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Function of *Y* Chromosomes in *Rumex thyrsiflorus*

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Summary. 1. Numerous polyploid and diploid plants of *R. thyrsiflorus* with various numbers of X and Y chromosomes were obtained. Analysis of these plants confirmed earlier conclusions (Żuk, 1963) that Y chromosomes carry no sex-determining genes.

2. Investigation of pollen fertility of male plants without Y chromosomes, and with one or more Y chromosomes, seems to indicate that in the Y chromosomes of *R. thyrsiflorus* are located some factors reponsible for the fertility of male plants. This result is supported by cytological data (Żuk, 1969) indicating that Y chromosomes of *R. thyrsiflorus* are active in premeiotic stages of microsporogenesis. Meiosis in plants without Y chromosomes and with one Y chromosome was also studied. In all cases conjugation of chromosomes and meiotic divisions in PMC were normal. These data suggest that there are no genes located in Y chromosomes responsible for synapsis or the course of meiotic division in PMC of *R. thyrsiflorus*.

3. In a natural population of *R. thyrsiflorus* the male individuals are less numerous than the female ones. This is due to nonrandom fertilization. The gametes $YY + 6A = 8$ fertilize less frequently than do gametes $X + 6A = 7$. In crosses involving a male plant with chromosome constitution $XYYYY + 12A = 17$, which produces more uniform gametes as regards Y chromosomes, there were more male individuals than normally. This is interpreted as evidence that the preponderance of females in *R. thyrsiflorus* is connected with Y chromosomes.

Introduction

The data presented in this paper confirm the previous observation (Żuk, 1963) that the mechanism of sex determination in *R. thyrsiflorus* is of the *Drosophila* type. Sex in this species (♀ $XX + 12A = 14$ and ♂ $XYY + 12A = 15$) depends on the autosomes to X chromosomes ratio; Y chromosomes play no role in sex determination.

Cytological examination and autoradiographic study (Żuk, 1969a) revealed that Y chromosomes in *R. thyrsiflorus* are heterochromatic and late-replicating. In further study evidence was found (Żuk, 1969b) that these chromosomes may be genetically active during premeiotic stages of microsporogenesis.

New data obtained from observation of the fertility of plants without Y chromosomes or with one Y chromosome, and of the sex ratio in the progeny of male plants with additional Y chromosomes, suggest that Y chromosomes are responsible for the fertility of male plants and for the proportion of male and female plants in natural populations of this species.

Material and Methods

Seeds of *R. thyrsiflorus* were collected from the natural population growing on flood dikes on the Vistula river near Puławy. Material for study of microsporogenesis was fixed in ethanol-acetic acid fixative (3:1). Squash preparations were prepared after staining in alcoholic hydrochloric acid carmine according to Snow (1963). Chromosome numbers were estimated in squash preparations of root tips treated for 4 hrs with 8-oxyquinoline (Tjio and Levan, 1950) before fixation in ethanol-acetic acid (3:1) and staining in aceto-orcein.

Results

1. Role of Y chromosomes in sex determination

In the course of the present investigation numerous diploid and polyploid plants with different combinations of sex chromosomes and autosomes were analysed. All these plants were obtained as progeny of specific crosses between diploids and polyploids occurring frequently in *R. thyrsiflorus*.

Table 1. *Sex and sex index of plants with additional Y chromosomes*

Karyotype	Number of plants investigated	Sex	Sex index ($X{:}A$ ratio)
$14 = XX + 12A$	30	♀	$2:2 = 1,0$
$15 = XXY + 12A$	38	♀	$2:2 = 1,0$
$15 = XXY + 12A$	1	⚥	$2:2 = 1,0$
$16 = XXYY + 12A$	4	♀	$2:2 = 1,0$
$17 = XXYYY + 12A$	1	♀	$2:2 = 1,0$
$15 = XYY + 12A$	30	♂	$1:2 = 0,5$
$16 = XYYY + 12A$	17	♂	$1:2 = 0,5$
$17 = XYYYY + 12A$	4	♂	$1:2 = 0,5$
$18 = XYYYYY + 12A$	1	♂	$1:2 = 0,5$

In Table 1, karyotypes and sex of 76 diploid plants with additional Y chromosomes are given. With one exception all diploid plants with two X chromosomes were pure female with no indication of intersexuality in spite of having one, two or three additional Y chromosomes. The sex index of these plants (ratio of X chromosomes to autosomes) was 1.0. One exceptional plant with karyotype $XXY + 12\,A = 15$ was intersexual and sterile. The reason remains obscure, as according to the sex index = 1.0, female sex should have been expected.

