Structure and Function of Sex Chromosomes in *Rumex thyrsiflorus*

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INTRODUCTION

Rumex thyrsiflorus is a dioecious species of the *Acetosa* group. It grows on the lowlands, mostly along roads, river banks, railway embankments and dry meadows. The species is common in Siberia, North-East, Central and South-East Europe.

R. thyrsiflorus and the closely related species *R. acetosa, R. tuberosus* and *R. arifolius* form in the genus *Rumex* a distinct group of dioecious species under the common name of *Acetosa*. The mechanism of sex determination in this group is relatively well known by now. It is a system of the X/A type also called *Drosophila* type. Doubtlessly a mechanism of sex determination of this type is evolutionarily far advanced. Therefore the group *Acetosa* may be considered as an interesting example of the evolution of dioecism in higher plants.

It is generally considered that bisexualism is a more primitive state than dioecism (W e s t e r g a a r d, 1958; O h n o, 1967; D a r l i n g t o n, 1965). Thus, bisexualism is as a rule the starting point for evolution towards dioecism which may be evolutionarily advanced to various degrees, as shown on the following examples.

Urtica dioica is a plant with a rather primitive mechanism of sex determination. In this species no sex chromosomes have been found. In natural populations there occurr, beside male and female plants, also bisexual specimens. Moreover, plants which in one year were bisexual may in the next prove to be male or female. It also happens that plants recognized as male or female may in the following season become bisexual. In the progeny of crosses between bisexual individuals, and those between bisexual and male or female individuals, no regularity is found in the segregation of sex-determining factors, the proportion of ♂ and ♀ plants being quite fortuitous (Ż u k, unpublished data). It would therefore seem that in *Urtica dioica* sex chromosomes are not yet differentiated, and the sex-determining factors are distributed in various chromosomes. The sex of the individual is not definitively determined genetically so that it may be modified by external factors.

An example of a plant with a more developed mechanism of sex deter-

mination is *Rumex acetosella* (L ö v e, 1944) or *Melandrium album* (W e s t g a a r d, 1958). It was established conclusively in these species that they possess chromosomes X and Y. The mechanism of sex determination is of X/Y type and depends on the ratio of X and Y chromosomes. The factors strongly determining male sex are localized in chromosomes Y, so that one Y chromosome is an effective counterpart to several X chromosomes. Intersexual individuals arise only as the result of definite chromosome combinations, and not owing to changes, however drastic, in the external conditions.

At the next stage of evolution the role of chromosome Y in sex determination decreases. *R. hastatulus* may be quoted here as example. In one of the races of this species, female individuals have a karyotype $8a + XX = 10$, and the male ones $8a + XY = 10$ (where a-autosomes). B a r t- k o w i a k (in preparation) obtained a number of polyploid *R. hastatulus* plants with a varying number of X and Y chromosomes. Analysis of these plants demonstrated that the sex of the plant depends both on the ratio of Y chromosomes to X chromosomes, and on the ratio of X chromosomes to autosomes. Similar results were obtained by S m i t h (1955, 1963, 1964, 1969) for the race of *R. hastatulus* with the $6a + XX = 8$ ♀ and $6a + XYY = 9$ ♂ karyotypes.

Still more advanced evolutionally is the type of sex determination mechanism in *R. thyrsiflorus* and related species. In this species sex is exclusively dependent on the ratio of X chromosomes and autosomes. Chromosomes Y do not take part in sex determination. The next evolutional stage would be the loss of chromosomes Y and the occurrence of the X/O mechanismas found in certain groups of insects. So far, however, this type of sex determination mechanism has not been observed in higher plants. Therefore, the mechanism of X/A type found in *R. thyrsiflorus* may be considered as evolutionally most advanced as compared with all those known in higher plants. Thus, investigations on the mechanism of sex determination and the function of sex chromosomes in *R. thyrsiflorus* seems to be particularly interesting.

This plant is a good object both for genetic and cytological investigations. Plants bred from seeds flower within 4—5 months, and each female plant bears several thousand seeds, this making possible production of a numerous progeny in a relatively short time. Controlled pollination is exceptionally simple and effective owing to the complete dioecism of the plant. The species is easily cultivated both in glasshouse and on experimental plots.

R. thyrsiflorus has a small number of chromosomes. In female plants two X chromosomes and twelve autosomes may be distinguished giving a total of $2n = 14$; in male plants there is one X chromosome, two Y chromosomes and twelve autosomes making a total of $2n = 15$ (M e u r m a n, 1925; Ś w i e t l i ń s k a, 1963; Ż u k, 1963; L ö v e, 1968). The chromoso-

mes are relatively large. The X chromosomes, in the set, measure about 7—8 μ, and the size of the smallest pair of autosomes is of the order 3—4 μ. Sex chromosomes (X, Y_1 and Y_2) are easy to distinguish. The X chromosomes are metacentric and large, Y chromosomes are somewhat smaller, yet distinctly larger than the autosomes, and as a rule sub- or matecentric.

I. MECHANISM OF SEX DETERMINATION IN R. *THYRSIFLORUS*

The obtention of natural polyploids in the progeny of R. *thyrsiflorus* x R. *arifolius* and R. *acetosa* hybrids (Ś w i e t l i ń s k a, 1963; Ż u k 1963; Ś w i e t l i ń s k a and Ż u k, 1965) allowed to investigate in detail the mechanism of sex determination in the *Acetosa* group. By repeated crossing of the polyploids obtained with diploid individuals of R. *thyrsiflorus,* a number of triploid and aneuploid plants was obtained with a varying number of X and Y chromosomes. Also diploid male and female plants occurred with additional Y chromosomes. The author also succeeded in breeding several „supermale" plants with one X chromosome and a triploid number of autosomes as well as several „superfemale" plants with three X chromosomes and a diploid number of autosomes.

Analysis of individuals with different ratios of X and Y chromosomes and autosomes throws more light on the system of sex determination in R. *thyrsiflorus.* According to previously obtained data (Ż u k, 1963; Ż u k 1970b), the sex of the individual is determined by the quantitative ratio of X chromosomes and autosomes, X/A, termed sex index. In female plants (2A + XX) the sex index is equal to 1, and in male plants (2A + XYY) the sex index is 0.5. Plants with a sex index between 1 and 0.5 are intersexual, e.g. in the case ♀♂ (3A + 2X + 2Y) the index is 0.66. Analogous data were obtained for R. *acetosa* by Japanese investigators (O n o, 1935; Y a m a m o t o, 1938) thus indicating that this system of sex determination is common to all species of the *Acetosa* group.

It is interesting to compare the data obtained for R. *thyrsiflorus* with those of other organisms, the mechanism of sex determination of which is well known, such as *Melandrium* and *Drosophila.* The table 1 lists the karyotypes and sex of R. *thyrsiflorus, Melandrium* and *Drosophila* individuals with the same combinations of sex chromosomes and autosomes. As seen, the individuals of *Drosophila* and *Rumex* with the same ratio of sex chromosomes and autosomes are of the same sex. Thus, individuals No. 16 (karyotype 2A + XXYY) are female, individuals No. 4 (3A + XX) and No. 22 (3A + XXY) are intersexual in both these species. It results from the Table that both in *Drosophila* and R. *thyrsiflorus* sex is determined by the ratio of X chromosomes to autosomes, and the presence of 1, 2 or even 3 Y chromosomes has no effect on sex expression. In this system Y chromosomes are completely inactive in sex determination.

