

# Mechanism of Sex Determination in *Rumex hastatulus* Baldw.

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**Summary.** The results presented indicate that the sex determination mechanism in the Texas race of *R. hastatulus* [ $\varphi 2n = 10 (XX + 8A)$ ;  $\delta 2n = 10 (XY + 8A)$ ] is intermediate between the  $X/Y$  and  $X/A$  systems. In this race, sex is determined to some extent by the  $X/A$  balance, but the  $Y$  chromosome also affects sex expression, maleness or intersexuality being correlated with different ratios of  $X$  and  $Y$  chromosomes.

The results obtained for the Texas race are fully compatible with data presented by Smith (1963) for the North Carolina race [ $\varphi 2n = 8 (XX + 6A)$ ;  $\delta 2n = 9 (XY_1 Y_2 + 6A)$ ]. It may be concluded that evolution of the karyotype in this species is not accompanied by changes in the mechanism of sex determination.

## Introduction

*R. hastatulus* is a dioecious annual plant, endemic in the southeastern United States. Within the species there are three distinct chromosome races (Smith 1964, 1969a, Jackson and Smith 1969), connected with different geographic localities and, for convenience, designated *T* (Texas), *NC* (North Carolina) and *SIM* (Southern Illinois-Missouri). These races differ in chromosome morphology and in their somatic and sex chromosome constitution. On the basis of crosses between individuals of the different races and cytogenetic analysis of the hybrids, Smith (1969a) concluded that, apart from structural differences, the sex chromosomes of all races are homologous. His investigations also suggested that the *NC* race arose from the *T* race as the result of karyotype evolution involving reciprocal translocation between autosomes and the sex chromosomes of both races (Smith 1964, 1969a). The *SIM* race, on the other hand, probably arose by natural hybridization of representatives of the *NC* and *T* races (Jackson and Smith 1969).

The mechanism of sex determination in the *NC* race was studied by Smith (1957, 1963, 1969b), who found it to be intermediate between the  $X/A$  and  $X/Y$  systems. The *T* race was considered to be the most primitive one, but its mode of sex determination was unknown. In view of the hypothesis that the *T* race gave rise to the *NC* race, it would be interesting to find whether, despite the karyotype rearrangements mentioned, the sex determination mechanism of both races is the same. The subject of the present investigation was, therefore, analysis of the cytogenetical mechanism determining sex in the *T* race.

## Material and Methods

Seeds of the 10-chromosome Texas race collected from its natural populations in the United States were kindly provided by Professor A. Löve.

Studies of the sex-determining mechanism were based on polyploids induced by colchicine treatment. Appropriate crosses between polyploid and diploid plants were

made to obtain individuals with different ratios of  $X$  and  $Y$  chromosomes and different number of autosome sets.

Polyploids were produced by treating the shoot apices of young diploid seedlings (about 0.5–1 cm long) with 0.1 percent colchicine solution for 48 hours.

Karyotypes of di- and polyploid plants were determined on metaphasal plates from the root tips. After 4-hours pretreatment with a solution of 8-oxyquinoline (Tjio and Levan 1950) the root tips were fixed in acetic alcohol (1:3) for 2–24 hours. The material was stained with a mixture of 2 per cent acetoorcein and 1 *N* hydrochloric acid (9:1) for 10–15 min. and then squashed in 1 per cent acetoorcein.

The course of meiosis was studied in PMC's of the diploid as well as polyploid plants. Flower buds were fixed in Carnoy's fixative for 24 hours. For staining, the alcoholic – hydrochlorid acid carmine method was used (Snow 1963). Stained anthers were squashed in 45 per cent acetic acid.

## Results

### 1. Chromosome complement of *R. hastatulus*

The diploid number of chromosomes in both sexes is 10. Female plants (Fig. 1a) have the chromosomal constitution  $2n = 10 (XX + 8A)$ , male plants (Fig. 1b)  $2n = 10 (XY + 8A)$ . The sex chromosomes,  $X$  and  $Y$ , differ considerably from each other in morphology and can be easily distinguished. The

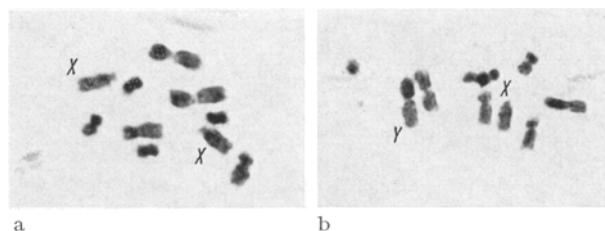


Fig. 1. Metaphasal plates from root tips of the female (a) and male (b) plants.  $\times 1000$

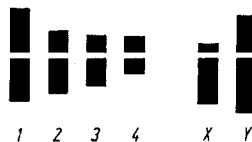


Fig. 2. Karyotype of the *T* race of *R. hastatulus*

X chromosome is short and acrocentric, whereas the Y chromosome is the longest of the complement and more or less submetacentric.