All plants with one X chromosome and an additional one, two or three Y chromosomes were male; their sex index was 0.5. Thus the chromosome balance 1 X to 12 autosomes determines male sex. The presence of additional Y chromosomes does not change the sexuality of plants. Bearing in mind the inertness of Y chromosomes in sex determination, plants with one X and with one, two or three additional Y chromosomes cannot be considered as supermale. In *R. thyrsiflorus* supermale plants are those with one X chromosome and a higher than diploid number of autosomes.

In Table 2, sex and the sex indexes of polyploid plants with various numbers of autosomes and sex chromosomes are given. It can be seen that all plants with sex index around 0.7 were intersexual. Plants with sex index 0.5, or close to 0.5, were male. As in diploid male plants, additional Y chromosomes had no influence on sex expression.

One plant was obtained with only one X, one Y chromosome, 12 autosomes and an additional fragment (Fig. 1). The sex index of this plant was 0.5, but it was not a pure male because some flowers also developed stigmata. This plant was the offspring of a diploid female plant and a male plant with a "new" heterochromatic S chromosome (Żuk, 1969c). An abnormal gamete with only one Y chromosome and an additional fragment was produced by this male parent. Observation of chromosome conjugation in an $XY \times 12\,A + f = 14$ plant revealed the association of this fragment with an autosomal bivalent, suggesting an autosomal origin for the fragment. However, information about the origin of the $XY + 12\,A + f = 14$ karyotype is not sufficient to explain the intersexuality of the plant in question. Thus, among 76 diploids and 28 polyploids there were only two points which did not confirm the general conclusion that Y chromosomes carry no sex determining genes.

Additional evidence indicating that Y chromosomes play no role in sex determination in *R. thyrsiflorus* was obtained from examination of translocation between the Y chromosome and the autosome. By DEB treatment a small fragment of the Y chromosome was translocated to an *i*-type autosome (Fig. 2). In the progeny of the cross between a normal female plant ($XX + 12\,A = 14$) and a diploid male plant carrying this translocation, several plants with two X chromosomes and the new chromosome (composed of *i*-type chromosome and a small fragment of Y chromosome) were obtained. The translocated fragment of the Y chromosome had, as expected, no effect on the sexuality of plants. However, there were some other effects of this translocation like more vigorous growth of plants and considerable changes in leaf morphology. These effects may be considered to be the result of the "position effect".

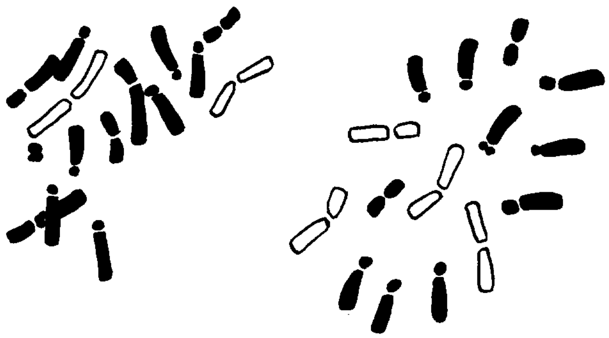

Fig. 1. Plant with karyotype $14 = XY + 12\,A + f$

Fig. 2. Male plant $2n = 15 = XYY + 12\,A$ with translocation between Y chromosome and autosome

Fig. 3. Intersexual plant with karyotype $21 = XX + 19\,A$

Fig. 4. Plant with karyotype $23 = XX + 21\,A$

Figs. 1—4. Metaphasal plates from root tips. × 1700

2. *Y chromosomes and male fertility*

In order to estimate the effect of Y chromosomes on male fertility, male plants with various numbers of Y chromosomes were examined. Pollen fertility was estimated on acetocarmine smears and was correlated with the number of Y chromosomes (Table. 2). Pollen fertility of normal diploid male plants was about 90%. Plants lacking Y chromosomes (Nos. 1 and 2 in Table 2) were completely male-sterile. Plant No. 1 was intersexual with the sex index = 0.62. The sterility of this plant was probably due both to its intersexuality (unbalanced X chromosomes to autosomes ratio) and to the lack of Y chromosomes. In plant No. 2 most of the flowers were pure male and only occasionally were intersexual flowers with stigmata observed. It may be assumed that the sterility of this plant was caused mainly by the lack of Y chromosomes.