The system of sex determination in *Melandrium* is different. As shown in the table all individuals with a chromosome Y in their karyotype are male, independently of the number of X chromosomes and autosomes. Only tetraploid plants with four chromosomes X, and only one Y chromosome exhibit a tendency to intersexuality.

Among higher plants sex determination in *Melandrium* is the best known (W e s t e r g a a r d, 1940, 1948, 1953, 1958; W a r m k e and B l a- k e s l e e, 1940; N y g r e n, 1957). In the course of extensive studies it was established that in *Melandrium* the Y chromosome determines male sex and the X chromosome female sex. Besides, some of the autosomes carry the male factors determining male sex, and others female factors determining female sex. According to W e s t e r g a a r d, the sex depends on the interaction of two gene complexes: (1) the „male" and „female" genes carried by all chromosomes and present in both sexes, (2) sex-determining genes localised in the differential segments of the Y chromosome. The presence of the Y chromosome determines the development of the individuals in the male direction, and the absence of this chromosome determines the development in female direction.

The hypothesis of W e s t e r g a a r d is contradicted by G o l d - s c h m i d t (1958). According to this author W e s t e r g a a r d's interpretation explains only one exceptional case, that of *Melandrium,* it does not, however, elucidate numerous well known examples of intersexuality in other organisms. G o l d s c h m i d t considers as more common the mechanism of sex determination consisting in a balance between the autosomal determinants of male and female sex with those present in chromosome X. He quotes data indicating the presence in *Melandrium* of such a mechanism. The only fact which according to G o l d s c h m i d t requires further interpretation in *Melandrium* is the exceptionally strong action of the Y chromosome.

The case of *Melandrium,* however, in spite of the suggestions of G o l d s c h m i d t, cannot be treated as an exception, since a similar mechanism of sex determination has also been found in *Rumex angiocarpus, R. tenuifolius, R. acetosella, R. graminifolius* (L ö v e, 1944, 1957), *Spinacia oleracea* (J a n i c k & S t e f e n s o n, 1955), *Acnida* (M u r r a y, 1940) and in *Carica papala* (H o f m a y r, 1945, quoted after W e s t e r- g a a r d, 1958). The data of S m i t h (1963, 1964, 1969) suggest that also in *R. hastatulus* the chromosome Y contains male determining factors. As regards animals W e l s h o u s and R u s s e l l (1959) have found sex- -determining factors in the murine Y chromosome. The presence of male sex-determining factors in the Y chromosome has been conclusively proved in man (D r o n a m r a j u, 1965; L e w i s and J o h n, 1968).

In the light of these data Westergaard's interpretation of the mechanism of sex determination in *Melandrium* seems correct, and the case of this species is no exception as was suggested by G o l d s c h m i d t. This

hypothesis, however cannot be applied for explaining the mechanism of sex determination in *R. thyrsiflorus*. The data obtained for this species, particularly analysis of the intersex individuals obtained, do not support the existence of some trigger mechanism releasing the development of one or the other sex. It seems easier and simpler to explain the mechanism of sex determination by a balance established between the male factors in the autosomes and the female factors present in the X chromosome. Assuming the trigger mechanism we may expect that with a certain definite ratio of chromosomes X to autosomes the plant will be intersexual. On the other hand, plants with a sex index above or below this limiting value should be purely male or female. Experimental findings do not confirm this supposition. A number of intersexes has been obtained with various sex chromosome/autosome ratios so that the sex index varied in these plants within the limits 0.75—0.57. It was found that, as the sex index shifts towards the value 1.0 characteristic for female sex, the pistils in the flowers are better developed, and the anthers more rudimentary. On the other hand, if the sex index is shifted towards the value 0.5 (characteristic for male sex), the anthers are better developed and the pistils reduced to rudimentary organs. Thus, in *Rumex* there is no characteristic value of the sex index, the transgression of which would release a trigger mechanism determining the development of the individual towards one or the other sex.

II. MECHANISM OF SEX DETERMINATION AND POLYPLOIDY

In natural populations of *Rumex* species of the *Acetosa* group, polyploid individuals occur quite often (O n o, 1935; Y a m a m o t o, 1938; Ż u k, 1963). A high percentage of polyploids has also been found in interspecific hybrids. It has been demonstrated that polyploid individuals arise as the result of unreduced gamete formation by the parent plants. The tendency to such gametes formation is relatively frequent in the *Acetosa* group (Ż u k, 1963). The question therefore arises, why, in spite of this tendency, a polyploid dioecius line has not been established in this group in the course of evolution.

It would seem that the situation observed in the group *Acetosa* is analogous in various respects to that in the animal kingdom. M ü l l e r (1925) advanced a theory according to which the rare occurrence of polyploidy in animals is connected with their bisexuality. For initiating a polyploid line in bisexual organisms two polyploid individuals ♂ and ♀ are necessary. The chances of their simultaneous appearance in a population are very small. If such an eventuality even occurs, the fertility of these individuals is limited owing to disturbances in sex chromosome conjugation. For instance, in a tetraploid male individual with a sex chromosome composition XXYY, instead of two bivalents X—Y and X—Y, the biva-.

lents XX and YY will be formed because the chromosomes X and Y will rather have a tendency of conjugating with a similar one. Owing to such a conjugation a sex chromosome segregation of the type XY—XY will be most frequent. The gametes formed will thus mostly have an XY composition and in the progeny intersexes partially infertile or completely sterile will prevail.

An analogous situation is found in *R. thyrsiflorus*. In the tetraploid male plants with a 24a + XXYYYY = 30 karyotype, the sex chromosomes segregate generally as follows: XYY—XYY. The gametes formed exhibit usually the XYY composition. Therefore in the progeny of the cross between a tetraploid female plant (24a + XXXX = 28), and a tetraploid male plant (24a + XXYYYY = 30) most frequent are karyotypes 24a + XXXYY = 29. These plants are intersexual and mostly completely sterile, their progeny gives no possibility of establishing a polyploid line (Ż u k, 1963).

According to L ö v e (1969), however, this barrier may be circumvened. This author believes that in the *Acetosa* group of the *Rumex* genus there occurs a phenomenon known as internal autopolyploidy which does not cause disturbances in sex expression. The author supposes that the initial form for the evolution of the *Acetosa* group was *R. hastatulus* which has a set of chromosomes XX in the female and XYY in the male and only six autosomes. L ö v e believes that the same sex chromosomes occur now in the *Acetosa* group, only the number of autosomes has doubled to 12. A number of data, however, does not confirm the close relationship between *R. hastatulus* and the species of the subsection *Acetosa*. In *R. hastatulus*, namely, the chromosomes are much smaller and of different morphology than in the species of the *Acetosa* group. Also the external morphology of this species resembles rather that of *R. acetosella* than the species of the *Acetosa* group. S i n g h (1964) was the first to obtain hybrids between *R. hastatulus* and *R. acetosella*, whereas crosses between *R. tuberosus* and *R. hastatulus* could not be produced (B a r t k o w i a k, unpublished).

More convincing seems to be the view of M ü l l e r that dioecism is an essential obstacle in establishing a polyploidal line. This view seems correct both as far as animals and plants are concerned. The chances of establishment of a polyploid line depend on the type of the mechanism of sex determination. It would appear that a sex determining mechanism of the *Drosophila* type counteracts most strongly the establishment of polyploidy. A good example for this rule may be found in *Rumex*. In this genus 34 species have been cytologically examined (D a r l i n g t o n and W y l i e, 1955), and among these as many as 21 (62%) proved to be polyploid. Polyploid species occur, however, in some subgenera and sections of the genus *Rumex* very often, whereas in others they are very rare. They are often found in systematic groups including hermaphoroditic

species and in the dioecious subspecies *Acetosella* where a sex-determining mechanism of the *Melandrium* type has developed. On the other hand, in the subsection *Acetosa* not one polyploid species has been detected, although, as reported by L ö v e (1957) about 20 species and subspecies belonging to this section have been examined.

