j + 3t + v + 7i$); 75 — ($14 = 2X + t + v + 10i$)

Fig. 76. Intersexual plant No. I from the cross 239 ($29 = 3X + 2Y + 3j + 3v + 18i$). Paraffin preparations. Magnific.: 1575 $\times$

Fig. 77—85. Meiosis in PMCs from the plant $F_1/17$ of the cross 239

77 — diakinesis; 78—80 — A I; 81, 82 — restitution nucleus; 83 — metaphase of restitution nucleus; 84 — anaphase of restitution nucleus; 85 — diad from the division of a restitution nucleus. Paraffin preparations. Magnific.: 1575 $\times$

86

87

88

89

Fig. 86—89. Meiosis in PMCs in a plant $F_1/17$ of the cross 239

86 — metaphase of restitution nucleus, beside interkinesis; 87 — metaphase of restitution nucleus, beside A II; 88 — metaphase of restitution nucleus, beside a tetrad; 89 — anaphase of restitution nucleus, beside A II. Paraffin preparations
Magnific.: 1575 $\times$

Fig. 90—95. Meiosis in PMCs in a plant $F_1/17$ of the cross 239

90, 91 — chromatin bridges at M II maintained from first division; 92—95 — A II with chromatin bridges persisting from first division

Fig. 96—100. Pollen from the plant $F_1/17$ of the cross 239

96 — normal pollen; 97—100 — pollen grains resulting from abnormal meiosis. Paraffin preparations. Magnific.: 1575 $\times$

Table 8

The frequencies of diads in *R. thyrsoflorus* and in F₁ hybrids of the cross 239

Plant	Diads	Tetrads	Total	% of diads
<i>R. thyrsoflorus</i>	2	315	317	0,6
F ₁ /5	5	127	132	3,8
F ₁ /13	3	132	135	2,0
F ₁ /17	161	920	1081	14,9

tion the diads were the most numerous in the plant F₁/17, which led to the supposition that the pollen grains of this plant would not be uniform. (Phot. 34). To check this supposition measurements were made of pollen grains of the plants F₁/5, F₁/13, F₁/17, *R. thyrsoflorus*, and *R. arifolius* (Table 9).

Table 9

Pollen diameters in *R. thyrsoflorus*, *R. arifolius* and in F₁ hybrids

Plant \ Pollen diameter in μ	15	17,5	20	22,5	25	27,5	30	32,5	35	37,5	40	42,5	45	N	M
F ₁ /5	1	2	3	7	9	8	4	1	2		1			38	25.65
F ₁ /13		1	3	10	10	6	1			1				32	24.10
F ₁ /17			1	10	23	25	23	19	8	6	12	6	3	136	30.80
<i>R. thyrsoflorus</i>	5	12	8	4	1									30	18.67
<i>R. arifolius</i>	6	8	7	6	3									30	19.91

The expected variability in the size of pollen grains was fully confirmed by the measurements. The pollen grains of the three individuals from the F₁ generation and in particular of plant F₁/17 were markedly larger than of the parental species.

Mitosis was examined in 35 plants of the F₂ generation. The chromosome composition of these plants was

$$\left. \begin{array}{l} \text{♀ } 2X \\ \text{♂ } X + 2Y \end{array} \right\} + 12a$$

the number of chromosomes of types j and v ranging 2 to 0 (Fig. 70, 71, 72).

Meiosis having been examined in some male F₂ plants it was found that the occurring irregularities led to the formation of restitution nuclei and the production of diploid gametes; these irregularities were not frequent.

In the backcross $F_1 \times R. arifolius$ mitosis was examined in 20 plants. The chromosome number in root tips was diploid (Fig. 74, 75). The irregularities in the course of meiosis of these plants were considerable.

Among the progeny of the backcross $R. thyrsiflorus \times F_1$ there was one plant with both male and female flowers: this plant was marked No. I. After checking the chromosome number it was found that the plant was

Fig. 101. Diagram of the origin of tetraploid intersexual plant No. I from the cross 239 with the karyotype $29 = 3X + 2Y + 3j + 3v + 18i$

a tetraploid with the karyotype $29 = 3X + 2Y + 3j + 3v + 18i$ (Fig. 76). The remaining 15 plants of this population had the diploid chromosome number (Fig. 73). The parental plants of the intersex must have, therefore, been diploids and, thus, the intersex must have developed out of unreduced gametes. The chromosome set of the intersex, which was $29 = 3X + 2Y + 3j + 3v + 18i$, showed that presumably it developed from a gamete produced by the female plant *R. thyrsiflorus* Puławy,

with the chromosome set $14 = 2X + 2j + 2v + 8i$, and from a gamete produced by the male plant of the F_1 generation with the chromosome set $15 = X + 2Y + j + v + 10i$.

Table 10
Progeny of the tetraploid plant No. I

No.	Year	Plant	Karyotype	Sex index	Sex	Pollen fertility
1	1959	I	$29 = 3X + 2Y + 3j + 3v + 18i$	1.33:1	σ^+	0%
2	1960	I/a	$29 = 3X + 2Y + 3j + 3v + 18i$	1.33:1	σ^+	not studied
3	1960	I/b	$22 = 2X + 2Y + 2j + 3v + 13i$	1.50:1	$* \sigma^+ \rightarrow \sigma^+$	0%
4	1961	I/14	$21 = 3X + 2v + 16i$	1.00:1	σ^+	not studied
5		I/10	$22 = 3X + Y + 3j + 2v + 13i$	1.00:1	—	" "
6		I/34	$21 = 2X + 2Y + 2j + 2v + 13i$	1.42:1	σ^+	0%
7		I/19	$20 = 2X + j + 3v + 14i$	1.50:1	—	not studied
8		I/37	$20 = 2X + 2j + 2v + 14i$	1.50:1	σ^+	" "
9		I/5	$21 = 2X + Y + j + 2v + 15i$	1.50:1	—	" "
10		I/40	$21 = 2X + Y + 2j + v + 15i$	1.50:1	$\sigma^+ \rightarrow \sigma^+$	0%
11		I/4	$21 = 2X + Y + 2j + 2v + 14i$	1.50:1	—	not studied
12		I/36	$22 = 2X + 2Y + j + v + 16i$	1.50:1	—	" "
13		I/11	$22 = 2X + 2Y + j + v + 16i$	1.50:1	—	" "
14		I/3	$22 = 2X + 2Y + j + 2v + 15i$	1.50:1	σ^+	" "
15		I/16	$22 = 2X + 2Y + j + 2v + 15i$	1.50:1	—	" "
16		I/21	$22 = 2X + 2Y + j + 2v + 15i$	1.50:1	$\sigma^+ \rightarrow \sigma^+$	0%
17		I/30	$22 = 2X + 2Y + 2j + 2v + 14i$	1.50:1	$\sigma^+ \rightarrow \sigma^+$	0.5%
18		I/6	$22 = 2X + 2Y + 2j + 3v + 13i$	1.50:1	σ^+	1.8%
19		I/13	$22 = 2X + 2Y + 2j + 3v + 13i$	1.50:1	σ^+	0.8%
20		I/35	$23 = 2X + 3Y + j + 2v + 15i$	1.50:1	—	not studied
21		I/20	$23 = 2X + 3Y + 2j + 2v + 14i$	1.50:1	—	" "
22		I/9	$22 = 2X + Y + j + v + 17i$	1.58:1	σ^+	0%
23		I/39	$22 = X + 3Y + 2j + v + 15i$	3.00:1	—	not studied
24		I/31	$23 = X + 4Y + j + v + 16i$	3.00:1	σ^+	not formed
25		I/8	$23 = X + 4Y + j + 2v + 15i$	3.00:1	—	not studied
26		I/32	$24 = 5(X + Y) + 2j + 3v + 14i$	—	—	" "

* Plants with sex index 1.5:1, although form only male flowers, morphologically resemble intersexes.

The irregularities leading to the formation of restitution nuclei and, thus, giving unreduced gametes were fully described for the plant $F_1/17$. The chromosome analysis of the tetraploid in question showed that the male gamete must have had the formula $15 = X + 2Y + j + v + 10i$. Such a gamete could have developed only if the first meiotic division had been obstructed to give a restitution nucleus. (If the second meiotic division had been abolished the male plant would have produced gametes with the chromosome set $14 = 2X + 12a$ or $16 = 4X + 12a$). In the case of this intersex it was also possible to establish which chromosomes came from *R. thyrsoflorus* and which from *R. arifolius*. From the scheme showing how this tetraploid intersex developed (Fig. 101) it was possible to establish that the chromosomes $3X + 3j + 3v + 12i$ were derived from *R. thyrsoflorus* and the chromosomes $2Y + 6i$ from *R. arifolius*.