Triploid intersexual plants with the sex index ranging from 0.74 to 0.62 were also male-sterile. Among them, plants with only one Y chromosome did not produce morphologically fertile pollen at all; in some plants with two Y chromosomes a small

Table 2. *Pollen fertility, sex and sex index of plants with various numbers of Y chromosomes*

No.	Karyotype	Pollen fertility	Sex	Sex index $X:A$ ratio
1	$XX + 19\,A = 21$	0%	♂♀	0.62
2	$XX + 21\,A = 23$	0%	♂→♂♀	0.57
3	$XXY + 16\,A = 19$	0%	♂♀	0.74
4	$XXY + 17\,A = 20$	0%	♂♀	0.71
5	$XXY + 17\,A = 20$	0%	♂♀	0.71
6	$XXY + 18\,A = 21$	0%	♂♀	0.67
7	$XXY + 18\,A = \; = 21$	0%	♂→♂♀	0.67
8	$XXY + 19\,A = 22$	0%	♂♀	0.62
9	$XXY + 19\,A = 22$	0%	♂→♂♀	0.62
10	$XXY + 19\,A = 22$	0%	♂♀	0.62
11	$XXYY + 17\,A = 21$	0%	♂♀	0.71
12	$XXYY + 18\,A = 22$	0%	♂→♂♀	0.67
13	$XXYY + 18\,A = 22$	0%	♂♀	0.67
14	$XXYY + 18\,A = 22$	0%	♂♀	0.67
15	$XXYY + 18\,A = 22$	0%	♂→♂♀	0.67
16	$XXYY + 18\,A = 22$	0,5%	♂→♂♀	0.67
17	$XXYY + 18\,A = 22$	1,8%	♂♀	0.67
18	$XXYY + 18\,A = 22$	1,8%	♂♀	0.67
19	$XXYY + 18\,A = 22$	1,8%	♂♀	0.67
20	$XY + 12\,A = 14$	47%	♂	0.50
21	$XXY + 22\,A = 25$	0%	♂	0.54
22	$XXY + 23\,A = 26$	0%	♂	0.53
23	$XXY + 23\,A = 26$	0%	♂	0.53
24	$XXY + 23\,A = 26$	35%	♂	0.53
25	$XXYY + 22\,A = 26$	14%	♂	0.54
26	$XXYY + 23\,A = 27$	60%	♂	0.53
27	$XXYY + 24\,A = 28$	30%	♂	0.48
28	$XXYYY + 24\,A = 29$	50%	♂	0.50
29	$XXYYYY + 24\,A = 30$	80%	♂	0.50

amount of good pollen grains was found and their pollen fertility was between 0.5 and 1.8%.

The diploid plant No. 20, with only one Y and one X chromosome, showed 47% pollen fertility, but after pollinating several normal diploid female plants only a few fertile seeds were obtained. The male plant No. 24, with two X and one Y chromosomes and 23 autosomes, presented a similar case of morphologically normal pollen being ineffective in fertilization of female plants. Three other polyploid male plants with one Y chromosome were completely male-sterile. The sterility of these plants seems to be caused by the lack of one Y chromosome. This conclusion is supported by the data on pollen fertility for male polyploid plants with two or more Y chromosomes (plants Nos. 25—29). These plants produced 14 to 80% morphologically good pollen. The observation that plants with two or more Y chromosomes are more fertile than those with one Y chromosome, or then those without Y chromosomes, suggests that Y chromosomes carry some factors responsible for pollen fertility.

To obtain more information on the effect of Y chromosomes on male fertility, the meiosis of two plants without Y chromosomes and of six plants with only one Y chromosome was examined. The plant with chromosome constitution $XX + 19\,A = 21$ (Fig. 3) was intersexual, the other plant without Y chromosomes ($XX + 21 = 23$, Fig. 4) was male but occasionally intersexual flowers were also formed. In the first plant, chromosome conjugation in the meiotic prophase and the first metaphase was normal in most PMC's (Fig. 5). No disturbances were observed either in the first or second meiotic division, and normal tetrads were formed (Fig. 6). In some PMC's, however, trivalents and univalents, or even a complete lack of chromosome conjugation, were observed. These disturbances led to formation of monads or dyads instead of tetrads (Fig. 7). In spite of the regular course of meiosis in most PMC's, this plant, as mentioned before, was completely male-sterile and degeneration of pollen grains took place before pollen mitosis (Fig. 8). In the second plant without the Y chromosome, only non-viable pollen was produced in spite of normal meiosis in most PMC's (Fig. 9, 10).