According to the list prepared by G a l l i e n (1959), it results that in the animal kingdom the sex determination mechanism of *Drosophila* type is most common. Since there is no doubt that such a type of mechanism is an essential obstacle to the establishment of polyploid lines, it may be assumed that one of the causes of the rare occurrence of polyploidy in animals is their mechanism of sex determination. The same seems to be true in the case of dioecious *Rumex* species in the section *Acetosa*.

III. CYTOLOGICAL ANALYSIS OF SEX CHROMOSOMES

1. Localization of heterochromatin segments

In the interphase nuclei of *R. thyrsiflorus* numerous chromocentres are easily visible formed by the heterochromatic segments of the chromosomes. Detailed observations allowed to establish that the number of
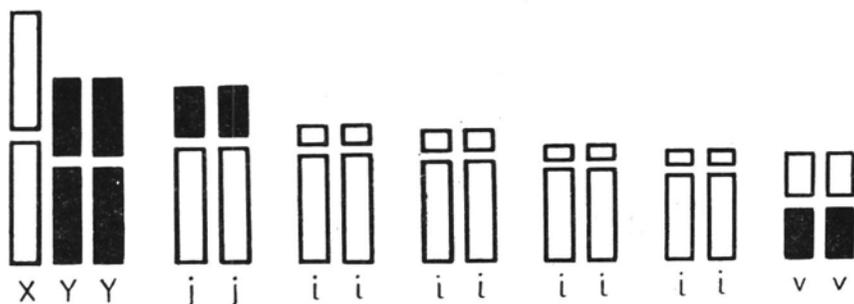

Fig. 1. Localization of heterochromatic segments in chromosome complement of *R. thyrsiflorus*.

heterochromatic bodies is much higher in male than in female plants in the interphase nuclei. In the male individual, two large heterochromatic bodies are seen which are lacking in female plants. Since the karyotype of female and male plants differs only in the sex chromosomes (XX in ♀ and XYY in ♂ individuals), therefore the presence of large heterochromatic bodies in male plants must be connected with the presence of Y chromosomes. It may be deduced indirectly from this that Y chromosomes are partly or completely heterochromatic.

In the prophase of mitotic division the heterochromatic parts of the particular chromosomes are much more condensed than the euchromatic ones, and they stain much more intensely. These differences become still more pronounced if the material examined is pretreated with oxyquinoli-

Table 1

Relations between chromosome composition and sex in *Melandrium* *,
Drosophila * and *Rumex*

Chromosomes	*Melandrium*	*Drosophila*	*Rumex* thyrsiflorus
1. 2A+XX	♀	♀	♀
2. 2A+XXX	♀	♀	♀
3. 3A+X	—	♂	—
4. 3A+XX	♀	intersex	intersex
5. 3A+XX	♀	♀	♀
6. 4A+XX	♀	♂	—
7. 4A+XXX	♀	intersex	—
8. 4A+XXXX	♀	♀	♀
9. 4A+XXXXX	♀	—	—
10. 2A+XY	♂	♂	♂
11. 2A+XYY	♂	♂	♂
12. 2A+XYYY	—	—	♂
13. 2A+XYYYY	—	—	♂
14. 2A+XYYYYY	—	—	♂
15. 2A+XXY	♂	♀	♀
16. 2A+XXYY	—	♀	♀
17. 2A+XXYYY	—	—	♀
18. 3A+XY	♂	—	—
19. 3A+XYY	♂	—	♂
20. 3A+XYYY	—	—	♂
21. 3A+XYYYY	—	—	♂
22. 3A+YXY	♂	intersex	intersex
23. 3A+XXYY	—	—	intersex
24. 3A+XXXY	♂	♀	♀
25. 3A+XXXYY	—	—	♀
26. 4A+XY	♂	—	—
27. 4A+XXY	♂	—	♂
28. 4A+XXYY	♂	—	♂
29. 4A+XXYYY	—	—	♂
30. 4A+XXYYYY	—	—	♂
31. 4A+XXXY	♂	—	—
32. 4A+XXXYY	♂	—	intersex
33. 4A+XXXXY	♂→♀	—	—
34. 4A+XXXXXY	♂	—	—
35. 5A+XXXYYYY	—	—	intersex

* According Bacci (1965)

ne (T j i o and L e v a n, 1950). It could be demonstrated (Ż u k, 1969) by this method that in *R. thyrsiflorus* both Y chromosomes are actually heterochromatic. It was also found that large segments of some autosomes are heterochromatic. The distribution of the heterochromatic segments in the karyotype of *R. thyrsiflorus* is illustrated in Fig. 1. So far among higher plants, beside *R. thyrsiflorus*, heteropycnotic Y chromosomes have been revealed only in *Humulus japonicus* (J a c o b s e n, 1957).

Investigations on the morphological variability of sex chromosomes (Ż u k, 1969) and autosomes (Z a b o r o w s k a, 1969) in natural *R. thyrsiflorus* populations have revealed a certain regularity. Chromosomes X, namely, from various populations are morphologically uniform, whereas Y chromosomes are much more variable. The cytologically examined plants could be grossly divided into four groups as regards the morphology of Y chromosomes. In the first group both chromosomes Y are of the same length and metacentric. Those of the second group are also metacentric but they differ in size. Of the Y chromosomes of group three one is large and metacentric, and the other is smaller and submetacentric. In the last group one Y chromosome is large and submetacentric, the second smaller and metacentric. The morphological variability of Y chromosomes in *R. thyrsiflorus* probably results from the fact that they are heterochromatic. Data for autosomes (Z b o r o w s k a, 1969) indicate that, analogously, the partly heterochromatic autosomes are morphologically more variable. A similar variability of Y chromosomes was also observed in man in various ethnic groups (D r o n a m r a j u, 1965), this being probably also connected with the heterochromatic state of these chromosomes.

2. Chromosome replication

By the application of autoradiographic methods for the study of chromosome replication it has been demonstrated that the heterochromatic segments replicate later than the euchromatic ones (L i m a de F a r i a, 1959; T a y l o r, 1960; E v a n s, 1964; K u s a n a g i, 1966; D a r l i n g t o n. 1966; S c h m i d t, 1967; L i m a de F a r i a and J a w o r s k a, 1968; Ż u k, 1969). It was also found that in most of the mammals investigated including man, and in insects, sex chromosomes X and Y replicate later than other chromosomes of the complement, this being due to their heterochromatic state (L i m a de F a r i a, R e i t u l u and B e r g m a n (1961). L i m a de F a r i a (1964) was the first to demonstrate that chromosome Y replicates in man later than the other chromosomes. He also discovered that in women one of the X chromosomes which undergoes physiological heterochromatisation also replicates late. C h u and R u s - s e l l (1965) investigated chromosome replication in mice and noted a late replication of Y chromosome. In *Cricetulus griseus* chromosomes X and Y form distinct chromocentres in interphase, and replicate later than the euchromatic chromosomes (T a y l o r, 1960; H s u, 1964; P f l u e g e r and Y u n i s, 1966). According to S c h m i d t (1967) the sex chromosomes of *Mesocricetus auratus* behave in a similar way. The investigations of W o l f et al. (1965) demonstrated that the sex chromosomes in *Microtus agrestis* are larger than in any other known mammals. A late replication of chromosomes X and Y was also observed in this species. The phenomenon was noted in rats (T a k a g i and M a k i n o, 1966), in rabbits (K i n s e y, 1966),

in the opossum (S i n h a, 1966; S c h n e i d e r and R i e k e, 1967). The whole chromosome Y of *Drosophila*, and the segment of chromosome X on both sides of the centromere replicate later than the euchromatic autosomes (B a r i g o z z i *et al.,* 1966; B e r e n d e s, 1966).