In 1959 the tetraploid plant No. I was pollinated with pollen from *R. thyrsoflorus*: this cross produced only two well developed seeds, which gave two plants No. I/a and I/b. In 1960 the same plant gave 65 well developed seeds from free pollination: from these seeds 32 plants were obtained, but 9 of them perished soon after they were transferred to the experimental plot.

The chromosome sets and the sex index, i. e. the ratio of the number of autosomes to the number of X-chromosomes ($2A : X = 2 : 1$, $2A : 2X = 1 : 1$), established for the progeny of the plant No. I are listed in Table 10 together with the sex and the pollen fertility of the particular plants. The karyotypes of these plants are shown in Fig. 102—119 and Phot. 21—24.

In 1961 isolating bags were placed on 15 flower shoots of the plant No. I: 37 well developed seeds were obtained from self-pollination. Out of nine of these seeds 7 germinated on a Petri dish. These plants had the following karyotypes:

Karyotypes	Number of plants
$21 = 2X + Y + j + 2v + 15i$	1
$22 = 2X + j + 2v + 17i$	1
$23 = 2X + 2Y + j + v + 18i$	2
$23 = X + Y + 2j + 3v + 16i$	1
$29 = 3X + 2Y + 3j + 3v + 18i$	2

Since it was found that plant No. I produced no fertile pollen it seems that the seeds on it must have developed in the course of apomixis. This supposition was supported by the fact that two of the plants in the progeny obtained from the self-pollination of plant No. I had the karyotype identical with the parent. The question whether this

Fig. 102—107. Root tips metaphases from polyploid plants (cross 239)

102 — plant I/a ($3X + 2Y + 3j + 3v + 18i$); 103 — plant I/b ($2X + 2Y + 2j + 3v + 13i$); 104 — plant I/10 ($3X + Y + 3j + 2v + 13i$); 105 — plant I/19 ($2X + j + 3v + 14i$); 106 — plant I/37 ($2X + 2j + 2v + 14i$); 107 — plant I/5 ($2X + Y + j + 2v + 15i$). Fig. 102: paraffin preparation; Fig. 103—107: smears. Magnific.: 1575 $\times$

Fig. 108—113. Root tips metaphases from polyploid plants (cross 239)

108 — plant I/40 ($2X + Y + 2j + v + 15i$); 109 — plant I/4 ($2X + Y + 2j + 2v + 14i$); 110 — plant I/11 ($2X + 2Y + j + v + 16i$); 111 — plant I/16 ($2X + 2Y + j + 2v + 15i$); 112 — plant I/30 ($2X + 2Y + 2j + 2v + 14i$); 113 — plant I/13 ($2X + 2Y + 2j + 2v + 13i$). Smears. Magnific.: 1575 $\times$

Fig. 114—119. Root tips metaphases from polyploid plants (cross 239)

114 — plant I/35 ($2X + 3Y + j + 2v + 15i$); 115 — plant I/20 ($2X + 3Y + 2j + 2v + 14i$); 116 — plant I/9 ($2X + Y + j + v + 17i$); 117 — plant I/39 ($X + 3Y + 2j + v + 15i$); 118 — plant I/8 ($X + 4Y + j + 2v + 15i$); 119 — plant I/32 ($5XY + 2j + 3v + 14i$). Smears. Magnific.: 1575 $\times$

actually was a case of apomixis or not could be finally solved only by suitable embryological examinations.

c) Cross 240 (*R. arifolius* Krościenko $\times$ *R. thyrsiflorus* Puławy)

Among the investigated eight plants of the F_1 generation the following karyotypes were found:

Karyotypes	Number of plants
$14 = 2X + 12a$	6
$15 = X + 2Y + 12a$	1
$29 = 3X + 2Y + 2j + 2v + 20i$	1

Judging from the morphological traits the other plants of the F_1 generation also were diploids.

The teraploid individual was found to be an intersex: it was marked No. II. This plant probably developed from a diploid gamete of the female plant *R. arifolius* with the chromosome set $14 = 2X + 12i$ and a diploid gamete of the male plant *R. thyrsiflorus* with the chromosome set $15 = X + 2Y + 2j + 2v + 8i$. This plant thus had 12 autosomes derived from *R. arifolius* and 12 from *R. thyrsiflorus*; moreover, $2X$ came from *R. arifolius* and $X + 2Y$ from *R. thyrsiflorus*.

Table 11
Progeny of the tetraploid plant No. II

No.	Plant	Kariotype	Sex index	Sex	Pollen fertility
1	II	$29 = 3X + 2Y + 2j + 2v + 20i$	1.33:1	♂ +	0%
2	II/11	$29 = 3X + 2Y + 2j + 2v + 20i$	1.33:1	♂ +	not studied
3	II/6	$21 = 2X + Y + 2j + v + 15i$	1.50:1	♂ +	0%
4	II/4	$22 = 2X + 2Y + j + v + 16i$	1.50:1	♂ +	not studied
5	II/13	$22 = 2X + 2Y + j + 2v + 15i$	1.50:1	♂ +	0%
6	II/8	$22 = 2X + 2Y + 2j + v + 15i$	1.50:1	♂ +	1,8%
7	II/13	$22 = 2X + 2Y + 2j + v + 15i$	1.50:1	♂ +	0%
8	II/10	$22 = 2X + 2Y + 2j + 2v + 14i$	1.50:1	♂ +	0%
9	II/5	$22 = 2X + Y + v + 18i$	1.58:1	♂ +	0%
10	II/16	$23 = X + 4Y + 2j + 3v + 13i$	3.00:1	♂	not studied
11	II/7	$24 = X + 3Y + 2j + v + 17i$	3.33:1	♂	not formed

Fig. 120—125. Root tips metaphases from plants of the cross 240

120 — plant II ($3X + 2Y + 2j + 2v + 20i$); 121 — plant F_1 ($14 = 2X + 12a$); 122 — plant II/6 ($2X + Y + 2j + v + 15i$); 123 — plant II/11 ($3X + 2Y + 2j + 2v + 20i$); 124 — plant II/10 ($2X + 2Y + 2j + 2v + 14i$); 125 — plant II/4 ($2X + 2Y + j + v + 16i$). Smears. Magnific.: 1575 $\times$

Fig. 126—128. Root tips metaphases from polyploid plants (cross 240)

126 — plant II/5 ($2X + Y + v + 18i$); 127 — plant II/16 ($X + 4Y + 2j + 3v + 13i$); 128 — plant II/7 ($X + 3Y + 2j + v + 17i$). Smears. Magnific.: $1575 \times$

In 1960 the plant No. II produced 100 well developed seeds from free pollination: of these 30 were planted giving 19 plants. The karyotypes of the plants were determined and their sex indexes were calculated. These data together with the sex and the pollen fertility of the particular plants are assembled in Table 11. The karyotypes are shown in Fig. 120—128 and Phot. 25.

Tre chromosome numbers of the remaining nine plants ranged from 22 to 29.

3. Meiosis in Triploid and Tetraploid Plants

a) Triploids

Meiosis was examined in three individuals with following chromosome sets:

$$1/6 \quad 22 = 2X + 2Y + 2j + 3v + 13i$$

$$1/13 \quad 22 = 2X + 2Y + 2j + 3v + 13i$$

$$1/b \quad 22 = 2X + 2Y + 2j + 3v + 13i$$

All these three individuals were the progeny of the plant No. I the karyotype of which was $29 = 3X + 2Y + 3j + 3v + 18i$.

At diakinesis and anaphase I the conjugation of chromosomes was not complete univalents, bivalents, and rare trivalents being formed. The number of univalents in one PMC ranged from two to six and of bivalents from seven to ten; the trivalents were very rare. At anaphases I and II the parting of chromosomes was very difficult, many chromatin bridges were formed, and chromosomes lying outside the spindle were frequent. The result of these irregularities was that diads, triads, pentads, and hexads were formed instead of the regular tetrads; the frequencies of these abnormalities are listed in Table 12.

Table 12

Disturbances in the microsporogenesis in triploid hybrids between
R. thyrsoflorus and *R. arifolius*

Plant	Diads	Triads	Tetrads	Pentads	Hexads	Total
I/6	13	26	75	10	1	125
	10.4%	28%	60%	8%	0.8%	
I/13	8	27	84	16	1	136
	5.9%	19.8%	61.8%	11.8%	0.7%	
I/b	7	16	86	13	2	124
	5.6%	12.9%	69.4%	10.5	1.6%	

In the examined triploid plants pollen mother cells with the meiotic process completely disorganized were quite frequently found. In such cells there were about 40 univalents distributed at random throughout the cell while alongside tetrads had already developed. Such cell degenerated and did not form pollen grains.