In the diploid male plants lacking one Y chromosome, usually seven bivalents were formed in diakinesis, six by autosomes and one by the sex chromosomes XY. Segregation of chromosomes was regular. X and Y pollen grains with chromosome constitutions $X + 6\,A = 7$ and $Y + 6\,A = 7$ were formed. This pollen was morphologically normal in 47% only.

In five polyploid plants with one Y chromosome no major disturbances of meiosis were observed. However, in spite of fairly regular meiosis, three plants produced only completely degenerated pollen (Fig. 10); in the other two, 35% and 47% of morphologically good pollen were found (Fig. 12). The fact that some plants with one Y chromosome are able to produce normal pollen grains and some do not, seems to suggest that Y chromosomes differ in some respect in their function of controlling male fertility.

The observation that plants without Y chromosomes or with one Y chromosome are sterile, in spite of a normal course of meiosis, suggests that Y chromosomes are necessary for normal post-meiotic development of pollen.

3. *Effect of Y chromosomes on sex ratio*

Both in natural and in cultivated populations of *R. thyrsiflorus* male individuals are less frequent than female plants (sex ratio usually about 1:9). Female plants ($XX + 12\,A = 14$) produce only one type of gametes: $X + 6\,A = 7$. Male plants with karyotype $XYY + 12\,A = 15$ produce gametes with chromosome constitution $YY + 6\,A = 8$ determining male sex, and gametes $X + 6\,A = 7$ determining female sex, in equal proportions. The preponderance of female plants in the population suggests non-random fertilization or lower viability of male zygotes.

The data discussed in an earlier publication (Żuk, 1963) indicate that fertilization in *Rumex* is not random, and male-determining gametes fertilize egg cells less frequently than do those determining female sex. As male-determining gametes are $YY + 6\,A = 8$, the possible reason for their lower viability, as compared with $X + 6\,A = 7$ gametes, may be either


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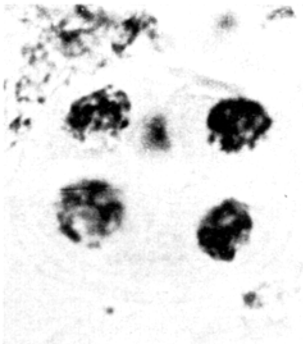
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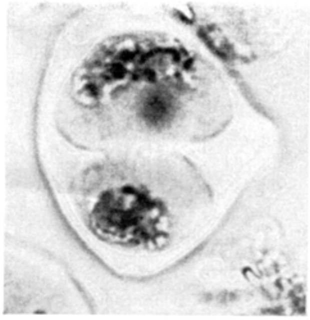
Fig. 5. MI in plant with karyotype $21 = XX + 19\,A$. ×1700

Fig. 6. Tetrad in plant with karyotype $21 = XX + 19\,A$. ×1700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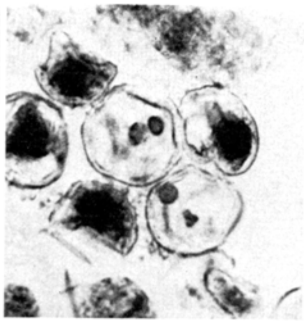
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Fig. 7. Dyad in plant with karyotype $21 = XX + 19\,A$. ×1700

Fig. 8. Abortive pollen grains in plant with karyotype $21 = XX + 19\,A$. ×700


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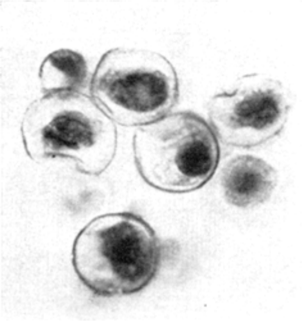
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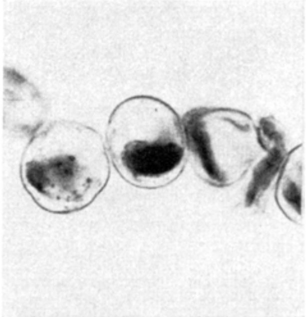
Fig. 9. MI in plant with karyotype $23 = XX + 21\,A$. ×1700