Up till now analogous investigations on sex chromosome replication in plants have not been undertaken. *R. thyrsiflorus* owing to its rather large chromosomes and easily distinguishable sex chromosomes X, Y_1 and Y_2 is a convenient object for this kind of studies. Chromosome replication was studied in this plant according to the standard technique (Ż u k, 1969). Seeds germinated on moist filter paper were incubated for one hour in a 3H-thymidine solution, samples taken at 2-h intervals were fixed up to 26 h.

After 2 and 4 h all the metaphases in the fixed material were unlabelled, this indicating that the cells had completed DNA replication before their contact with radioactive thymidine. The first labelled metaphase appeared in the fixed material 6 h after the beginning of incubation in ^{3}H-thymidine. Labelling could be found on the metaphase plates only in Y chromosomes and short segments of some autosomes. The occurrence of labelling coincided fully with the previously localized heterochromatin. These cells completed chromosome replication incubation in ^{3}H-thymidine. Labelling could be found on the metawere the first to show labelling in mitosis. Since in these cells only the heterochromatic segments of the chromosomes are labelled, it may be concluded that they replicate somewhat later than the euchromatic ones. In preparations fixed 8 and 10 h after the beginning of incubation only heterochromatin was labelled on some metaphase plates but on others all chromosomes were uniformly labelled. Most cells which had reached the metaphase stage at a later period had all the chromosomes uniformly labelled. On some metaphase plates, however, one of the Y chromosomes was almost unlabelled. These cells were probably in the beginning of phase S during incubation, it would thus seem that one of the Y chromosomes starts replication later than the other.

One may conclude therefrom that both Y chromosomes replicate longer than the X chromosomes and autosomes; one Y chromosomes begins replication simultaneously with the autosomes, and the other one a little later. Since both Y chromosomes complete their replication at the same time, the replication time of one Y chromosome must be somewhat longer than that of the other one.

Autoradiography allowed to determine more precisely the replication time of heterochromatic Y chromosomes and of euchromatic chromosomes. It appeared that one Y chromosome replicates within 7.8 h, the other one in 8 h and euchromatin in the course of 5.8 h. Thus, replication of heterochromatic Y chromosomes lasts two hours longer than that of euchromatic ones.

It is interesting to compare these data with those for man and for the hamster. The replication time of heteropycnotic X chromosomes in human fibroblast cells was investigated by C o m i n g s (1967), and that of the heterochromatic chromosome Y in *Cricetulus griseus* by H s u (1964). In both cases heterochromatic chromosomes began replication much later than the euchromatic ones and completed it later. The entire replication time of heterochromatin was, however, much shorter than for euchromatin. Thus, the situation in *Rumex* in this respect is quite different, since euchromatin replication lasts here a shorter time than that of heterochromatin.

3. Replication of heterochromatin

As already mentioned, the Y chromosomes in *R. thyrsiflorus* form in the interphase compact, intensively staining bodies. The question arises, how does the replication of these chromosomes occur. There seem to be three possibilities: (1) the heterochromatic Y chromosomes become completely despiralised before and during DNA replication; (2) DNA synthesis is possible without despiralisation; (3) the short submicroscopic segments of the compact Y chromosomes despiralise, replicate and immediately return to their initial state.

In order to elucidate which of these systems is most probable, germinated seeds were incubated for a short time in a radioactive thymidine solution and fixed immediately afterwards. Autoradiographic preparations were obtained in which all cells which were in S period during incubation were labelled, whereas cells in other phases of the cell cycle, i.e. before replication (G_1), after replication (G_2) and during mitosis (M) were not labelled. The observations revealed that heterochromatic bodies subsist in the course of the entire interphase, that is both before, after and during DNA replication. In the labelled cells radioactive thymidine incorporation was noted both into the euchromatin and into the heterochromatic bodies. The presence of heterochromatic bodies in S phase and their ability of incorporating radioactive thymidine indicate that the heterochromatic Y chromosomes are in a compact state in the course of replication. This would mean that in this case despiralisation of the entire heterochromatic block or its large segment is not a necessary condition for DNA synthesis. These data, do not exclude, however, the hypothesis of K l i n g e r, S c h w a r z a c h e r and W e i s s (1967) and of Z a k h a - r o v and E g o l i n a (1968) that short submicroscopic segments of condensed heterochromatin are despiralised during replication, and return to their initial state rapidly after this process. More precise observation of the heterochromatic blocks in S phase plainly confirms this possibility. It seems, namely, that in *R. thyrsiflorus* the heterochromatic blocks have a more fuzzy appearance and are less sharply outlined during DNA replication.

4. Sex chromosome conjugation

The conjugation of sex chromosomes was investigated in normal diploid male plants with a karyotype $12a + XYY = 15$, in plants with karyotype $12a + XYYY = 16$, $12a + XYYYY = 17$ and $12a + XYYYYY = 18$ (Ż u k, 1970a). In a normal male plant sex chromosomes form a trivalent Y_1XY_2. The homologous segments of sex chromosomes X, Y_1 and Y_2 are very short, so that in the prophase of meiosis the sex chromosomes in the trivalent associate end-to-end, the chromosome X occupying as a rule a central position. The X chromosomes are completely homologous with one another, and as observed in plants with karyotypes $18a + XX = 21$ and $21a + XX = 23$, they conjugate side-by-side, and form a bivalent with two terminal chiasmata in diakinesis. According to the conjugation pattern in meiosis the homology between the sex chromosomes X and Y_1Y_2 is as follows: the X chromosomes are homologous to one another on their whole length, at the end of one arm a short segment homologous to chromosome Y_2 is localised, while the second arm exhibits a segment homologous to chromosome Y_2. Chromosomes Y_1 and Y_2 are completely nonhomologous to one another.

In plants with additional Y chromosomes normal sex conjugation is disturbed. Instead of the characteristic trivalent Y_1—X—Y_2, frequently univalents, bivalents X—Y or Y—Y and tetravalents X—Y—Y—Y may be found. Association between Y chromosomes may occur as the result of conjugation between homologous segments (in the case of bivalents Y_1—Y_1 or Y_2—Y_2), or also owing to the tendency of heterochromatin to fusion. Disturbed sex chromosome conjugation results in their irregular segregation into daughter nuclei. In normal conjugation (trivalent Y_1 — X — Y_2) sex chromosome segregation is as a rule of the type X $\longleftrightarrow Y_1Y_2$. In plants with a karyotype $12a + XXYY = 16$ segregation was mostly of the XY $\longleftrightarrow$ YY type.