The autosome complement consists of four pairs (Fig. 2). Two pairs are long; one of them is nearly metacentric, the other submetacentric. The other two pairs are short; again one is metacentric and the other submetacentric.

### 2. Induction of polyploids

By means of colchicine treatment six polyploid plants were obtained; two of them were male and four female. The level of ploidy in male plants was examined in *PMC*'s. Cytological analysis revealed that the microsporocytes of these plants contained 20–21 chromosomes. Presumable female polyploids were selected on the basis of morphological traits. The crosses between polyploids were performed, and the karyotypes as well as the sex of their progeny were examined. Karyotype analysis proved that in the female parent plants the number of chromosomes was also approximately tetraploid. In the progeny, some plants were aneuploids with 18–22 chromosomes, but the majority were tetraploids with chromosome number  $4n = 20$ . Among the tetraploids the following karyotypes were found:

- (a)  $20 (4X + 16)$  — female plants
- (b)  $20 (2X + 2Y + 16)$  — male plants
- (c)  $20 (3X + Y + 16)$  — intersexual or male plants.

The karyotypes (a) and (b) represent strictly doubled chromosome complements of the female and male diploids respectively, and such plants were chosen for the next crosses. The karyotype (c) (Fig. 3) displayed a doubled autosome set, but untypical sex chromosome constitution.

The following crosses were then made:

- a)  $\text{♀ } 4n \times \text{♂ } 4n$

In the progeny of this cross mostly tetraploids were identified; a small proportion of plants were

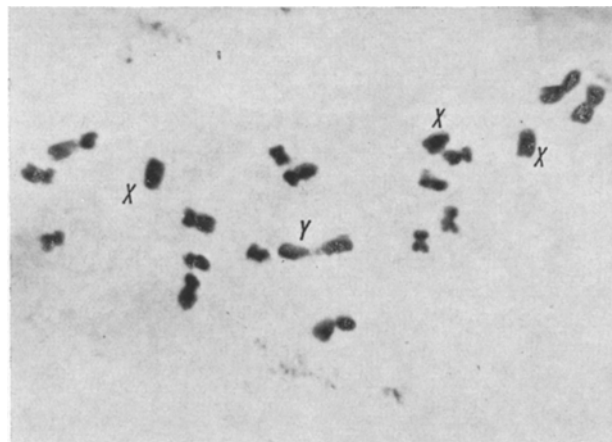


Fig. 3. Metaphasal plate of the intersexual plant with chromosome constitution  $4n = 20 (3X + Y + 16)$ .  $\times 1000$

aneuploids. Individuals with karyotype  $4n = 20 (3X + Y + 16)$  made up the main group of tetraploids. The predominance of  $XXX$ Y types seems to indicate that most gametes formed by tetraploid male plants are of the XY type. It is also possible that male gametes with the XY constitution fertilize more frequently than do the XX or YY gametes.

- b)  $\text{♀ } 4n \times \text{♂ } 2n$

The progeny of this cross again consisted mainly of tetraploids and some aneuploids with a chromosome number approximating to the tetraploid one. However, from this cross three triploids and one pentaploid plant were also obtained.

- c)  $\text{♀ } 2n \times \text{♂ } 4n$

Analysis of the karyotypes in the progeny of this cross revealed that all plants were diploid except for one individual with the constitution  $3n = 16 (2X + Y + 13)$ .

Thus, obtaining triploids in *R. hastatulus* proved to be difficult. The progeny of crosses between di- and tetraploids was expected to be predominantly triploid. Seed setting in such crosses was considerably reduced. Moreover, the progeny of the  $4n \times 2n$  cross was mainly tetraploid, whereas in the  $2n \times 4n$  cross diploid plants were obtained. The results of the latter cross can be explained by the fact that frequent irregularities were found in meiosis of male tetraploids. Consequently, some proportion of haploid ( $n = 5$ ) gametes may be formed and, presumably, these are far more efficient in fertilization than gametes with  $n = 10$ . In contrast, the occurrence of tetraploids in the progeny of the  $4n \times 2n$  cross seems to indicate that in diploid male plants unreduced gametes may be formed. However, neither meiotic disturbances nor the presence of unreduced gametes were detected in *PMC*'s of diploid male plants. Nevertheless, it is possible that such gametes are produced very infrequently, and they may be more efficient in fertilization than haploid ones.