Fig. 10. Abortive pollen grains in plant with karyotype $23 = XX + 21\,A$. ×700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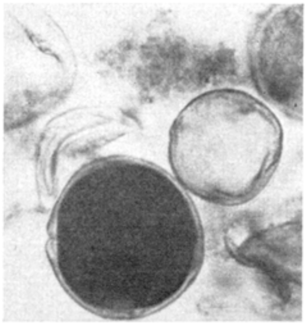
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Fig. 11. Abortive pollen grains in plant with karyotype $26 = XXY + 23\,A$. ×700

Fig. 12. Pollen grains in plant with karyotype $14 = XY + 12\,A + f$. ×700

Table 3. *Number of ♀ and ♂ individuals in progeny of crosses between Y chromosome hyperploid plants*

No. of cross	Karyotypes of parental forms ♀	♂	Progeny number of ♀ individuals	number of ♂ individuals
	$XX+12\,A = 14$	$XYY+12\,A = 15$	249 [90%]	29 [10%]
1/65	$XXYY+12\,A = 16$	$XYY+12\,A = 15$	53 [70%]	23 [30%]
1/66	$XX+12\,A = 14$	$XYYY+12\,A = 16$	16 [67%]	8 [33%]
2/66	$XXY+12\,A = 15$	$XYY+12\,A = 15$	20 [77%]	6 [23%]
3/66	$XX+12\,A = 14$	$XYYYY+12\,A = 17$	20 [83%]	4 [17%]
4/66	$XXYY+12\,A = 16$	$XYYY+12\,A = 16$	23 [74%]	8 [26%]
5/66	$XXYY+12\,A = 16$	$XYYYY+12\,A = 17$	28 [46%]	33 [54%]
8/66	$XX+12\,A = 14$	$XYYYY+12\,A = 17$	31 [54%]	26 [46%]

the presence of two Y chromosomes or the absence of the X chromosome. To elucidate this problem several crosses between plants with additional Y chromosomes were performed. From the data presented in Table 3, it may be seen that in all experimental crosses the percentage of male individuals was higher than in the cross between a normal female plant with karyotype $XX + 12\,A = 14$ and a male plant with karyotype $XYY + 12\,A = 15$. The most interesting were the progeny of the crosses Nos. 5/66 and 8/66 with an equal number of male and female plants. The explanation of this result may be as follows: the chromosome constitution of the male parent in the crosses 5/66 and 8/66 was $XYYYY + 12\,A = 17$; it is to be expected that in a plant with 2 additional Y chromosomes the most frequent pattern of sex chromosome segregation will be $XYY - YY$, and thus, both male and female determining gametes will contain two Y chromosomes and be uniform in this respect. Consequently, fertilization in these two particular crosses was random giving a sex ratio very close to 1:1 in the progeny. From this experiment the conclusion can be drawn that the presence of two Y chromosomes, rather than the absence of the X chromosome, in the gametes determining male sex is responsible for nonrandom fertilization and the preponderance of female plants in the population.

Discussion

In the course of the present investigation the observation was made that in *R. thyrsiflorus* heterochromatic and late-replicating Y chromosomes which play no role in sex determination are not genetically inert. There is some evidence that in *R. thyrsiflorus*, and probably in other dioecious species of the *Acetosa* group, Y chromosomes are responsible for the fertility of male plants and control the sex ratio in populations.

It was found that male plants with only one Y chromosome or without Y chromosomes ($XY+12A = 14$, $XX + 19\,A = 21$, $XX + 21\,A = 23$ and $XXY + 23\,A = 26$) were normal in viability and appearance. In microsporogenesis of such plants no major abnormalities were observed. However, in spite of the normal course of meiosis, these plants were to a various degree male-sterile. Thus, sterility of male plants lacking the normal number of Y chromosomes suggests that in Y chromosomes some factors responsible for pollen fertility are localized. This fits well with the observation that, in the premeiotic stage of microsporogenesis, Y chromosomes are in a diffuse stage which coincides with intensive RNA synthesis (Żuk, 1969b). Thus the genetic function of Y chromosomes becomes more clear in view of the present observation that they control the fertility of male plants.

The role of Y chromosomes in the fertility of male individuals is well established in *Drosophila* (Brosseau, 1960; Dronamraju, 1965; Lewis and John, 1968; Henning, 1968; Hess and Meyer, 1968). Recently a factor influencing male sterility was detected in the Y chromosome of mice (Krzanowska, 1969). Thus the role of Y chromosomes in male fertility seems to be a more general phenomenon.