The chromosome number in the gametes of these triploids ranged

6 to 21. In the 12 analysed metaphase plates in pollen grains from the individual No. I/6 the following chromosome sets were found:

Number of chromosomes	Number of plates	Number of chromosomes	Number of plates
$10 = 2X + 8a$	1	$6 = X + Y + 6a + \text{fragm.}$	1
$12 = 2X + 10a$	1	$21 = X + 2Y + 18a$	1
$8 = X + 7a$	1	$12 = X + 2Y + 9a$	1
$11 = X + 10a$	2	$12 = Y + 11a$	1
$12 = X + 11a$	1	$14 = 2Y + 12a$	1
$13 = X + 12a$	1		

As is to be seen from this list the number of X- and Y chromosomes ranged from 0 to 2.

The course of meiosis in the plants No. I/13 and No. I/b was on the whole similar as in the individual No. I/6.

A more detailed analysis of the behaviour of the sex chromosomes X and Y in PMCs was impossible because the buds of the triploid plants did not fix well and it was difficult to obtain good slides.

b) Tetraploids

Plants No. I and II with the karyotype $29 = 3X + 2Y + 24a$

The course of meiosis in plant No. I was very irregular. At diakinesis and anaphase I hardly any bivalents were formed and more than 20 univalents could be found (Phot. 19). In the majority of cases the further stages of meiosis never occurred, the chromosomes parted forming several groups, and the pollen mother cells degenerated (Phot. 20).

Besides, some data about the segregation of X-chromosomes could be deduced from the chromosomal constitution of progenies of these plants. If the X-chromosomes segregate at random one can calculate following frequencies of zygotes after pollination with pollen of diploid plants:

	Calculated frequencies in %	Obtained frequencies in %
Zygotes with: no X	6,25	—
1X	25,00	15,20
2X	37,50	78,40
3X	25,00	6,30
4X	6,25	—

It seems that in the tetraploid plants No. I and No. II the segregation of the X-chromosomes was almost always of the type X — 2X.

Plant No. F₃ 241/75, with the karyotype $30 = 2X + 4Y + 24a$

As has been stated previously this plant developed from the union of unreduced gametes produced by F₂ plants in the progeny of the Cross 241.

Fig. 129—148. Meiosis in PMCs from the F_3 tetraploid plant of the cross 241 ($30 = 2X + 4Y + 24a$)
 129—134 — diakinesis (uni-, bi- and polyvalents); 135—143 — trivalents; 144—148 — tetrads and polyvalents. Paraffin
 preparations. Magnific.: 1575 $\times$

The unreduced gametes were the result of the absence of the second meiotic division and it, therefore, seems plausible to suppose that in this tetraploid two of the four homologous chromosomes could have come from *R. arifolius* and two from *R. thyrsiflorus*, or that all four came from only one of the parental species. It follows, therefore, that at diakinesis and anaphase I the most frequent configurations should have been bivalents or tetravalents. The analysis of the diakinesis stage showed, however, that the most frequent were the univalents, bivalents, and trivalents, whereas tetravalents and polyvalents of higher orders were relatively rare (Fig. 129—148, Phot. 27). At metaphase I the bivalents and multivalents arranged themselves on the plane of the equator and the spindle was formed (Fig. 149—151). The parting of chromosomes at anaphase I was difficult and some of them remained on the spindle without passing to the poles (Fig. 152—155, Phot. 28). Sometimes the bivalents and the multivalents did not part and all moved to one of the poles (Fig. 155). Owing to the irregularities in the parting of chromosomes at anaphase I either one restitution nucleus or more than two nuclei were formed at interkinesis.

Sometimes at anaphase II some of the chromosomes were not included in the plates (Fig. 163—165, Phot. 30) or instead of two, three plates appeared (Fig. 166); there also were bridges between the plates (Fig. 162).

Quite frequently chromatin bridges were formed at anaphase II and occasionally some chromosomes were lagging on the spindle and not moving to the poles (Fig. 167, 168, Phot. 29).

The result of these irregularities was that diads, triads, pentads, hexads, septads, and even octads instead of the regular tetrads were the final products of meiosis (Fig. 169—175, Phot. 31, Table 13).

From the data in this table it is to be seen that in the plant F_3 242/75 the most frequent besides the tetrads were the pentads and the hexads.

Table 13

Disturbances in the microsporogenesis in a diploid and tetraploid plants from F_3 of the cross no. 241

Plant	Diads	Triads	Tetrads	Pentads	Hexads	Septads	Octads	Total
F_3 241/75 $30 = 2X + 4Y + 24a$	2 1%	6 3%	80 40.4%	70 35.4%	30 15.2%	8 4%	2 1%	198
F_3 241/23 $15 = X + 2Y + 12a$		1 0.6%	174 99.4%					175

The analysis of meiosis in the plant F_3 241/75 was made with special attention to the behaviour of the sex chromosomes. The F_1 progeny was obtained by crossing a female plant *R. arifolius* producing gametes $X + 6a$ with a male plant *R. thyrsiflorus* producing gametes $X + 6a$

Fig. 149 — 164. Meiosis in PMCs from tetraploid F_3 plant (cross 241)

149—151 — M I; 152 — 155 — A I with lagging bivalents; 156 — 161 — interkinesis; 162 — 164 — different disturbances at M II. — 2800 $\times$; paraffin preparations

Fig. 165 — 176- Meiosis in PMCs from tetraploid F_3 plant of the cross no. 241 ($30 = 2 \times + 2y + 24a$)
 165, 166 — different disturbances at M II; 167, 168 — A II; 169 — 175 — tetrads, pentads, sexads, septads and octads;
 176 — complete breakdown of meiosis. — 2800 $\times$; paraffin preparations

and $2Y + 6a$. In F_1 and in the further generations of crosses all the Y-chromosomes were, therefore, derived from *R. thyrsiflorus*, whereas the X-chromosomes could come either from *R. thyrsiflorus* or *R. arifolius*.

The plant F_3 241/75 thus obtained all the four Y's from *R. thyrsiflorus* consisting of two identical pairs. In meiosis the Y-chromosomes should, therefore, conjugate with each other in pairs, forming bivalents, or with the corresponding ends of the X-chromosomes. In this way the sex chromosomes could form any configuration, from bivalents to hexavalents.

Owing to various technical difficulties (the large number of chromosomes and the impossibility to obtain well fixed slides) the observations were incomplete and the error in the results may be considerable. It was, however, possible to establish that $Y + X + Y$ and $X + X$ were the more frequent combinations; tetravalents $Y + X + X + Y$ and pentavalents were also found (Fig. 130, 131, 132, 134, 146, 147, 148).

To obtain more information about what happened with the sex chromosomes after meiosis 12 metaphase plates of the somatic divisions of pollen were analysed:

Number of chromosomes	Number of plates
$14 = 2Y + 12a$	2
$18 = 2Y + 16a$	1
$12 = X + 11a$	1
$12 = X + Y + 10a$	1
$14 = X + 2Y + 11a$	1
$15 = X + 2Y + 12a$	1
$14 = X + 3Y + 10a$	2
$12 = 2X + Y + 9a$	1
$14 = 2X + Y + 11a$	1
$17 = 2X + Y + 14a$	1

V. THE DETERMINATION OF SEX

The cytological analysis of *R. arifolius*, *R. thyrsiflorus*, and of the polyploids found among the hybrids of these two species allowed to make some conclusions on the nature of the mechanism of sex determination in these species.

Table 14 lists karyotypes, sex indexes and sex of different diploid and polyploid plants.