Of the three triploid plants obtained from the  $4n \times 2n$  cross, two were intersexual and one was female. These individuals failed to yield progeny, as the female plant developed its inflorescence at a different time from the intersexes. This plant also did not set seeds after pollination with pollen from diploid or tetraploid male plants. The triploid intersexes, on the other hand, were probably female sterile because no seeds were set after self-pollination.

### 3. Analysis of sex expression in the polyploids

In contrast to diploids, many intersexes were found among the polyploid plants. Analysis of sex expression showed the presence of three types of intersex:

- a) intersexes with a predominance of male characters\* ( $\text{♀ } M$ ). Such individuals usually had male in-

\* for convenience designated as "male" or "female" intersexes respectively

florescences in which some hermaphrodite flowers were present. Intersexes of this kind appeared most often, but they formed seeds only occasionally.

b) intersexes with a predominance of female characters (♀ *F*). They had typical female inflorescences but with a small number of hermaphrodite flowers. In these intersexes seed setting was much lower than in normal female plants.

c) intersexes with inflorescences composed of female and male, as well as hermaphrodite, flowers. These individuals appeared sporadically. The numbers of each kind of flower in their inflorescences were counted and on this criterion the plants were classified as male or female intersexes.

In some of the intersexual plants, hermaphrodite flowers occurred only on offshoots.

#### 4. Meiosis in the diploid and tetraploid plants

No disturbances were found in the course of meiosis in *PMC*'s of the diploid plants. Conjugation of the *X* and *Y* chromosomes was, in all cases, end to end. The sex chromosomes form a distinctly asymmetric bivalent (Fig. 4) which can be easily distinguished. In the metaphase of the first meiotic division, in 66 cells out of 100 studied the chromosomes of the *XY* bivalent segregated earlier than the pairs of autosomes (Fig. 5). Tetrad formation was regular and mean pollen fertility was 98 per cent.

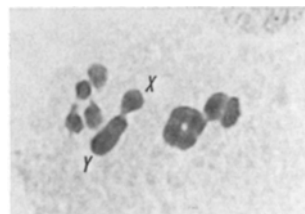


Fig. 4. Diakinesis in the diploid male plant. Note asymmetric sex bivalent.  $\times 800$

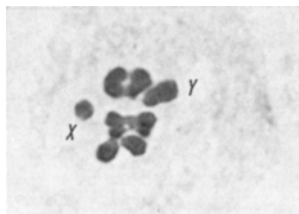


Fig. 5. *PMC* of diploid plant in metaphase I. Note precocious separation of the *XY* bivalent.  $\times 800$

Cytological examination of microsporogenesis in tetraploids revealed many irregularities at all stages of meiotic division. In diakinesis mainly bivalents were observed, tetravalents occurring only occasionally. At this stage, many asynaptic cells were found with sex chromosomes as well as autosomes unpaired. In metaphase I, uni- and tetravalents were observed. During anaphase I the separation of some bivalents was delayed. At this stage chromatic bridges were also found. Some of the bridges remained till the second meiotic division and were observed in metaphase and telophase II. In metaphase II, individual chromosomes occurred outside the spindle. Lagging chromosomes and chromosomes outside the spindle were also found during anaphase II. In the second meiotic division, some of the chromosomes formed separate spindles giving an increased number of division figures in the cell. Microsporocytes in telophase II consisted of 3–8 nuclei, usually differing in size. The mean pollen fertility was 66 per cent.

The frequency of irregularities was relatively high and included about 30 per cent of cells in each stage of the meiotic division. Such disturbances could be responsible for the formation of gametes with abnormal chromosome numbers, and these in turn would lead to the occurrence of aneuploids in the progeny of tetraploid plants.

Special attention was given to the pattern of sex chromosome conjugation in tetraploids. It appeared that, in plants with karyotype  $4n = 20 (2X + 2Y + 16)$ , the *X* chromosomes in most cases were paired with *Y* chromosomes, giving two asymmetric bivalents in each cell. In several cells per 100 examined, such bivalents were not found. In these cells the sex chromosomes were probably paired "homologously" (that means *XX* and *YY*), but sex bivalents could not be identified with certainty. In one case, a trivalent *XXY* and a *Y* chromosome as the univalent were found. On the other hand, in plants with karyotype  $4n = 20 (3X + Y + 16)$ , only one asymmetric bivalent (*XY*) was observed, the two paired *X* chromosomes again being indistinguishable. Tetravalents *XXXX* were found in several cases.