End-to-end association is the most common way of sex chromosomes conjugation in meiosis in mammals (H a y w a r d, 1962; U t a k o j i, 1966; M i t t w o c h, 1967; S o l a r i, 1969) and in dioecious plants (J a c o b s e n, 1957; W e s t e r g a r d, 1958; S m i t h, 1964). It has been demonstrated in a number of organisms that end-to-end association of X—Y chromosome pairs is due to the formation of chiasmata between the small homologous segments of these chromosomes (O h n o, K a p l a n and K i n o s i t a, 1959; U t a k o j i, 1966; S o l a r i, 1969). The homologous segments of X and Y chromosomes in *Rumex* are very short, below the resolving power of autoradiography, this method shows late replication of Y chromosomes on their entire length. Actually, however, Y chromosomes must have minute euchromatin segments at the ends, which makes possible the conjugation with the completely euchromatic X chromosomes. Thus, we may imagine the Y chromosomes in *R. thyrsiflorus* as composed of large

heterochromatic, and tiny euchromatic segments homologous to the X chromosome.

As mentioned above, in *R. thyrsiflorus* the association of sex chromosomes may be due not only to the formation of chiasmata between the homologous segments of X and Y chromosomes, it may also be the result of heterochromatin fusion (Żuk, 1970a). If in a plant with karyotype $12a + XYYY = 16$ a „sex tetravelent" is formed, then the conjugation between the chromosomes Y_1XY_2 results from the partial homology between the chromosomes Y_1, X and Y_2. On the other hand, conjugation of this trivalent with the additional Y chromosome (that is between two Y's) must be the result of fusion of heterochromatin, because the homologous segment on one Y chromosome is already „occupied" owing to conjugation with the X chromosome.

Association of nonhomologous chromosomes during meiosis owing to fusion of heterochromatin has been observed in a number of cases (Riley and Low, 1965, Żuk, 1969). Lately Maguire (1969) has found that the distribution of large heterochromatic knobs in premeiotic interphase nuclei of maize is not random. It was found that two large K10 heterochromatic knobs are much nearer to one another than random expectations predict.

It would seem therefore, that the tendency of heterochromatin to clump and its nonrandom distribution in interphase, and particularly in premeiotic interphase is a rather common phenomenon, and may explain the "conjugation" of nonhomologous heterochromatic chromosomes.

IV. THE FUNCTION OF Y CHROMOSOMES

It results from the above presented data that in *R. thyrsiflorus* both Y chromosomes are heterochromatic and have no effect on sex determination. Thus it would seem that the Y chromosomes of this species have lost their genetic activity and are some kind of rudimentary organ. In this situation their elimination in the course of evolution would have no disfavourable effect on the expansion of the species. There is reason to believe that in the *Acetosa* group of the genus *Rumex* dioecism has established very long ago (Löve, 1969). The fact that in the course of this long period the Y chromosomes have not been eliminated would seem to indicate that they have some function essential for the species. Analysis of plants with various numbers of sex chromosomes has allowed to elucidate some of these functions.

1. Control of fertility of male plants

A number of intersexual and male plants lacking Y chromosomes or possessing only one chromosome has been obtained. The course of meiosis

in these plants, and their ability of producing normal pollen was investiga-
ted (Ż u k, 1970b). It was found that plants without Y chromosomes are
completely male sterile although chromosome conjugation and the entire
course of meiosis in these plants were normal. Neither were disturbances
in chromosome conjugation and meiosis observed in plants with a single
chromosome Y, only the amount of normal pollen was often reduced.
Some plants with one Y chromosome exhibited a rather high fertility,
but others were completely male-sterile. On the contrary, male plants
with the same polyploidy level but possessing two Y chromosomes always
produced a greater or smaller amount of morphologically normal pollen.

These observations allow the supposition that Y chromosomes contain
some factors controlling the normal pollen development. The fact that,
in plants lacking the Y chromosome or having but a single one, meiosis
is normal suggests that the presence of Y chromosomes is not necessary
for the normal course of meiosis, it is, however, indispensable for the
postmeiotic pollen development. The observation that some plants with
a single Y chromosome are completely sterile, and some relatively fertile,
may indicate that one of these chromosomes plays a more important role
in the control of fertility of male plants than the other.

2. Control of the sex ratio in populations

Some observations indicate that in *R. thyrsiflorus* Y chromosomes play
a significant role in the regulation of the ratio of male and female plants
in the population (Ż u k, 1970b). In natural populations of this species
male individuals are much less numerous than the female ones (sex ratio
ca. 1:9 in favour of female plants). The female plants with karyotype
$12a + XYY = 15$ produce equal numbers of gametes with a $6a + XY = 8$
and $6a + X = 7$ composition determining male and female sex, respecti-
vely. The predominance of female plants may either indicate a greater
chance of fertilization with pollen determining female sex, or a lower
viability of male zygotes. Data discussed in an earlier publication (Ż u k,
1963) suggest rather nonrandomness of fertilization favouring female sex
determining gametes. The cause of the lower effectiveness in fertilization
of the $6a + YY = 8$ than of the $6a + X = 7$ gametes may be either the
presence of two Y chromosomes or the absence of chromosome X. In
order to elucidate which of these eventualities is more probable, a num-
ber of crosses was performed between plants with additional Y chromo-
somes. In the progeny of these crosses the percentage of male individuals
was higher than in the progeny of crosses between plants with a normal
karyotype. These results may be interpreted as follows. The male indivi-
dual used for the cross had a karyotype $12a + XYYYY = 17$, thus, with
as many as four Y chromosomes. One may suppose that in such a plant
gametes with the composition $6a + XYY = 9$ and $6a + YY = 8$ will be

most frequent. Therefore both male and female sex determining gametes will each contain two Y chromosomes, thus they will not differ in this respect. Consequently, the chance of fertilization of the gametes determining male and female sex will be equal, and in the progeny the sex ratio should be close to 1:1. The results obtained confirm these assumptions, and it would seem that it is rather the presence of two Y chromosomes than the absence of the X chromosome in the gametes determining male sex that is the cause of nonrandom fertilization leading to a prevalence of female individuals in the population.

The question arises why such a system of regulation of the sex ratio has been established in the course of evolution. L e w i s and J o h n (1968) call attention to the fact that dioecism is a system preventing selfpollination. It is, however, a very wasteful system in which most of the gametes are not utilized. This can be corrected in some extent by a change in the sex ratio in favour of female individuals. In R. thyrsiflorus one male plant produces a sufficient amount of pollen for fertilising a large number of female plants. Therefore, if the sex ratio is changed in favour of female plants in the population, the reproductive ability of the species is increased. Such a system ensures the possibility of exploatation of a wider area and ressources of the environment by female seed-producing plants. Thus, it is a very profitable system from the point of view of evolution, this finding its expression in the wide spread of this species and its ability of adaptation to various environments.

3. The phenotypic effect of additional Y chromosomes

The obtention of male and female individuals with a varying number of additional Y chromosomes allowed to investigate their effect on the fertility and morphology of the plants (Ż u k, in press). Normal male R. thyrsiflorus plants with karyotype $12a + XYY = 15$ produce 85—90 per cent of normally developed pollen. In Y chromosome hyperploid plants, the percentage of normal pollen also varied within these limits. Plants with a distinctly reduced pollen fertility were relatively rare. It may be assumed on the whole that additional Y chromosomes do not exert a negative influence on pollen development.

In the case of female plants with additional Y chromosomes the situation is somewhat different, since seed setting and their germination power were markedly reduced. Crosses performed between normal diploid plants yielded usually about 1000 seeds per cross. In crosses of normal male plants with female ones having additional Y chromosomes the number of seeds was distinctly reduced varying within the limits of 14—80 per cent.