The data listed in Table 14 seems to indicate that the Y-chromosomes played no part in the determination of the sex of the investigated species. The sex of a plant depended not on the presence or the absence of

the Y chromosome, but on the ratio of the autosomes to the X-chromosomes, i. e. on the sex index. In female plants the index equalled or was less than 1:1 and in the male plants it equalled or was higher than 2:1; when the value of the index was between 1:1 and 2:1 the plant was an intersex. E. g. the three plants with the karyotypes 3A + XX,

Table 14

Karyotype, sex and sex indexes of *R. thyrsiflorus*, *R. arifolius* and of their polyploid hybrids

No	Plant	Karyotype	Sex	Sex index
1	<i>R. thyrsiflorus</i>	2A+XX	♀	1:1
2	<i>R. thyrsiflorus</i>	2A+XY Y	♂	2:1
3	<i>R. arifolius</i>	2A+XX	♀	1:1
4	<i>R. arifolius</i>	2A+XY Y	♂	2:1
5	Polyploid hybrids	3A+XXX	♀	1:1
6		3A+XX	♂ ₊	1.5:1
7		3A+XXY	♂ ₊	1.5:1
8		3A+XXY	♂ ₊ → ♂ ₊	1.5:1
9		3A+XXYY	♂ ₊	1.5:1
10		3A+XXYY	♂ ₊ • ♂ ₊	1.5:1
11		3A+XYYY Y	♂	3:1
12		4A+XXXX	♀	1:1
13		4A+XXYYYY	♂	2:1
14		4A+XXXYY	♂ ₊	1.33:1
15		4A+XXXYY	♀ → ♂ ₊	1.33:1
16		2A+5+XXYY	♂ ₊	1.42:1
17		3A+1+XXY	♂ ₊	1.58:1
18		3A+2+XYYY	♂	3.33:1

3A + XXY, and 3A + XXYY all had the same sex index 1,5 : 1 and were all intersexes regardless of either the number of the Y-chromosomes or their absence in the chromosome set. Sometimes only male flowers were produced by the individuals with such karyotypes but even so the morphological structure of the inflorescences and of the flower

buds resembled the intersexes: we may, thus, assume that these instances did not contradict the general principle.

The conclusion here made ought to be valid for both *R. thyrsoiflorus* and *R. arifolius*, since the polyploid plants obtained experimentally were a combination of both these species: if one of the parental species had a different mechanism of sex determination, e.g. X/Y, then entirely different results would have been obtained in the experiments.

In the case of the experimentally obtained polyploid plants, for which the chromosome sets had been established, the number of female, bisexual, and male flowers on each individual was counted and from these counts their index of intersexuality was calculated in the same way as had been done by Ono (1935). It was assumed that when a plant had only male flowers its index was — 100, when it had only female flowers its index was 100, and when half of the flowers were male and half female or all the flowers were bisexual the index was 0.

When the number of male flowers amounted to 40 per cent and of the bisexual ones to 60 per cent the index was 40. When the proportions between the differently sexed flowers were 10 per cent male, 70 per cent bisexual, and 20 per cent female, the index was 10, but when the proportions were 20 per cent male, 70 per cent bisexual, and 10 per cent female, the index was — 10.

In 19 out of the 24 polyploid plants listed in Table 15 the index of intersexuality agreed with the sex as established from the karyotype.

The plants No. I/b, I/40, I/21, and I/30 produced only male flowers though the value of the sex index for these four plants was 1,5 : 1. The sex index of plant No. II/11 was 1,33 : 1, but the plant produced only female flowers.

Morphologically and structurally the inflorescences and the flower buds of the individuals I/b, I/40, I/21, and I/30 resembled the intersexes: the flower buds of these plants were much smaller than in the diploid male individuals.

The magnitude of these deviations from the expected sexual condition of the polyploid plants being significant it seems probable that the irregularities were caused by genetic factors and were not merely due to phenotypic variability. Probably the distribution of the genes for maleness and possibly also for the femaleness in the autosomes was not uniform and their particular combinations could have caused the segregation of the abnormal types described above.

The comparison of the karyotypes of the other plants listed in Table 15 with the intersexuality index of these plants confirmed this supposition. The variability of the frequency with which male, female, and bisexual flowers occurred on individuals with the same sex index was too high to have been caused only by external factors. The index

Table 15

Karyotypes, sex index, sex and intersexuality index of polyploid hybrids *R. thyrsiflorus* x *R. arifolius*

No.	Plant	Karyotype	Sex index	Sex	Flowers				Intersexuality index
					bottom of the inflorescence	middle of the inflorescence	top of the inflorescence	total	
1	2	3	4	5	6	7	8	9	10
1	I	29 = 3X + 3Y + 3j + 3v + 18i	1.33:1	♀ ♂ + ♂	29 = 87.9 ⁰ / ₁₀₀ — 4 = 12.1 ⁰ / ₁₀₀	39 = 83 ⁰ / ₁₀₀ — 8 = 17.0 ⁰ / ₁₀₀	46 = 97.9 ⁰ / ₁₀₀ — 1 = 2.1 ⁰ / ₁₀₀	104 = 88.9 ⁰ / ₁₀₀ — 13 = 11.1 ⁰ / ₁₀₀	+ 77.8
2	I/b	22 = 2X + 2Y + 2j + 3v + 13i	1.50:1	♀ ♂ + ♂	— — 31 = 100 ⁰ / ₁₀₀	— — 31 = 100 ⁰ / ₁₀₀	— — 39 = 100 ⁰ / ₁₀₀	— — 101 = 100 ⁰ / ₁₀₀	—100
3	I/14	21 = 3X + 2v + 16i	1.00:1	♀ ♂ + ♂	17 = 100 ⁰ / ₁₀₀ — —	15 = 100 ⁰ / ₁₀₀ — —	16 = 100 ⁰ / ₁₀₀ — —	48 = 100 ⁰ / ₁₀₀ — —	+100
4	I/34	21 = 2X + 2Y + 2j + 2v + 13i	1.42:1	♀ ♂ + ♂	51 = 100 ⁰ / ₁₀₀ — —	29 = 97.5 ⁰ / ₁₀₀ 1 = 2.5 ⁰ / ₁₀₀ —	25 = 80.6 ⁰ / ₁₀₀ 6 = 19.4 ⁰ / ₁₀₀ —	115 = 94.3 ⁰ / ₁₀₀ 7 = 5.7 ⁰ / ₁₀₀ —	+ 88.6
5	I/37	20 = 2X + 2j + 2v + 14i	1.50:1	♀ ♂ + ♂	8 = 100 ⁰ / ₁₀₀ — —	11 = 100 ⁰ / ₁₀₀ — —	7 = 100 ⁰ / ₁₀₀ — —	26 = 100 ⁰ / ₁₀₀ — —	+100
6	I/40	21 = 2X + Y + 2j + v + 15i	1.50:1	♀ ♂ + ♂	— — 16 = 100 ⁰ / ₁₀₀	— — 34 = 100 ⁰ / ₁₀₀	— — 25 = 100 ⁰ / ₁₀₀	— — 75 = 100 ⁰ / ₁₀₀	—100
7	I/3	22 = 2X + 2Y + j + 2v + 15i	1.50:1	♀ ♂ + ♂	1 = 3.8 ⁰ / ₁₀₀ 25 = 96.2 ⁰ / ₁₀₀ —	13 = 33.3 ⁰ / ₁₀₀ 26 = 66.7 ⁰ / ₁₀₀ —	3 = 13.0 ⁰ / ₁₀₀ 20 = 87 ⁰ / ₁₀₀ —	17 = 19.3 ⁰ / ₁₀₀ 71 = 80.7 ⁰ / ₁₀₀ —	— 80.7

1	2	3	4	5	6	7	8	9	10
8	I/21	$22 = 2X + 2Y + 2j + 2v + 14i$	1.50:1	♀ ♂ + ♂	— — $23 = 100\%$	— — $21 = 100\%$	— — $32 = 100\%$	— — $86 = 100\%$	—100
9	I/30	$22 = 2X + 2Y + 2j + 2v + 14i$	1.50:1	♀ ♂ + ♂	— — $19 = 100\%$	— — $21 = 100\%$	— — $30 = 100\%$	— — $70 = 100\%$	—100
10	I/6	$22 = 2X + 2Y + 2j + 3v + 13i$	1.50:1	♀ ♂ + ♂	— — $28 = 80\%$ $7 = 20\%$	— — $28 = 68.3\%$ $13 = 31.7\%$	— — $25 = 69.4\%$ $11 = 30.6\%$	— — $81 = 72.3\%$ $31 = 27.7\%$	— 27.7
11	I/13	$22 = 2X + 2Y + 2j + 3v + 13i$	1.50:1	♀ ♂ + ♂	— — $10 = 52.6\%$ $9 = 47.4\%$	— — $14 = 43.7\%$ $18 = 56.3\%$	— — $9 = 25\%$ $27 = 75\%$	— — $33 = 37.9\%$ $54 = 62.1\%$	— 62.1
12	I/9	$22 = 2X + Y + j + v + 17i$	1.58:1	♀ ♂ + ♂	— — $3 = 18.8\%$ $13 = 81.2\%$	— — $21 = 100\%$	— — $3 = 9.7\%$ $28 = 90.3\%$	— — $6 = 8.8\%$ $62 = 91.2\%$	— 91.2
13	I/31	$23 = X + 4Y + j + v + 16i$	3.00:1	♀ ♂ + ♂	— — $22 = 100\%$	— — $33 = 100\%$	— — $43 = 100\%$	— — $98 = 100\%$	—100
14	II*	$29 = 3X + 2Y + 2j + 2v + 20i$	1.33:1	♀ ♂ + ♂	— — —	— — —	— — —	— — $98 = 98\%$ $2 = 2\%$	— 2
15	II/11	$29 = 3X + 2Y + 2j + 2v + 20i$	1.33:1	♀ ♂ + ♂	$23 = 100\%$ — —	$17 = 100\%$ — —	$23 = 100\%$ — —	$63 = 100\%$ — —	+100
16	II/6	$21 = 2X + Y + 2j + v + 15i$	1.50:1	♀ ♂ + ♂	— — $32 = 100\%$	— — $1 = 2.3\%$ $42 = 97.7\%$	— — $35 = 100\%$	— — $1 = 0.9\%$ $110 = 99.1\%$	— 99.1