5. Analysis of the sex determination mechanism

To identify the sex-deciding mechanism, the sex expression of polyploids differing in the number of *X* and *Y* chromosomes, as well as in the number of autosome sets, was analyzed.

Altogether, the sex and the karyotypes of 238 polyploid plants were examined, and 30 different karyotypes were found. The most frequent (143 plants) were tetraploids. They exhibited most frequently the karyotype  $4n = 20 (3X + Y + 16)$ , but such individuals were usually intersexual. Tetraploids with the constitution  $4n = 20 (4X + 16)$  were female, whereas plants with the chromosome constitution  $4n = 20 (2X + 2Y + 16)$  were male. As well as tetraploids, three triploid and one pentaploid plant were obtained. The remaining group (91 individuals) consisted of aneuploids with karyotypes differing in the sex chromosome constitution and in the number of somatic chromosomes. Table 1 presents the karyotypes and the sex expression of the polyploids.

From the data listed, it appears that sex in *R. hastatulus* is controlled to some extent by the *X/A* balance, but the *Y* chromosome also takes a definite part in sex determination. From the compilation presented below\*, plants with *X/A* ratio equal to 1 are female:

$2n = 10 (2X + 8)$	$X/A = 1$
$3n = 15 (3X + 12)$	$X/A = 1$
$4n = 20 (4X + 16)$	$X/A = 1$
$5n = 25 (5X + 20)$	$X/A = 1$

\* besides polyploids, diploids were also compared

On the other hand, when the  $X/A$  ratio equals 0.5 the plants are male:

$$2n = 10 (X + Y + 8)$$

$$X/A = 0.5$$

$$4n = 20 (2X + 2Y + 16)$$

$$X/A = 0.5.$$

When the  $X/A$  ratio lies between 0.5 and 1, the plants are intersexual:

$$3n = 15 (2X + Y + 12)$$

$$X/A = 0.66$$

$$4n = 20 (3X + Y + 16)$$

$$X/A = 0.75.$$

Comparison of the intersexes with  $X/A$  ratio equal to 0.66 and 0.75 leads to some conclusions concerning the role of the  $Y$  chromosome in sex determination. Triploids with the karyotype  $3n = 15 (2X + Y + 12)$  were intersexes with a predominance of male characters. Tetraploids with the karyotype  $4n = 20 (3X + Y + 16)$  were also, in most cases (55 individuals), male intersexes; moreover 14 individuals with such a karyotype were even purely male. In this group, only 8 plants were intersexes with a predominance of female characters. These data may be interpreted as an indication that, apart from the  $X/A$  balance, the  $Y$  chromosome also has a marked influence on the expression of male characters.

Analysis of most of the aneuploids also supports these conclusions concerning the influence of the  $X/A$  balance and the  $Y$  chromosome in sex determination. In this case, however, the situation is much more complicated because the differences in sex chromosome constitution are superimposed on the differences in the number of autosomes. Consequently, among aneuploids, intersexes also occur in cases where the  $X/A$  ratio is 0.5 or 1. However, these examples can be explained as follows:

a) the plant with karyotype  $4n = 19 (2X + Y + 16)$  and  $X/A$  ratio equal to 0.5 was a female intersex. The increased expression of female characters can be explained by the lack of one  $Y$  chromosome compared with the constitution of normal tetraploid male plants;

b) plants with karyotype  $4n = 21 (4X + Y + 16)$  were female intersexes in spite of the  $X/A$  ratio being equal to 1. The shift of sex expression towards

Table 1. Relation between chromosome constitution and sex expression in polyploids of *R. hastatulus*