Both male and female plants with one additional Y chromosome are

indistinguishable in external morphology from normal individuals. On the other hand, the presence of two or more additional Y chromosomes distinctly modifies the plant's appearance. The leaves, particularly those of seedings are curled, light green with a modified shape of their blades. The number of leaves and flower stems is greatly reduced. The plants are distinctly lower than the normal ones. It was found that the more numerous are the additional chromosomes Y the more dwarfed are the plants. Even one additional Y chromosome which does not change the leaf morphology reduces visibly the length of the plant.

It was also observed that Y chromosome hyperploid plants are less viable than normal ones. *R. thyrsiflorus* is a perennial species and normal diploid plants can be cultivated for several years, whereas those with additional Y chromosomes die much sooner.

Hyperploid individuals in respect to the Y chromosome have been obtained in a number of organisms such as *Drosophila* (D r o n a m r a j u, 1965; M i t t w o c h, 1967; L e w i s and J o h n, 1968; G r e l l, 1959, 1969), in humans (C h u, 1964; J a c o b s, B r u n t o n and M e l v i l l e, 1965), mice (C a t t a n a c h, 1961; R u s s e l l and C h u, 1961; M i t t w o c h, 1967) and *Melandrium* (W e s t g a a r d, 1958). In all the above named organisms with the exception of *Drosophila,* individuals with additional Y chromosomes are male, because these chromosomes are strongly male sex determining. In *Drosophila,* in contrast, Y chromosomes have no effect on sex and the individuals with additional chromosomes Y may be male or female. The presence of supranumerary Y chromosomes was found to reduce fertility in *Drosophila* similarly as it does in *R. thyrsiflorus.* The negative effect of Y chromosomes has also been demonstrated in humans. According to the data of J a c o b s, B r u n t o n and M e l v i l l e (1965) it would seem that men with additional Y chromosomes are excessively tall, mentally underdeveloped an behave agressively. Female mice with a sex chromosome composition XXY instead of XX, though they cannot be morphologically distinguished from normal speciemens, are sterile.

According to M i t t w o c h (1967), relatively small modifications in the phenotype of mice with a changed sex chromosome composition may be explained by the fact that in this species sex chromosomes both X and Y are partly heterochromatic, and hence the changes in their number are less drastic than for instance in man. A similar situation seems to occur in *Rumex:* both Y chromosomes here are heterochromatic, therefore the presence of one additional chromosome does not noticeably influence the morphology of the plant or its ability of producing normal pollen. The effect of two or three additional chromosomes Y is already quite conspicuous, probably owing to the cumulation of minute effects not noticeable in the case of one additional Y chromosome.

V. ANALYSIS OF THE GENETIC ACTIVITY OF Y CHROMOSOMES

Data indicating that factors controlling such essential functions as fertility of male plants may be localized in the Y chromosomes prompted a more detailed analysis of their activity. It was demonstrated by cytological analysis and autoradiographic studies that Y chromosomes are practically completely heterochromatic and late replicating.

A view has been advanced that late replication and the formation of condensed heterochromatic bodies by the whole chromosome or some of its segments is connected with the lack of genetic activity, whereas a loose diffused state of chromatin is evidence of its activity (G r u m - b a c h, M o r i s h i m a and T a y l o r, 1963; H s u, S c h m i d t, S t u b b - l e f i e l d, 1964; B e r l o w i t z, 1965; B r o w n, 1966; F r e n s t e r (1966). Some authors ascribe the lack of genetic activity in heterochromatin to differences in the content of composition of histone proteins between hetero- and euchromatin. B l o c h (1966) developed a method of double staining of histones with eosin and fast green. In this method the chromatin of some nuclei stains pink, and of others green. The differences in staining are due to higher affinity of fast green for arginine-rich, and of eosin to lysine-rich histones. C l a y p o o l and B l o c h (1967) demonstrated, moreover, that the affinity to eosin stains is associated with cessation or slowing down of RNA synthesis. Thus, the method of histone double staining allows to obtain indirect information concerning the genetic activity of chromatin.

The results of other authors, however, are not univocal in this respect. B e r l o w i t z (1965) for instance found quantitative differences in histone content between hetero- and euchromatin, he did not, however, note any differences in the lysin/arginin ratio between these two types of histones. C o m i n g s (1967) compared electrophoretically histones originating from cells differing significantly in active and inactive chromatin content, but he found no differences between them. On the other hand, D o l b e a r e and K o e n i n g (1968) found distinct differences, both physical and chemical, between diffusive and compact chromatin.

Heterochromatic Y chromosomes in *Rumex* forming visible compact bodies in interphase nuclei allow to verify by cytochemical methods whether there exist in this plant differences in composition or content of histone proteins between hetero- and euchromatin.

The above described double staining method for histones developed by B l o c h was utilised for this purpose. It was found (Ż u k, 1969) that, upon double staining of mitotic interphase nuclei, the heterochromatic blocks stain pink with eosin, whereas euchromatin stains with fast green. One may assume on this basis that heterochromatin in *Rumex* contains more lysin-rich histones than does euchromatin.

As already mentioned, the affinity to eosin may indicate a cessation

B l o c h, 1967). This would be in agreement with the prevailing assumption that RNA is synthesized much more intensively by loose than by condensed chromatin (A l l f r e y and M i r s k y, 1962; F r e n s t e r et al., or slowing down of RNA synthesis (B l o c h, 1966; C l a y p o o l and 1963; B e r l o w i t z, 1965; F r e n s t e r, 1966). This postulate found confirmation in investigations using autoradiography in the electron microscope (L i t t a u, A l l f r e y, F r e n s t e r and M i r s k y, 1964). It results from the data obtained that the despiralisation of the chromosome thread is a necessary condition for the activity of genes localised in the given segment. This relation is not absolute, however, and as reported by C l e v e r (1968), exceptions are known when RNA synthesis was inhibited, although the chromosomes were not condensed, or was continued, in spite that the chromosomes were in condensed state.

Thus, according to the data discussed, cytological observations may provide some information concerning chromosome activity. Therefore the heterochromatic bodies of R. thyrsiflorus formed by Y chromosomes in various tissues were examined (Ż u k, 1969). Two large heterochromatic bodies may be found in about 60 per cent of meristematic cell nuclei from root tips. Also in most tapetal nuclei three or four heterochromatic bodies are usually well visible. Similarly, in the nuclei of cells from the flower stem usually two heterochromatic bodies can be detected. It may, therefore, be supposed that in these tissues the heterochromatic Y chromosomes are not genetically active.

A different picture is found in the last premeiotic interphase. At this stage distinct intensely staining heterochromatic bodies are not observed in pollen mother cells. This would indicate that at this stage heterochromatic Y chromosomes are not condensed but have a looser structure like the euchromatic ones. It is possible that in this stage the diffusive character of the Y chromosomes is associated with their genetic activity.

The cytological observations suggesting the activity of Y chromosomes in the premeiotic interphase were confirmed by cytochemical investigations using the method of double staining with eosin and fast green.

It was found that the chromatin of pollen mother cells stains green in the last interphase before meiosis, and only few chromatin grains exhibit affinity towards eosin. This picture is quite different from that in other tissues where intensive pink staining of the heterochromatic bodies formed by chromosomes Y was noted.