1	2	3	4	5	6	7	8	9	10
17	II/4	22 = 2X + 2Y + j + v + 16i	1.50:1	♀ ♂ + ♂	— 10 = 27.8% 26 = 72.2%	— 10 = 28.6% 25 = 71.4%	— 2 = 7.7% 24 = 92.3%	— 22 = 22.7% 75 = 77.3%	— 77.3
18	II/13	22 = 2X + 2Y + j + 2v + 15i	1.50:1	♀ ♂ + ♂	— 15 = 62.5% 9 = 37.5%	— 20 = 71.4% 8 = 28.6%	— 12 = 70.6% 5 = 29.4%	— 57 = 72.2% 22 = 27.8%	— 27.8
19	II/8	22 = 2X + 2Y + 2j + 2v + 15i	1.50:1	♀ ♂ + ♂	— 20 = 62.5% 12 = 37.5%	— 11 = 42.3% 15 = 57.7%	— 7 = 21.9% 25 = 78.1%	— 38 = 42.2% 52 = 57.8%	— 57.8
20	II/3	22 = 2X + 2Y + 2j + v + 15i	1.50:1	♀ ♂ + ♂	— 33 = 52.4% 30 = 47.6%	— 13 = 28.3% 33 = 71.7%	— 11 = 22.4% 38 = 77.6%	— 57 = 36.1% 101 = 63.9%	— 63.9
21	II/0	22 = 2X + 2Y + 2j + 2v + 14i	1.50:1	♂ ♂ + ♂	— 34 = 100%	— 42 = 100%	— 43 = 93.5% 3 = 6.5%	— 119 = 97.5% 3 = 2.5%	— 2.5
22	II/5	22 = 2X + Y + v + 18i	1.58:1	♀ ♂ + ♂	— 31 = 96.9% 1 = 3.1%	— 47 = 92.2% 4 = 7.8%	— 34 = 94.4% 2 = 5.6%	— 114 = 94.2% 7 = 5.8%	— 5.8
23	II/16	23 = X + 4Y + 2j + 3v + 13i	3.00:1	♀ ♂ + ♂	— 16 = 100%	— 28 = 100%	— 25 = 100%	— 65 = 100%	—100
24	II/7	24 = X + 3Y + 2j + v + 17i	3.33:1	♀ ♂ + ♂	— 19 = 100%	— 31 = 100%	— 30 = 100%	— 80 = 100%	—100

* The inflorescence was too short to divide into 3 parts.

** With intersexual habitus

of intersexuality in plants with the sex index equal 1,5 : 1 ranged — 99.1 to 88.6 and in plants with the sex index equal 1.33 : 1 it ranged — 2 to 77.8.

Another noteworthy circumstance in this connection is a certain regularity manifested by the distribution of the female and male flowers on some intersex plants: near the top of the inflorescences the number of the male flowers increased and the number of female flowers decreased. On the average the proportion of the male flowers in the lower part of the inflorescences of these intersexes was 43.9 per cent, in the middle of the inflorescences 54.7 per cent, and near the top 53.8 per cent.

Summing up it seems that in *R. thyrsiflorus* and *R. arifolius* the factors determining the male sex are carried by the autosomes, whereas the factors determining the female sex are contained in the X-chromosomes and probably also in the autosomes. The Y-chromosomes do not contain sex-determining genes.

When the karyotype of an individual is $2X + 12a$ the factors determining the female sex predominate and the individual is female. The plants with the karyotype $X + 2Y + 12a$ are male because in this case the factors determining the male sex are dominant.

Mention must still be made here of the sex ratio, in the genus *Rumex*, i. e. of the proportions between the female and male individuals in a population.

Even superficial observations showed that in *R. thyrsiflorus* and *R. arifolius* the female individuals were much more numerous than the male ones. Counts of the female and male individuals made in natural populations of *R. thyrsiflorus*, *R. arifolius*, and *R. acetosa* as well as on the experimental plots fully confirmed these provisional observations. The relevant data are listed in Table 16.

These data indicate that in all instances the female plants were much more numerous than the male ones. For *R. acetosa* the ratio of the male to the female plants was in the natural populations 29,1 : 70,9, for *R. thyrsiflorus* this ratio was somewhat lower, i. e. 25.6 : 74.4, and for *R. arifolius* it was the lowest, i. e. 7.2 : 92.8. For the crosses the ratio ranged 3.1 : 96.9 to 39.8 : 60.2. The researches of Ono (1935) and Yamamoto (1938) on *R. acetosa* and the associated with these researches, investigations on *R. thyrsiflorus* and *R. arifolius* showed that the male plants of these species produced pollen determining the male and the female sexes in equal amounts. There must be, therefore, some mechanism which causes the great differences between the numbers of the male and the female plants and the deviation of this ratio from the expected value of 1 : 1.

In the course of this investigation chromosome count in root tips of 90 germinated seeds of *R. thyrsiflorus* from Ząbki were made to determine the karyotype. Among these plants 25 had the karyotype $2X + 12a$ and only five the karyotype $X + 2Y + 12a$. This means that the domination in the natural populations of the female plants was caused either by the degeneration of a certain amount of the zygotes

Table 16

The numbers of male and female individuals in *F. thyrsiflorus*, *R. arifolius*, *R. acetosa* and in hybrids *R. thyrsiflorus* $\times$ *arifolius*

Population	♂	♂ +	♀	%		
				♂	♂ +	♀
<i>R. thyrsiflorus</i> Ząbki	224	—	651	25.6	—	74.4
<i>R. thyrsiflorus</i> (different populations cultivated)	29	—	249	10.4	—	89.6
<i>R. arifolius</i> Bieszczady	9	1	45	16.4	1.8	81.6
<i>R. arifolius</i> Gorze	1	4	115	0.8	3.3	95.9
<i>R. arifolius</i> Gorze	4	—	85	4.5	—	95.5
Total	14	5	245	5.3	1.9	92.8
<i>R. arifolius</i> (different cultivated populations)	7	—	28	20.0	—	80.0
<i>R. acetosa</i> Ząbki	794	—	1937	29.1	—	70.9
<i>R. acetosa</i> (different cultivated populations)	6	—	23	26.9	—	73.1
Cross 239 — F ₁	8	—	43	15.7	—	84.3
F ₂	69	—	592	10.4	—	89.6
F ₁ $\times$ <i>R. arifolius</i>	16	—	164	8.9	—	91.1
<i>R. thyrsiflorus</i> $\times$ F ₁	60	1	150	28.4	0.5	71.1
Total	153	1	949	13.9	0.1	86.0
Cross 240 — F ₁	3	1	25	10.3	3.4	86.3
Cross 241 — F ₁	33	—	50	39.8	—	60.2
F ₂	26	—	121	17.7	—	82.3
F ₃	16	—	43	27.1	—	72.9
Total	75	—	214	26.0	—	74.0
Cross 313 — F ₁	5	—	124	3.9	—	96.1
F ₂	4	—	127	3.1	—	96.9
F ₃	1	—	10	9.1	—	90.9
Total	10	—	261	3.7	—	96.3

$X + 2Y + 12a$, or by a certain degree of selective pollination. In the latter case it is plausible to suppose that the pollen tubes bearing the female sex determinants germinate earlier and grow quicker thus fertilizing the egg-cells more frequently. A similar interpretation of this effect was also advanced by Lewis (1942).

VI. DISCUSSION

As has been said at the beginning of this work many authors have found in the natural populations of *R. acetosa* single, tri- and tetraploid individuals with the following karyotypes:

♀ $3n = 21$ ($3X + 18a$)	Ono (1928), Yamamoto (1938), Kihara and Yamamoto (1931), Kihara and Yamamoto (1931),
♂ $3n$	Kihara and Yamamoto (1931),
♂ $3n = 22$ ($2X + 2Y + 18a$)	Ono and Shimotomai (1923), Ono (1929), Kihara and Yamamoto (1931), Yamamoto (1931), Ono (1935), Yamamoto (1938), Löve (1942),
♂ $4n = 29$ ($3X + 2Y + 24a$)	Ono and Shimotomai (1928).