No.	Karyotype	Number of plants studied	Total	$X/A$	Number of plants with different sex			
					$\varnothing$	$F$	$M$	$\delta$
1.	15 (3X + 12)	1	3	1.00	1	—	—	—
2.	15 (2X + Y + 12)	2		0.66	—	—	2	—
3.	20 (4X + 16)	42	143	1.00	42	—	—	—
4.	20 (2X + 2Y + 16)	24		0.50	—	—	1	23
5.	20 (3X + Y + 16)	77		0.75	—	8	55	14
6.	25 (5X + 20)	1	1	1.00	1	—	—	—
7.	16 (2X + Y + 13)	1	91	0.61	—	—	1	—
8.	18 (2X + 2Y + 14)	2		0.57	—	—	—	2
9.	18 (3X + Y + 14)	4		0.85	—	2	2	—
10.	19 (2X + Y + 16)	1		0.50	—	1	—	—
11.	19 (2X + 2Y + 15)	3		0.53	—	—	—	3
12.	19 (3X + Y + 15)	5		0.80	—	1	2	2
13.	19 (4X + 15)	4		1.06	4	—	—	—
14.	20 (2X + Y + 17)	6		0.47	—	—	1	5
15.	20 (3X + 2Y + 15)	8		0.80	—	—	3	5
16.	20 (4X + Y + 15)	6		1.06	—	5	1	—
17.	20 (5X + 15)	1		1.33	1	—	—	—
18.	21 (2X + 2Y + 17)	1		0.47	—	—	—	1
19.	21 (3X + Y + 17)	14		0.70	—	1	11	2
20.	21 (3X + 2Y + 16)	10		0.75	—	—	2	8
21.	21 (4X + Y + 16)	2		1.00	—	2	—	—
22.	21 (4X + 2Y + 15)	2		1.06	—	—	2	—
23.	21 (4X + 17)	6		0.94	6	—	—	—
24.	21 (5X + 16)	1		1.25	1	—	—	—
25.	22 (2X + 2Y + 18)	3		0.44	—	—	—	3
26.	22 (3X + Y + 18)	1		0.66	—	—	1	—
27.	22 (3X + 2Y + 17)	3		0.70	—	—	1	2
28.	22 (4X + Y + 17)	1		0.94	—	1	—	—
29.	22 (4X + 2Y + 16)	3		1.00	—	—	3	—
30.	22 (4X + 18)	3		0.89	3	—	—	—
Total			238		59	21	88	70

male characters can here be explained by the presence of the additional  $Y$  chromosome in the karyotype which is characteristic for tetraploid female plants. The influence of the  $Y$  chromosome is pronounced, because plants with the same karyotype but devoid of the  $Y$  chromosome,  $4n = 20 (4X + 16)$ , are always purely female;

c) plants with karyotype  $4n = 22 (4X + 2Y + 16)$  which have an  $X/A$  ratio equal to 1 were male intersexes. The change of sex expression in favour of male characters is caused probably by the influence of two supernumerary  $Y$  chromosomes as compared with the constitution of normal tetraploid female plants. Comparison of such aneuploids with those of constitution  $4n = 21 (4X + Y + 16)$  suggests that the coexistence of one  $Y$  chromosome with four  $X$  chromosomes results in a more pronounced expression of female characters, whereas two  $Y$  chromosomes with the same number of  $X$  chromosomes strengthens the expression of male characters.

Thus, all the examples discussed above confirm the conclusion that sex in *R. hastatulus* is controlled not

only by the  $X/A$  balance, but also by the male-determining influence of the  $Y$  chromosome.

Another group of examples consists of the aneuploids which were female or male plants irrespective of the  $X/A$  ratio within the limits 0.5–1, for instance:

a) female plants with karyotypes

$$4n = 21 (4X + 17) \quad X/A = 0.94$$

$$4n = 22 (4X + 18) \quad X/A = 0.89$$

b) male plants with karyotypes

$$4n = 18 (2X + 2Y + 14) \quad X/A = 0.57$$

$$4n = 19 (2X + 2Y + 15) \quad X/A = 0.53.$$

These cases seem to be incompatible with the finding that sex in *R. hastatulus* is dependent on the  $X/A$  balance. However, it should be pointed out that in these individuals the deviations from the  $X/A$  ratio equal to 1 (in female plants) or 0.5 (in male plants) are not significant. Besides, in these individuals, the number of autosomes was changed when compared with karyotypes of normal tetraploid plants. Thus the examples mentioned here can be explained also by the lack or the presence of additional autosomes.

Moreover, as shown in Table 1, sometimes different plants, in spite of having the same karyotype, have a different sex expression. Such individuals are usually aneuploids, and differences in their sex expression can also be explained by an autosomal effect or by a change of balance between genes acting in sex chromosomes and in particular types of autosome.

Nevertheless, analysis of aneuploid plants also demonstrates a reciprocal cooperation of the  $X/A$  and  $X/Y$  systems in sex determination. If the karyotypes and sex of aneuploids with the same number of autosomes but different constitution of  $X$  and  $Y$  chromosomes (Table 2) are compared, some regularities can be detected. Within particular groups of the compared karyotypes the number of autosomes remains the same, but as the result of changes in the ratio of  $X$  to  $Y$  chromosomes the  $X/A$  balance is shifted. As may be concluded from Table 2, the decrease in the number of  $Y$  in favour of  $X$  chromosomes within each group results in a shift of the  $X/A$  ratio towards 1 and induces the stronger expression of female characters (and vice versa). This is another proof that sex in *R. hastatulus* is determined not only by the number of  $Y$  chromosomes, but also by changes in the  $X/A$  balance.