These findings indicate that in the premeiotic interphase chromatin including Y chromosomes is genetically active, and intensive RNA, synthesis should take place. Autoradiographic data confirm this suggestion. After incubation of flower stems for 48 h in a ^{3}H-uridine solution intensive labelling of the nuclei in premeiotic phase and at the very beginning of meiosis was found on the autoradiograms. A high degree of ^{3}H-uridine incorporation is evidence of intensive RNA synthesis in these cells. Thus,

the data indicating a genetic activity of Y chromosomes before meiosis coincide with the results of genetic observations which point to the localisation of the male fertility factors in the Y chromosomes. It may, therefore, be assumed that in the last interphase before meiosis, derepression of genetic information of Y chromosomes takes place. This information, as indicated by genetic data, is realised only during postmeiotic pollen development. Such a system seems logical, because in the course of meiotic division RNA synthesis does not occur. In R. *thyrsiflorus* male plants, two kinds of gametes $6a + X = 7$ and $6a + XY = 8$ are formed, therefore Y chromosomes are present only in one half of the gametes. If the information concerning pollen fertility would be derepressed after meiosis, only the pollen containing Y chromosomes would be fertile. Derepression of genetic information of the Y chromosomes before meiotic division ensures a normal development of both types of gametes.

The Y chromosome activity in R. *thyrsiflorus* should be confirmed by more extensive studies. If further data concerning the above advanced assumptions are obtained, it will be an interesting example of the regulation of genetic activity at the chromosome level. In this regulation system both heterochromatic Y chromosomes would be inactive during the entire ontogenetic development, with the exception of the stage immediately preceding meiosis in which they exhibit a looser structure and synthesise RNA.

An analogous example of fertility control by Y chromosomes is *Drosophila*. In this organism the role of chromosome Y in male fertility was confirmed by numerous authors (C o o p e r, 1959; B r o s e a u, 1960; D r o n a m r a j u, 1965; H e s s, 1967; H e s s and M e y e r, 1968; H e n n i n g, 1967, 1968). Recently the factor controlling male fertility was localised in the Y chromosome of mouse (K r z a n o w s k a, 1968). Thus, the role of Y chromosomes in fertility of male individuals seems to be a more general phenomenon.

SUMMARY AND CONCLUSIONS

1. Female *Rumex thyrsiflorus* plants have two X chromosomes and twelwe autosomes ($2n = 14$). The male plants have only one X, two Y chromosomes and twelve autosomes ($2n = 15$). The sex chromosomes are easily distinguishable. X chromosomes are metacentric and larger than the others in the set, Y chromosomes are metacentric or submetacentric, somewhat smaller than the X one but markedly larger than the autosomes.

2. Examination of a number of diploid and polyploid plants with a varying number of chromosomes X and Y demonstrated conclusively that Y chromosomes do not participate in sex determination. The sex is dependent on the ratio of X chromosomes to autosomes (sex determina-

tion mechanism of X/A type). This mechanism may be considered as the most evolutionally advanced among the higher plants studied to date.

3. In the *Acetosa* section no dioecious polyploid line was established in the course of evolution, in spite of the sporadic occurrence of polyploid individuals in the populations. The essential obstacle in the starting and establishement of such a line is dioecism and the system of sex determination associated with it. In tetraploid male plants with karyotype 24a + XXYYYY = 30, sex chromosomes mostly undergo segregation in the following way XYY$\longleftrightarrow$XYY. That is why, after crossing a female tetraploid plant (24a + XXXX = 28) with a tetraploid male plant (24a + XXYYYY = 30), as a rule only sterile intesexes are obtained with karyotype 24a + XXXYY = 29 which give no chance of establishing a polyploid line. It may, therefore, be assumed that dioecism based on the sex determination system of X/A type is the main obstacle in the arising and establishment of a polyploid line.

4. It was found that both Y chromosomes are heterochromatic. Analysis of the morphological variability of X and Y chromosomes in natural populations showed a greater variability of Y than of X chromosomes. The latter being euchromatic. The presence of short heterochromatic segments was also located in some autosomes. These partially heterochromatic autosomes were also found to be more variable than the remaining ones (Z a b o r o w s k a, 1969). There is, thus, a distinct relation between the presence of heterochromatic segments in the chromosome, and its morphological variability.

5. Autoradiography demonstrated that both the heterochromatic Y chromosomes replicate later than the euchromatic chromosomes X and autosomes. In interphase, mitotic nuclei chromosomes Y generally form two large heterochromatic bodies clearly visible also during DNA replication. It would seem that during DNA replication there occurs in heterochromatin despiralisation of small submicroscopic segments which, after the end of replication, immediately return to their initial state.

6. In male *R. thyrsiflorus* plants sex chromosomes XY$_1$Y$_2$ conjugate end-to-end forming a characteristic long trivalent Y$_1$XY$_2$. It may be assumed on the basis of the pattern of sex chromosome conjugation that Y chromosomes are composed of big differential heterochromatic segments, and very short euchromatic segments homologous to the X chromosomes. The latter segments are so short that they cannot be localised by autoradiographic methods.

7. In Y chromosome hyperploid plants of *R. thyrsiflorus*, associations between Y chromosomes during meiosis may be due to conjugation between homologous segments, or may be the result of heterochromatic fusion.

8. The phenotypic effect of hyperploidy was studied in respect to Y chromosomes. Individuals with additional chromosomes Y are lower, with

a poorer foliation, less vigorous and viable than normal plants. The fertility of these plants is reduced. These effects are the more pronounced the more numerous are the additional Y chromosomes. One additional Y chromosome modifies the phenotype but slightly, probably in view of the heterochromatic nature of these chromosomes.

9. Studies of the course of meiosis and of the pollen morphology in male plants lacking Y chromosome, with a single or with more additional Y chromosomes revealed that factors controlling fertility of male plants may be localised in chromosomes Y. Plants without Y chromosomes or possessing only a single one were completely sterile or produced only a low percentage of defective pollen, in spite of normal meiosis. This shows that the factors decisive for the fertility of male plants control the stages of postmeiotic pollen development.

10. It is believed that Y chromosomes influence the ratio of male and female plants in the population. In natural R. *thyrsiflorus* populations, female individuals are much more numerous than male ones. Normal diploid male individuals (12a + XYY) produce male and female sex-determining gametes with a chromosome composition 6a + YY and 6a + X, respectively. The low number of male individuals in the populations is connected with nonrandom fertilization, that is a lower chance of fertilization by gametes with a composition 6a + YY = 8. In crosses where the pollen-supplying male plant had a set 12a + XYYYY = 17, and both the male and female sex-determining gametes contained a Y chromosome, a sex ratio close to 1:1 was obtained. This finding shows that Y chromosomes influence the ratio of male and female individuals in the population.

11. Data indicating that Y chromosomes control such essential functions as pollen development prompted the analysis of the genetic activity of these chromosomes. The method of double staining of histones with fast green and eosin according to B l o c h (1966) was applied. It was found that in the mitotic interphase the heterochromatic bodies formed by chromosomes Y stain pink, whereas euchromatin stains green. This picture is proof of differences in the histone proteins between eu- and heterochromatin, and may be considered as indication of genetic inactivity of the heterochromatin. A different picture was observed in the last premeiotic interphase. At this stage Y chromosomes are in a diffuse state and do not stain pink with eosin as they did in somatic cells. It was also demonstrated by autoradiography that the diffuse stage of chromosomes Y coincides with intensive RNA synthesis by the pollen mother cells. It would seem that this premeiotic Y chromosome activity is necessary for normal pollen development.