Investigating the incidence of intersexes in the natural *R. acetosa* populations Takenaka (1955) found 3328 female diploids, 1960 male diploids, and seven intersexes: this means that for 300 male plants there was one intersex. Of these seven intersexes six had the karyotype $3n = 22$ ($2X + 2Y + 18a$) and one $4n = 29$ ($3X + 2Y + 24a$). Takenaka thinks that in the natural *R. acetosa* populations the polyploid female plants probably are more numerous than the intersexes, since the union $14(2X + 12a) \times 7(X + 6a)$ is more frequent than the union $14(2X + 12a) \times 8(2Y + 6a)$, but that the female triploids having the same phenotype as the normal female plants are very difficult to identify.

In natural populations in Poland only few polyploid intersexes were found. The mechanism of the formation of the diploid gametes has been explained neither by Takenaka nor by other workers. Only Ono (1929) having found in *R. acetosa* PMCs diploid M II plates suggested that they could have been the source of the diploid gametes and thus of the triploid and tetraploid plants.

From the results obtained in the present investigation it is possible to attempt a more detailed explanation of this question.

The observations have revealed that there are two ways in which diploid gametes can be formed: a) by the abolition of the first meiotic division in PMCs and b) by the abolition of the second meiotic division in PMCs. Examinations of the cytological preparations have shown that in the first instance the chromosome set of the diploid pollen is almost always $X + 2Y + 12a = 15$ and in the second $2X + 12a = 14$ or $4Y + 12a = 16$.

The course of macrosporogenesis was not investigated, but since the karyotype of the female plants always is $14 = 2X + 12a$, the diploid

gametes must always have the chromosome set $14 = 2X + 12a$ irrespectively of whether the first or the second meiotic division was abolished.

Thus when in the female and the male plants the first meiotic division is partly abolished the following karyotypes are to be expected in the progeny of these plants:

♀ \ ♂	Normal meiosis		Abolished first meiotic division
	7 (X + 6a)	8 (2Y + 6a)	15 (X + 2Y + 12a)
Normal meiosis 7 (X + 6a)	14 (2X + 12a)	15 (X + 2Y + 12a)	22 (2X + 2Y + 18a)
Abolished first meiotic division 14 (2X + 12a)	21 (3X + 18a)	22 (2X + 2Y + 18a)	29 (3X + 2Y + 24a)

When the second meiotic division does not take place the progeny should have the following karyotypes:

♀ \ ♂	Normal meiosis		Abolished second meiotic division	
	7 (X + 6a)	8 (2Y + 6a)	14 (2X + 12a)	16 (4Y + 12a)
Normal meiosis 7 (X + 6a)	14 (2X + 12a)	15 (X + 2Y + 12a)	21 (3X + 18a)	23 (X + 4Y + 18a)
Abolished second meiotic division 14 (2X + 12a)	21 (3X + 18a)	22 (2X + 2Y + 18a)	28 (4X + 24a)	30 (2X + 4Y + 24a)

As is to be seen in both cases there may be in the progeny individuals with the karyotypes

14 (2X + 12a)
 15 (X + 2Y + 12a)
 21 (3X + 18a)
 22 (2X + 2Y + 18a).

An individual with the karyotype 29 (3X + 2Y + 24a) can arise only when the first meiotic division is inhibited in both parental plants and individuals with the karyotypes 28 (4X + 24a) and 30 (2X + 4Y + 24a) only when the second meiotic division is inhibited.

The occurrence in natural populations of plants with the karyotype 29 (3X + 2Y + 24a) alongside with plants having the karyotypes 21 (3X + 18a) and 22 (2X + 2Y + 18a) and the fact that an individual with the karyotype 28 (4X + 24a) or 30 (2X + 4Y + 24a) was never found in these populations points to the abolition of the first meiotic division as the more likely cause for the appearance of polyploid plants

in nature. The rather frequent occurrence of restitution nuclei is a further confirmation for this conclusion.

The two lists of the possible karyotypes assembled above clearly show how unjustified was Takenaka's (1955) supposition that in the natural populations of *R. acetosa* the triploid female plants should be more numerous than the triploid intersexes. Admittedly, the unions between the gametes $14(2X + 12a)$ and $7(X + 6a)$ are more frequent than the unions between the gametes $14(2X + 12a)$ and $8(2Y + 6a)$, but the triploid intersexes with the karyotype $22(2X + 2Y + 18a)$ may also arise from the union between the gametes $7(X + 6a)$ and $15(X + 2Y + 12a)$.

The analysis of meiosis in PMCs of the individual $F_1/17$ (Cross 239) showed that the formation of the restitution nuclei in this plant was not caused by the failure of the chromosomes to conjugate. At metaphase I of this plant the chromosomes formed the regular six bivalents and one trivalent, whereas the univalents were very rare. At anaphase I the chromosomes began to part but then the process was interrupted and instead of two telophase nuclei only one restitution nucleus was formed as a result of the malformation of the spindle. Darlington (1937) points out that for the course of the meiotic division to be regular the conjugation of chromosomes is by itself insufficient the important thing being for it to take place at the proper time. The too early or too late relatively to the conjugation of the chromosomes development of the spindle causes irregularities consisting in the incomplete parting of the chromosomes. Several small nuclei or one large nucleus can be formed and when subsequently such a large nucleus divides unreduced gametes develop.

Unreduced gametes can also be produced as the result of the too early or too late formation of the spindle at anaphase II, since then the parting of the chromosomes is also incomplete.

Irregularities of this kind were observed in many plant and animal species. Darlington (1937) notes that they were found in such species as e.g. *Knophofia Nelsoni* (Moffet 1932), *Cannabis sativa* (Breslawetz 1935), *Impatiens pallida* (Smith 1935), *Zea Mays* (Baedle 1933), *Triticum turgidovillosum* (Berg 1934), *Drosophila pseudoobscura* (Dobzhansky 1934), *Lathyrus odoratus* (Baberge 1936, Upcott 1936).

Jensen (1936) observed the failure of the chromosomes to part at anaphase I and the formation of restitution nuclei in the hybrid *Rumex crispus* $\times$ *R. obtusifolius*: some of the chromosomes being lost gametes with the incomplete diploid number of chromosomes developed.

Darlington is of the opinion that under certain conditions all the flowering plants may on rare occasions produce unreduced gametes. The abolition of the first meiotic division may, therefore, be assumed

as one of the causes of the occurrence in nature of polyploid plants of *R. acetosa* and the related species; this may be due to the absence of synchronism between the coniugation of the chromosomes and the development of the spindle at anaphase I.

In the course of this investigation the absence of the second meiotic division was observed in the F_2 crosses of *R. arifolius* $\times$ *R. thyrsoflorus* (Cross 241). Whereas the failure of the first meiotic division to take place always was an exceptional event and in the neighbouring pollen mother cells meiosis followed its regular course, the abolition of the second meiotic divisions in some plants affected almost all the PMCs.

This kind of "monokinetik" meiosis has been observed before by Levan (1936—1937) in *Allium schoenoprasum* and *Allium nutans* by Avery, Satina and Rietsem (1959) in *Datura stramonium*, and by Świetlińska (1960) in the crosses *R. thyrsoflorus* $\times$ *R. acetosa*. The total absence of meiosis or sometimes only of the second meiotic division and the formation of diploid and tetraploid gametes was reported in *Musa* by Dodds and Pittendrigh (1945) and Dodds and Simmonds (1944).

Levan (1936, 1937) observed the formation of big pollen grains in *Allium schoenoprasum* and *Allium nutans*; he investigated this phenomenon in detail on a hyperpentaploid plant which produced pollen consisting in nearly 100 per cent of big grains. The prophase of this plant was regular and metaphase I was followed by a normal anaphase. Multivalents being frequent the chromosomes were often stretched out and lagged at anaphase I, though later they joined the telophase nuclei. The beginning of interkinesis was regular but later a membrane appeared and the typical resting stage began in which one or two small nuclei developed. This resting stage was long lasting the diads developing the typical pollen membranes. After a long time the prophase of the first pollen division began: the chromosomes became visible with the centromeres laying near each other. Levan believes that up to the prophase stage in the mitosis of pollen the centromeres were not divided. Each "pair" of chromosomes in the division of pollen corresponded to one anaphase chromosome of the first meiotic division. After a short time the chromosomes of each "pair" were split lengthwise and at metaphase the number of chromosomes was already doubled.