To analyze the influence of the  $Y$  chromosome on sex more precisely, polyploid plants with the same number of autosomes and  $X$  chromosomes, but with a different number of  $Y$  chromosomes, were compared (Table 3).

It follows from the data of Table 3 that, within particular groups of karyotypes, plants with the same  $X/A$  ratio have a different sex expression depending on the number of  $Y$  chromosomes. It can also be

Table 2. Interaction between  $X/A$  and  $X/Y$  systems in sex determination of aneuploids

No.	Karyotype	$X/A$ ratio	Sex expression
1.	18 (3 $X$ + $Y$ + 14)	0.85	♀ $F, M$
2.	18 (2 $X$ + 2 $Y$ + 14)	0.57	♂
3.	19 (4 $X$ + 15)	1.06	♀
4.	19 (3 $X$ + $Y$ + 15)	0.80	♀ ( $F$ ) $M \rightarrow \delta$
5.	19 (2 $X$ + 2 $Y$ + 15)	0.53	♂
6.	20 (5 $X$ + 15)	1.33	♀
7.	20 (4 $X$ + $Y$ + 15)	1.06	♀ $F (M)$
8.	20 (3 $X$ + 2 $Y$ + 15)	0.80	♀ $M \rightarrow \delta$
9.	21 (4 $X$ + 17)	0.94	♀
10.	21 (3 $X$ + $Y$ + 17)	0.70	♀ $M \rightarrow \delta$
11.	21 (2 $X$ + 2 $Y$ + 17)	0.47	♂
12.	21 (5 $X$ + 16)	1.25	♀
13.	21 (4 $X$ + $Y$ + 16)	1.00	♀ $F$
14.	21 (3 $X$ + 2 $Y$ + 16)	0.75	♀ $M \rightarrow \delta$
15.	22 (4 $X$ + 18)	0.89	♀
16.	22 (3 $X$ + $Y$ + 18)	0.66	♂ $M$
17.	22 (2 $X$ + 2 $Y$ + 18)	0.44	♂
18.	22 (4 $X$ + $Y$ + 17)	0.94	♀ $F$
19.	22 (3 $X$ + 2 $Y$ + 17)	0.70	♀ $M \rightarrow \delta$

Table 3. Influence of  $Y$  chromosomes on sex expression

No.	Karyotype	Num- ber of plants studied	Plants with different sex, %			
			♀	♂	$F$	$M$
1.	19(2 $X$ + $Y$ +16)	1	—	100.0	—	—
2.	20(2 $X$ +2 $Y$ +16)	24	—	—	4.1	95.9
3.	20(2 $X$ + $Y$ +17)	6	—	—	16.4	83.6
4.	21(2 $X$ +2 $Y$ +17)	1	—	—	—	100.0
5.	19(3 $X$ + $Y$ +15)	5	—	20.0	40.0	40.0
6.	20(3 $X$ +2 $Y$ +15)	8	—	—	37.5	62.5
7.	20(3 $X$ + $Y$ +16)	77	—	10.5	71.4	18.1
8.	21(3 $X$ +2 $Y$ +16)	10	—	—	20.0	80.0
9.	21(3 $X$ + $Y$ +17)	14	—	7.3	78.5	14.2
10.	22(3 $X$ +2 $Y$ +17)	3	—	—	33.3	66.6
11.	19(4 $X$ +15)	4	100.0	—	—	—
12.	20(4 $X$ + $Y$ +15)	6	—	83.6	16.4	—
13.	21(4 $X$ +2 $Y$ +15)	2	—	—	100.0	—
14.	20(4 $X$ +16)	42	100.0	—	—	—
15.	21(4 $X$ + $Y$ +16)	2	—	100.0	—	—
16.	22(4 $X$ +2 $Y$ +16)	3	—	—	100.0	—
17.	21(4 $X$ +17)	6	100.0	—	—	—
18.	22(4 $X$ + $Y$ +17)	1	—	100.0	—	—

observed that, in plants with two  $X$  chromosomes (rows 1–4 in Table 3), the influence of  $Y$  chromosomes is most pronounced, and the percentage of male individuals in this group is high. On the other hand, in the group of plants with three  $X$  chromosomes (rows 5–10 in Table 3), the male-determining influence of  $Y$  chromosomes is slightly weaker and the number of male individuals is proportionately lower. However, when the  $X/Y$  ratio within this group is shifted from  $XXX/Y$  to  $XXX/YY$ , the percentage

of intersexes decreases in favour of male plants. In contrast, in plants with four  $X$  chromosomes (rows 11–18 in Table 3), the  $Y$  chromosomes are not so effective in suppressing the development of female sex organs, and, therefore, no male plants occurred in this group. Nevertheless, it is clearly visible that with the change in the number of  $Y$  chromosomes from one to two, the sex expression is shifted towards female or male intersexes, respectively.