Among the male plants with one Y chromosome there were completely sterile individuals (pollen degenerated in early stages), and plants with morphological pollen fertility up to 47%. This fact may be interpreted as an indication that, in the course of evolution the $XY_1\,Y_2$ system in *R. thyrsiflorus* probably arose from the XY system by X-autosome translocation (Smith, 1964; Lewis and John, 1968). The new Y chromosome composed of an autosome and part of an X chromosome became heterochromatic in the course of evolution, but its "heterochromatisation" is now less advanced than that of the old Y chromosome. This hypothesis may explain the unequal contribution of two Y chromosomes in controlling the fertility of male plants.

Another role of Y chromosomes that needs consideration is their effect on the sex ratio in populations. In *R. thyrsiflorus*, the preponderance of female plants in populations is a general phenomenon. Deviation from the theoretically expected 1:1 sex ratio is considerable, the ratio being usually about 1:9. This abnormal sex ratio was not observed in the progeny of crosses in which the male parent had an excess of Y chromosomes. In the progeny of such crosses the sex ratio was closer to 1:1. It is possible that in such plants all or most of the gametes produced had two Y chromosomes. Thus, both the female-determining gametes (carrying the X chromosome) and the male-determining gametes (without the X chromosome) were uniform as regards the presence of Y chromosomes. The fact that in the progeny of such a male plant the sex ratio was 1:1 suggests that the presence of two Y chromosomes in normal male-determining gametes is responsible for their low efficiency in fertilization of egg cells, and in consequence for a marked deviation from the 1:1 sex ratio.

The question arising is why such a system of regulation of the sex ratio developed and persisted in evolution. As pointed out by Lewis and John (1968), dioecy is an outbreeding system in which, however, utilization of gametes may be wasteful. This wastage can be reduced, to some extent at least, by a change in the sex ratio in favour of female individuals. In *R. thyrsiflorus* one male plant produces enough pollen to fertilize many female plants. In the whole population the shift in the sex ratio in favour of female individuals can improve the reproductive efficiency of a species. Several male plants are sufficient for the fertilization of numerous female individuals and at the same time there is more space for female plants producing seeds.

The question arises as to how Y chromosomes control germination of pollen and the growth of the pollen tube. This control may be exercized through genetical information or merely by the physical presence of heterochromatin. It is possible that the presence of extra DNA may slow down metabolic processes (Ayonoadu and Rees, 1968), repressing germination and inhibiting the growth of the pollen tube.

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Zusammenfassung

Eine Analyse zahlreicher polyploider und diploider Pflanzen von *Rumex thyrsiflorus* mit verschiedener Anzahl von X- und Y-Chromosomen bestätigt frühere Schlußfolgerungen des Autors, daß Y-Chromosomen keine geschlechtsbestimmenden Gene tragen.

Die Ergebnisse von Untersuchungen der Pollenfertilität männlicher Pflanzen mit einem und mit mehreren *Y*-Chromosomen sprechen dafür, daß auf dem Y-Chromosom lokalisierte Faktoren die Fertilität der männlichen Pflanzen bestimmen. Cytologische Befunde, die auf eine Aktivität der *Y*-Chromosomen von *R. thyrsiflorus* in den prämeiotischen Stadien der Mikrosporogenese deuten, stützen diese Feststellung. Die Untersuchung der Meiose an Pflanzen ohne *Y*-Chromosom und mit einem *Y*-Chromosom ergab in allen Fällen eine normale Paarung der Chromosomen und normale meiotische Teilungen der Pollenmutterzellen. Daraus ist zu schließen, daß sich in den *Y*-Chromosomen keine Gene befinden, die für die Synapsis oder den Ablauf der meiotischen Teilung in den Pollenmutterzellen von *R. thyrsiflorus* verantwortlich sind.

In einer natürlichen Population von *R. thyrsiflorus* sind weniger männliche Individuen als weibliche vorhanden. Diese Tatsache ist auf präferentielle Befruchtung zurückzuführen. Gameten der Konstitution $YY + 6A = 8$ befruchten nicht so häufig wie Gameten der Konstitution $X + 6A = 7$. Bei Kreuzungen, an denen eine männliche Pflanze der Chromosomenkonstitution $XYYYY + 12A = 17$ beteiligt ist, die bezüglich *Y*-Chromosomen einheitlichere Gameten ergibt, sind mehr männliche Individuen als normal festzustellen. Das wird als Beweis dafür angesehen, daß das Überwiegen der weiblichen Individuen bei *R. thyrsiflorus* mit den *Y*-Chromosomen im Zusammenhang steht.

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