The pollen of this was never composed of the enormous grains alone and there were always some few small grains developed as the result of a regular meiosis. Sometimes the second prophase and the second metaphase occurred in the diads but at this the second division ended and the resting stage began. In Levan's opinion the "monokinetik" meiosis was not caused by the structural properties of the chromosomes but was under genotypic control.

The work of Satina and Blakeslee (1935, quoted after Ave-

ry, Satina, and Rietssem 1959) on *Datura* confirms Levan's supposition. These workers found in the F_1 generation of plants pollinated with pollen treated with radium a gene mutation causing the abolition of the second meiotic division of micro- and macrosporogenesis: in these plants cytokinesis began soon after the first telophase and diads instead of tetrads were formed causing the doubling of the chromosome number in the gametes. Satina and Blakeslee even succeeded in localizing the position of this gene in the 9—10 chromosome.

In the crosses *R. thyrsoiflorus* $\times$ *R. acetosa* Świetlińska (1960) observed the abolition of the second meiotic division in PMCs and the formation of diads. These meiotic irregularities occurred in the F_1 generation and were inherited by the F_2 generation and the backcrosses. On this evidence Świetlińska reached the conclusion that the abolition of the second meiotic division was brought about by genetic factors.

The population of *R. thyrsoiflorus* from Puławy must contain a gene or a complex of genes, which in combination with the genotype of *R. acetosa* cause the absence of the second meiotic division in the PMCs. Świetlińska thinks that these genes occur only in some populations of *R. thyrsoiflorus* since the female plants of this species belonging to other populations when crossed with the same male plant of *R. acetosa* gave a wholly diploid progeny.

The process of the formation of diads in the PMCs through the abolition of the second meiotic division, observed in the course of this work, closely resembles the "monokinetik" meiosis described by Levan (1936—1937) and Świetlińska (1960).

The first division and the first stages of interkinesis are regular but later the primary membrane develops between the nuclei and diads are formed. Like in *Allium* the second prophase and the second metaphase take place in the diads but this process, and here the similarity with *Allium* ends, can go further leading to the partial parting of chromosomes and the formation either of one elongated nucleus or of two nuclei with a membrane between them: a triad or a tetrad is then obtained. In such tetrads all the microspores lie in one plane. The division of the chromosomes in the diads is somewhat earlier than in *Allium* since even in the diads stage the double number of chromosomes can be distinctly seen. At metaphase of the mitotic division of pollen the chromosomes often lie in pairs in the same way as is described by Levan in *Allium*.

In view of these circumstances it is possible to assume, as is done by Levan (1936—1937) for *Allium*, that the abolition of the meiotic division in the F_2 generation of the cross *R. arifolius* $\times$ *R. thyrsoiflorus* is caused by genetic factors and not by the structural differences between the chromosomes.

The fact that the abolition of the second meiotic division occurs in

the crosses of *R. thyrsiflorus* $\times$ *R. acetosa* (Świetlińska 1960) as well as in the crosses of *R. arifolius* $\times$ *R. thyrsiflorus* confirms the supposition made by Świetlińska about the presence of the genes causing this effect in the populations of *R. thyrsiflorus*. However, the abolition of the second meiotic division in the crosses of *R. arifolius* $\times$ *R. thyrsiflorus* having only occurred in some individuals of the F_2 generation and not in the F_1 and F_3 generations the manifestation of this effect must depend on the meeting of the suitable sets of genes. The genes determining the abolition of the second meiotic division seem to be associated with certain individuals and not with the various populations, since in the crosses between the various individuals from the same populations the effect occurs only occasionally meiosis being regular in the majority of cases (Świetlińska and Żuk, unpublished).

In the progeny of crosses with the male plants producing diads there are more tetraploids than triploids, which means that also the female plants produce diads instead of tetrads.

The polyploid plants obtained in the course of this work have made possible an investigation of the sex-determination mechanism. The previous investigations on *R. acetosa*, mainly those by Ono (1935) and Yamamoto (1938), have shown that in this species the Y-chromosomes contain no sex-determining genes.

Yamamoto (1938) gives the full list of the diploid and polyploid *R. acetosa* plants for which the karyotypes have been exactly established and the sex has been identified. From Yamamoto's list the individuals with the karyotypes identical as those of the polyploids obtained experimentally in the course of this work have been chosen and their sexes have been compared (Table 17).

As is to be seen in Table 17 both investigations have yielded essentially similar results. Only the individual with the karyotypes $3A + XXY$ (No. 9) and $3A + XXYY$ (No. 10) in this investigation had all male flowers though morphologically they resembled intersexes, whereas Yamamoto (1938) found them to have both male and female flowers.

Certain differences are also apparent when comparing the intersexuality index calculated for the polyploid crosses *R. thyrsiflorus* $\times$ *R. arifolius* with the same index calculated by Ono (1935) for the autopolyploids of *R. acetosa*. In the plants with the sex index equal 1.5:1 the intersexuality index (the ratio of the male to the female flowers) ranged 100 to — 100 in the first case and — 2 to — 100 in the second, though the number of plants examined by Ono was greater.

These differences in the results can be explained assuming that both in *R. arifolius* and *R. thyrsiflorus* the sex genes are distributed, similarly as in *R. acetosa*, also in the autosomes. However, the magnitude of the influence exerted by these genes and their distribution are somewhat different in each of these species. This is why it is easier for the different

combinations of autosomes carrying different sets of sex genes to arise in the polyploid hybrids of *R. thyrsiflorus* $\times$ *R. arifolius* than in the autopolyploids of *R. acetosa*.

Table 17

Karyotypes and sex of *R. thyrsiflorus*, *R. arifolius* and of their polyploid hybrids compared with *R. acetosa* plants of the same karyotypes

	Plant	Karyotype	Sex	Sex of <i>R. acetosa</i> with the same karyo- type acc. to Yama- moto (1938)
1	<i>R. thyrsiflorus</i>	2A+XX	♀	♀
2	<i>R. thyrsiflorus</i>	2A+XXY	♂	♂
3	<i>R. arifolius</i>	2A+XX	♀	♀
4	<i>R. arifolius</i>	2A+XXY	♂	♂
5	Polyploid hybrids	3A+XXX	♀	♀
6		3A+XX	♂ ⁺	♂ ⁺
7		3A+XXY	♂ ⁺	♂ ⁺
8		3A+XXXY	♂ ⁺	♂ ⁺
9		3A+XXY	♂ ⁺ →♂ ⁺	♂ ⁺
10		3A+XXXY	♂ ⁺ →♂ ⁺	♂ ⁺
11		3A+XXXY	♂	♂
12		4A+XXXX	♀	♀
13		4A+XXYYYY	♂	♂
14		4A+XXXXY	♂ ⁺	♂ ⁺ ±♀
15		4A+XXXXY	♀→♂ ⁺	♂ ⁺ ±♀
16		2A+5+XXXY	♂ ⁺	♂ ⁺

This result is in agreement with the observations of Yamamoto (1938) who assumes that in *R. acetosa* the set of autosomes must contain, besides the male factors, also the female ones. Thus, in spite of the certain differences in details between the results of this investigations and the results reported by Ono (1935) and Yamamoto (1938), it seems that the mechanism of sex determination in *R. arifolius* and *R. thyrsiflorus* is essentially the same as in *R. acetosa*. This means that the factors determining the male sex are carried on the autosomes whereas the factors determining the female sex are carried on the X-chromosomes and the autosomes.

Some doubts may be caused by that conclusions referring to both *R. thyrsiflorus* and *R. arifolius* are drawn from the analysis of polyploid hybrids of these two species. If, however, the sex-determining mechanism of one of these species differed significantly, this would be at once manifested also by the polyploid hybrids of the two species.

So far as flowering plants are concerned the mechanism of sex determination has been most fully investigated in *Melandrium*. This plant was studied among others by Westergaard (1940, 1948, 1953, 1958), Warmke and Blakeslee (1940), and Nygren (1957). The investigations on *Melandrium* were reviewed and summed up by Westergaard (1940, 1953, 1958). In this species the male sex is determined by the Y-chromosomes and the female sex by the X-chromosomes. Some of the autosomes carry the male factors and others the female factors. The sex of an individual depends on the cooperation of two sets of genes: 1) the "male" and "female" genes carried on all chromosomes and occurring in both sexes, and 2) the genes determining the sex (the genes of the "trigger mechanism") present in various sections of the Y-chromosome. The presence of the Y-chromosome determines the development of an organism in the direction of the male sex and the absence of this chromosome towards the female sex. The effectiveness of the trigger mechanism is associated with the absence linkage between the complex of the suppressors of the female sex and the fundamental genes inducing the male sex carried by the Y-chromosome.