In view of the data indicating that in *R. hastatulus* sex is controlled by the  $X/A$  balance as well as by the influence of the  $Y$  chromosome, it can be assumed that the sex determination mechanism in this karyologic race is intermediate between the  $X/A$  and  $X/Y$  systems.

### Discussion

*Rumex* is of particular interest in the cytogenetics of sex determination because several different systems have developed within this genus. In the *Acetosa* group the  $X/A$  mechanism is in operation (Ono 1935, Yamamoto 1938, Löve 1957, Žuk 1963), whereas within the *Acetosella* group the  $X/Y$  mechanism was found (Löve 1944, Löve and Sarkar 1956, Löve 1957). Moreover, within a single taxonomic unit — *R. hastatulus* — both simple ( $XX/XY$ ) and multiple ( $XX/XY_1Y_2$ ) sex chromosome complexes have been described (Smith and Smith 1947, Smith 1955, 1964) for distinct karyologic races. According to Smith (1964, 1969a), the differentiation of sex chromosome complexes in *R. hastatulus* resulted from karyotype evolution within the  $T$  and  $NC$  races. By means of structural rearrangements the autosomal genetic material of the  $T$  race has been included into the sex chromosomes of the  $NC$  race.

Cytological examination of the 10-chromosome race of *R. hastatulus*, reported in the present paper, revealed that its karyotype closely resembles that of the  $T$  race described by Smith (1964), with one exception concerning the presence of a satellite in chromosome III.

According to Smith's observations, in meiosis of diploid plants of the  $T$  race sex chromosomes were associated with terminal or interstitial chiasmata. He concluded that pairing segments are not restricted only to the distal regions of the  $X$  and  $Y$  chromosomes, but the data presented here do not confirm this

observation. In the present material, sex chromosomes were always connected with terminal chiasmata and the separation of the  $XY$  bivalent was in most cases precocious. This observation strongly suggests that the homologous segments of sex chromosomes are short and terminal.

Analysis of sex chromosome conjugation in tetraploids obtained from the  $T$  race revealed that  $XY$  bivalents were much more frequent than  $XX$  and  $YY$  bivalents. This finding coincides with Smith's observation (1963) concerning the  $NC$  race in which the trivalents  $Y_1XY_2$  were formed more frequently than the bivalents  $XX$ ,  $Y_1Y_1$  and  $Y_2Y_2$ . It suggests that in tetraploids the conjugation of sex chromosomes is not random.

In colchicine-induced tetraploids of the  $NC$  race, Smith (1963) did not find many disturbances in the course of meiosis. The pollen fertility was high and aneuploids appeared only sporadically in the tetraploid progeny. In contrast, during meiosis in tetraploids of the  $T$  race, many disturbances were encountered which resulted in a high frequency of aneuploids. Meiotic disturbances, however, are common in colchicine-induced polyploids of other plants (Eigsti and Dustin 1955).

Table 4. Karyotypes and sex expression of *R. hastatulus* compared with sex expected on the basis of the  $X/A$  or  $X/Y$  systems