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Struktura i funkcja chromosomów płci u Rumex thyrsiflorus

Streszczenie

1. Rośliny żeńskie R. thyrsiflorus mają dwa chromosomy X i dwanaście autosomów (2n = 14). Rośliny męskie mają jeden chromosom X, dwa chromosomy Y i dwanaście autosomów (2n = 15). Chromosomy płci są łatwo wyróżnialne. Chromosomy X są metacentryczne i największe z kompletu, chromosomy Y są metacentryczne lub submetacentryczne, nieco mniejsze od chromosomów X, lecz wyraźnie większe od autosomów.

2. Analizując szereg diploidalnych i poliploidalnych roślin z różną liczbą chromosomów X i Y wykazano przekonywująco, że chromosomy Y nie biorą udziału w determinacji płci. Płeć osobnika zależy od stosunku chromosomów X do autosomów (mechanizm determinacji płci typu X/A). Taki mechanizm determinacji płci można uważać za najbardziej zaawansowany ewolucyjnie ze zbadanych dotychczas u roślin wyższych.

3. W sekcji Acetosa mimo sporadycznego występowania w populacjach osobników poliploidalnych nie ustaliła się w toku ewolucji żadna dwupienna linia poliploidalna. Istotną przeszkodą w zapoczątkowaniu i ustaleniu się takiej linii jest dwupienność i związany z nią system determinacji płci. U tetraploidalnych roślin męskich o kariotypie 24a + XXYYYY = 30 chromosomy płci segregują najczęściej w układzie XYY←→XYY. Dlatego też po skrzyżowaniu tetraploidalnej rośliny żeńskiej o kariotypie 24a + XXXX = 28, z tetraploidalną rośliną męską o kariotypie

24a + XXYYYY = 30 z reguły otrzymuje się sterylne interseksy najczęściej o kario-
typach 24a + XXXYY = 29 nie dające szans utrwalenia się poliploidalności. Można
zatem założyć, że dwupienność oparta na systemie determinacji płci typu X/A jest
istotną przeszkodą w powstaniu i ustaleniu się linii poliploidalnej.

4. Stwierdzono, że oba chromosomy Y są heterochromatynowe. Analiza morfolo-
gicznej zmienności chromosomów X i Y w naturalnych populacjach wykazała więk-
szą zmienność morfologiczną chromosomów Y niż chromosomów X, które są euchro-
matynowe. Obecność krótkich odcinków heterochromatynowych stwierdzono rów-
nież w niektórych autosomach. Stwierdzono, że te częściowo heterochromatynowe
autosomy są również bardziej zmienne niż pozostałe autosomy (Z a b o r o w s k a,
1969). Tak więc istnieje wyraźna zależność między obecnością odcinków heterochro-
matynowych w chromosomie, a ich zmiennością morfologiczną.

5. Stosując metodę autoradiografii wykazano, że oba heterochromatynowe chro-
mosomy Y replikują się później niż euchromatynowe chromosomy X i autosomy.
W mitotycznych jądrach interfazowych chromosomy Y tworzą zwykle dwa duże
węzły heterochromatynowe, wyraźnie widoczne również podczas replikacji DNA.
Przypuszcza się, że przy replikacji DNA w heterochromatynie następuje despiraliza-
cja małych submikroskopowych odcinków, które po zakończeniu replikacji natych-
miast wracają do stanu wyjściowego.

6. U roślin męskich R. thyrsiflorus w profazie mejotycznej chromosomy płci
XY_1Y_2 koniugują końcami tworząc charakterystyczny, długi triwalent Y_1XY_2. Na
podstawie obrazów koniugacji chromosomów płci zakłada się, że chromosomy Y skła-
dają się z dużych różnicujących odcinków heterochromatynowych i bardzo krótkich
odcinków euchromatynowych homologicznych do chromosomu X. Odcinki te są tak
krótkie, że nie można ich zlokalizować metodą autoradiografii.

7. Chromosomy Y u R. thyrsiflorus mogą łączyć się w czasie mejozy również
na innej zasadzie niż koniugacja między homologicznymi odcinkami. Między chro-
mosomami Y mogą powstać połączenia również w wyniku zlepiania heterochroma-
tyny między niehomologicznymi odcinkami.

8. Zbadano fenotypowy efekt hyperploidalności w stosunku do chromosomów Y.
Rośliny z dodatkowymi chromosomami Y są niższe, słabiej ulistnione, mniej bujne
i mniej żywotne niż normalne. Płodność takich roślin jest obniżona. Obserwowane
efekty są tym wyraźniej zaznaczone im więcej dodatkowych chromosomów Y wystę-
puje w kariotypie. Jeden dodatkowy chromosom Y modyfikuje fenotyp stosunkowo
nieznacznie, co przypisuje się heterochromatycznej naturze tych chromosomów.

9. Na podstawie badania przebiegu mejozy oraz morfologii pyłku u roślin męs-
kich bez chromosomu Y, z jednym chromosomem Y oraz z dodatkowymi chromo-
somami Y wykazano, że w chromosomach Y mogą być zlokalizowane czynniki kon-
trolujące płodność roślin męskich. Rośliny ♂ bez chromosomów Y lub z jednym Y
mimo normalnej mejozy były całkowicie bezpłodne lub wytwarzały znaczny procent
nienormalnie wykształconego pyłku. Wskazuje to, że czynniki decydujące o płod-
ności roślin męskich kontrolują stadia postmejotycznego rozwoju pyłku.

10. Przypuszcza się że chromosomy Y mają wpływ na stosunek roślin męskich
i żeńskich w populacji. W naturalnych populacjach R. thyrsiflorus osobniki żeńskie
są znacznie liczniejsze niż męskie. Normalne diploidalne osobniki męskie (12a +
XYY) wytwarzają gamety determinujące płeć ♂ o składzie chromosomów 6a + YY
oraz gamety determinujące płeć ♀ o składzie 6a + X. Mała liczebność osobników
męskich w populacjach związana jest z nielosowym zapłodnieniem, to jest z mniej-
szą szansą zapłodnienia przez gamety o składzie 6a + YY = 8. W krzyżówkach, gdzie
dostarczająca pyłku roślina męska miała skład 12a + XYYYY = 17 i zarówno gamety
determinujące płeć ♀, jak i ♂ zawierały chromosom Y, otrzymano stosunek płci

zbliżony do 1:1. Obserwacja ta wskazuje na wpływ chromosomu Y na ilościowy stosunek osobników męskich i żeńskich w populacjach.

11. Dane wskazujące, że chromosomy Y kontrolują tak istotne funkcje, jak rozwój pyłku skłoniły do analizy aktywności tych chromosomów. Zastosowano metodę podwójnego barwienia histonów zielenią szybką i eozyną wg B l o c h a (1966). Stwierdzono, że w interfazie mitotycznej węzły heterochromatynowe utworzone przez chromosomy Y barwią się na różowo, natomiast euchromatyna daje zabarwienie zielone. Taki obraz świadczy o zróżnicowaniu białek histonowych między eu- i heterochromatyną i może być interpretowany jako wskaźnik braku aktywności genetycznej. W tym stadium chromosomy Y znajdują się w stanie dyfuzyjnym i nie barwią się na różowo eozyną, jak to stwierdzono w komórkach somatycznych. Ponadto stwierdzono autoradiograficznie, że dyfuzyjne stadium chromosomów Y zbiega się z syntezą RNA przez komórki macierzyste pyłku. Wydaje się prawdopodobne, że to stadium przedmejotycznej aktywności chromosomów Y decyduje o normalnym rozwoju pyłku.