This view is criticized by Goldschmidt (1955) who points out that Westergaard's interpretation explains only one unusual case and that it advances no explanation whatever for many fully investigated instances of intersexuality in other organisms. Moreover, the evidence advanced by Goldschmidt indicates that in *Melandrium* there is a mechanism balancing the determinants of the male sex in the autosomes with the determinants of the female sex in the X-chromosome. The only fact that according to Goldschmidt requires a new interpretation is the exceptionally high influence exerted by the Y-chromosome.

The data assembled for *R. acetosa*, *R. thyrsiflorus*, and *R. arifolius* seem to indicate that in these species no trigger mechanism is in operation and that the mechanism of sex determination can in this case be more simply explained by the balance between the factors determining the male sex carried on the autosomes and the factors determining the female sex carried on the X-chromosome. On the other hand, much newly advanced evidence seems to suggest that *Melandrium* is not an exception, as is believed by Goldschmidt, a similar mechanism of sex determination having been found in *Rumex angiocarpus*, *R. tenuifolius*, *R. acetosella*, *R. graminifolius* (A. Löve 1944b, 1957), *Spinacia oleracea* (Janicka, Stefenson 1955), *Acnida* (Murray 1940), and

Carica papaya (Hofmeyer 1945, quoted after Westergaard 1958). Similarly, the results obtained by Smith (1958) also suggest that in *Rumex hastatulus* the Y-chromosomes carry the male character. Welshous and Russell (1959) found that in mice the factor determining the male sex was contained in the Y-chromosome.

Mention has already been made that polyploid plants quite frequently occur in the natural populations of *R. acetosa*. The fact that a tetraploid individual was obtained in the F_1 progeny of the cross *R. arifolius* $\times$ *R. thyrsiflorus* indicates that unreduced gametes can be produced also in these species. This means that polyploid plants may appear in the natural populations of *R. arifolius* and *R. thyrsiflorus*. The question, therefore, arises why no polyploid bisexual line has so far developed from among the species of the subsection *Acetosa*?

The results of this investigation on *R. thyrsiflorus* and *R. arifolius* as well as the results of Ono's (1935) work on *R. acetosa* are essentially in agreement with the results obtained for *Drosophila* (Müller 1925). In the polyploid plants from the genus *Rumex* subsection *Acetosa* with the karyotypes $29 = 3X + 2Y + 24a$, $29 = 2X + 3Y + 24a$, and $30 = 2X + 4Y + 24a$ the X-chromosomes usually conjugate with each other and part to the opposite poles; the progeny of these plants usually consists of sterile intersexes. This seems to be one of the main obstacles for the development of a bisexual polyploid line among the species of the subsection *Acetosa*.

VII. CONCLUSIONS

1. The karyotypes of *Rumex arifolius* and *R. thyrsiflorus* were studied. They show a distinct variation in the occurrence of different types of autosomes both within and between different populations.

2. Between the two species many F_1 and F_2 hybrids were obtained. The cytological examinations revealed irregularities of meiosis consisting in the abolition of the first meiotic division in some PMCs of the F_1 and F_2 crosses of *R. arifolius* $\times$ *R. thyrsiflorus*. These irregularities lead to the formation of restitution nuclei and of unreduced gametes. It was found that plants of the pure parental species also produced diads. It seems that the same mechanism (the formation of diploid gametes) was responsible for the occurrence under natural conditions of the triploid and tetraploid intersexes reported by Japanese authors for *R. acetosa* and also found in *R. arifolius* from the Gorce Mts. and the Bieszczady Mts., Poland.

3. It was found that in some plants of the F_2 progeny of one of the crosses diads were formed as the result of the abolition of the second meiotic division. The frequency of this irregularity was different in various individuals. The abolition of the second meiotic division was not

caused by structural differences between the chromosomes of *R. thyrsiflorus* and *R. arifolius* but by genetic factors.

4. The karyotypes were established in the F_1 , F_2 , F_3 , and backcross progeny of *R. arifolius* $\times$ *R. thyrsiflorus*: the triploid, tetraploid, and aneuploid individuals, which were identified in these progenies, developed as the result of the abolition of the first or the second meiotic division.

5. Observations were made of the morphological traits of the polyploid plants and in some cases the course of meiosis in the PMCs was examined.

6. The occurrence in the progeny of the crosses between *R. thyrsiflorus* and *R. arifolius* of polyploid plants with different proportions between the number of the X-chromosomes and the number of the autosomes as well as with a different number of Y-chromosomes has made possible the study of the sex determining mechanism in these species.

7. It was found that in *R. thyrsiflorus* and *R. arifolius* the factors determining the male sex were located in autosomes and that the factors determining the female sex were carried on the X-chromosomes. The Y-chromosomes carry no sex-determining genes.

8. In the natural populations of *R. acetosa*, *R. thyrsiflorus*, and *R. arifolius* as well as in the populations of the hybrids *R. thyrsiflorus* $\times$ *R. arifolius* the number of the female individuals was found to be much greater than of the male ones.

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Polish Academy of Sciences,
Department of General Genetics,
Warsaw, al. Ujazdowskie 4, Poland

Translated by A. Potocki

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Plate I

Phot. 1—6. Root tips metaphases

1 — *R. thyrsiflorus* Nowy Dwór ♀ ($2X + j + 2v + t + 8i$); 2 — *R. thyrsiflorus* Nowy Dwór ♀ ($2X + j + v + 10i$);
 3 — *R. thyrsiflorus* Puławy ♀ ($2X + 2j + 2v + 8i$); 4 — *R. arifolius* Gorce ♀ ($2X + j + 11i$); 5 — *R. arifolius* Gorce ♀
 ($2X + j + 11i$); 6 — intersexual plant No. I/a ($29 = 3X + 2Y + 24a$). Phot. 1—5: smears; Phot. 6: paraffin preparation.
 Magnific.: 1755 ×

Plate II

Phot. 7—11. Meiosis in PMCs in F_2 hybrids *R. arifolius* Krościenko $\times$ *R. thyrsiflorus* Nowy Dwór (cross 241)

7 — diads from plant No. F_2 241/1; 8 — tetrads from plant No. F_2 241/1; 9, 10, 11 — "M II" in disintegrating diads from plant No. F_2 241/15.

Phot. 12. Metaphasal plate of somatic division in pollen grain ($n = 16$), plant No. F_2 241/12
Paraffin preparations. Magnific.: $630 \times$

Plate III

Phot. 13—18. Meiosis in PMCs of the hybrid *R. thyrsiflorus* Puławy $\times$ *R. arifolius* Krościenko (plant No. 239/17).

13, 14 — restitution nuclei; 15 — anaphase of restitution nucleus; 16, 17 — diads; 18 — metaphase of restitution nucleus

Phot. 19—20. Meiosis in PMCs of tetraploid intersexual plant No. I (cross 239).

19 — numerous univalents at M I; 20 — degeneration of PMC. Paraffin preparations. Magnific.: 630 $\times$

Plate IV

21

22

23

24

25

26

Phot. 21—26. Root tips metaphases

21 — plant No. I/6 ($2X + 2Y + 2j + 3V + 13i$); 22 — plant No. I/10 ($3X + Y + 3j + 2v + 13i$); 23 — plant No. I/30 ($2X + 2Y + 2j + 2v + 14i$); 24 — plant No. I/36 ($2X + 2Y + j + v + 16i$); 25 — plant No. II/12 ($4XY + 18a$); 26 — plant No. F₂ 241 ($4X + 24a$). Phot. 21—25: smears; Phot. 26: paraffin preparation. Magnific.: Phot. 21—25 — 1575 $\times$, Phot. 26 — 630 $\times$

Plate V

Phot. 27—31. Meiosis in PMCs of F_3 hybrid (*R. arifolius* Krościenko $\times$ *R. thyrsiflorus*) with karyotype $4n = 2X + 4Y + 24a$ (cross 241).

27 — multivalents in diakinesis; 28, 29 — A I with irregular separation of chromosomes; 30 — different kinds of disturbances at M I; 31 — abnormal tetrad. Paraffin preparations. Magnific.: 1575 $\times$

Plate VI

Phot. 32—37. Phot. 32 — Pollen grains of *R. thyrsiflorus*. Phot. 33 — pollen grains of *R. arifolius*. Phot. 34 — pollen grains of F_1 hybrid No. 17 (cross 239). Phot. 35 — pollen grains of intersexual triploid plant ($22 = 2X + 2Y + 18a$). Phot. 36 — pollen grains of tetraploid plant ($30 = 2X + 4Y + 24a$). Phot. 37 — seeds: on the left — *R. thyrsiflorus*, in the middle — *R. arifolius*, on the right — tetraploid plant ($29 = 3X + 2Y + 24a$)