No.	Karyotype	$X/A$ ratio	Sex		
			observed	expected on the basis of $X/A$ system	expected on the basis of $X/Y$ system
1.	15 (3 $X$ + 12)	1.00	♀	♀	♀
2.	15 (2 $X$ + $Y$ + 12)	0.66	♀	♀	♂
3.	16 (2 $X$ + $Y$ + 13)	0.61	♀	♀	♂
4.	18 (2 $X$ + 2 $Y$ + 14)	0.57	♂	♀	♂
5.	18 (3 $X$ + $Y$ + 14)	0.85	♀	♀	♂
6.	19 (2 $X$ + $Y$ + 16)	0.50	♀	♂	♂
7.	19 (2 $X$ + 2 $Y$ + 15)	0.53	♂	♀	♂
8.	19 (3 $X$ + $Y$ + 15)	0.80	♀ — ♂	♀	♂
9.	19 (4 $X$ + 15)	1.06	♀	♀	♀
10.	20 (2 $X$ + $Y$ + 17)	0.47	♀ — ♂	♂	♂
11.	20 (2 $X$ + 2 $Y$ + 16)	0.50	♂	♂	♂
12.	20 (3 $X$ + $Y$ + 16)	0.75	♀ — ♂	♀	♂
13.	20 (3 $X$ + 2 $Y$ + 15)	0.80	♀ — ♂	♀	♂
14.	20 (4 $X$ + $Y$ + 15)	1.06	♀	♀	♂
15.	20 (4 $X$ + 16)	1.00	♀	♀	♀
16.	20 (5 $X$ + 15)	1.33	♀	♀	♀
17.	21 (2 $X$ + 2 $Y$ + 17)	0.47	♂	♂	♂
18.	21 (3 $X$ + $Y$ + 17)	0.70	♀ — ♂	♀	♂
19.	21 (3 $X$ + 2 $Y$ + 16)	0.75	♀ — ♂	♀	♂
20.	21 (4 $X$ + $Y$ + 16)	1.00	♀	♀	♂
21.	21 (4 $X$ + 2 $Y$ + 15)	1.06	♀	♀	♂
22.	21 (4 $X$ + 17)	0.94	♀	♀	♀
23.	21 (5 $X$ + 16)	1.25	♀	♀	♀
24.	22 (2 $X$ + 2 $Y$ + 18)	0.44	♂	♂	♂
25.	22 (3 $X$ + $Y$ + 18)	0.66	♀	♀	♂
26.	22 (3 $X$ + 2 $Y$ + 17)	0.70	♀ — ♂	♀	♂
27.	22 (4 $X$ + $Y$ + 17)	0.94	♀	♀	♂
28.	22 (4 $X$ + 2 $Y$ + 16)	1.00	♀	♀	♂
29.	22 (4 $X$ + 18)	0.89	♀	♀	♀
30.	25 (5 $X$ + 20)	1.00	♀	♀	♀

It follows, from the studies of Smith (1963), that the mechanism of sex determination within the NC race is based upon an  $X/A$  balance modified by male factors localized in the Y chromosome. Data obtained in the present study seem to indicate that such an intermediate mechanism of sex determination also operates in the T race. In Table 4, different karyotypes are presented, together with sex observed and sex expected on the basis of the  $X/Y$  or  $X/A$  mechanism.

It is evident from Table 4 that the sex-deciding mechanism in the T race is different from the  $X/A$  and  $X/Y$  systems. Among the plants examined four groups may be distinguished:

1. individuals, the sex of which is compatible both with the  $X/Y$  and  $X/A$  systems (rows 1, 8–13, 15–19, 23–24, 26 and 30 in Table 4)

2. individuals, the sex of which is compatible only with the  $X/A$  system (rows 2, 3, 5, 25 and 27 in Table 4)

3. individuals, the sex of which is compatible only with the  $X/Y$  system (rows 4, 7, 22 and 29 in Table 4)

4. individuals, the sex of which is compatible with neither of these two systems (rows 6, 14, 20, 21 and 28 in Table 4).

Thus it must be assumed that an intermediate sex-determining mechanism is in operation within the *R. hastatulus* race examined. This system is based upon reciprocal interaction between autosomes and sex chromosomes. The most evident proof supporting such a conclusion was obtained from analysis of sex determination in a large population of tetraploids. However, attention should be given to the fact that, among aneuploids, some plants were detected which did not follow the general rule. Namely, the sex expression of certain plants differed from that expected on the basis of the  $X/A$  ratio. For instance, some aneuploids, in spite of having an  $X/A$  ratio equal to 0.5 or 1, were intersexual and some aneuploids characterized by an  $X/A$  ratio between 0.5 and 1 were male or female. The studies of Smith (1963) concerning the NC race did not reveal such deviations. However, among polyploid plants of the NC race only a few aneuploids were obtained and it is possible that examination of more individuals would reveal irregularities of the same type. Moreover, the exceptions mentioned above for the T race do not seem to be fully incompatible with the action of the  $X/A$  system, because deviations from the expected sex expression in all cases could be explained by the lack or addition of Y chromosomes or autosomes when compared with the typical constitution of tetraploid plants. Nevertheless, in aneuploids too,

a strong correlation between the sex expression and changes in the  $X/A$  balance could be shown (Table 2).

On the basis of Smith's studies on the NC race, together with the data presented here concerning the T race, it can be assumed that the sex determination mechanism in both races of *R. hastatulus* is the same. It seems to indicate that differences in the constitution and morphology of sex chromosomes in this particular case are not accompanied by changes in the sex determination mechanism. The evolution of the karyotype and structural rearrangements of sex chromosome complexes can thus proceed independently of the evolution of the mechanism determining sex.

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