THE MECHANISM OF SEX DETERMINATION
IN RUMEX HASTATULUS[1]

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TWO cytogenetic mechanisms of sex determination, the X/Y system of Mel-
andrium and Bombyx, the X/A balance of Drosophila, occur both in the higher
animals and among the dioecious seed plants. WESTERGAARD (1948, 1958) does
not regard these as different systems but as two stages in the evolution of stable,
multigenic mechanisms which ensure dioecism. GOLDSCHMIDT (1955) rejects
WESTERGAARD's multigenic hypothesis and interprets both systems as manifesta-
tions of a fundamental F/M balance in development. Both the X/Y and the X/A
modes of sex determination occur among the dioecious species of the genus Rumex
which thus provides opportunities for investigating the cytogenetic relationships
of these differing mechanisms of sex determination within a single genus.

An X/Y mechanism controls sex expression in the dioecious Rumex species
which comprise the subgenus Acetosella (LÖVE 1944). These species exist in a
natural polyploid series: $2n = 14, 28, 42,$ and 56, but the male of each species is
heterogametic for a single Y chromosome, combined respectively with 1, 3, 5, and
7 X chromosomes. The expression of maleness results from the strong epistatic
effect of the Y chromosome. Females are homogametic with 2, 4, 6, or 8 X chromo-
somes corresponding to the level of polyploidy in the respective species.

An X/A balance controls the expression of sexuality in plants of dioecious
Rumex acetosa L. (ONO 1935; YAMAMOTO 1938). This species belongs to the
subgenus Acetosa and has 12 autosomes plus XX in females, plus XY_1Y_2 in males
$(2n = 14, 15)$. Several closely related species have the same chromosome numbers
and similar sex-chromosome complements. Besides the *R. acetosa* complex, the
subgenus Acetosa also contains a number of more distantly related dioecious
species. Two of these, *R. paucifolius* Nutt. and *R. hastatulus* Baldw., are allopatric
North American endemics which differ markedly from *R. acetosa* in both chromo-
some number and morphology.

Rumex paucifolius is a subalpine perennial of the Rocky Mountains and of the
Sierra Nevada of California. It exists in at least two chromosome races $(2n = 14,
28)$, both dioecious. The chromosomes are small and mostly metacentric, entirely
different from those of *R. acetosa*. The males are heterogametic, and as LÖVE

and SARKAR (1956) have suggested the presence of polyploidy may indicate an X/Y system.

Rumex hastatulus is a dioecious annual native to the southeastern United States. SMITH (1955) described the chromosomes and discussed the possible phylogenetic relationships of *R. hastatulus*. Females were homogametic with eight chromosomes $(2n = 6 + XX)$; males were heterogametic with nine chromosomes $(2n = 6 + XY_1Y_2)$. The sex chromosomes resembled those of *R. acetosa* both in morphology and in meiotic behavior, but the autosomes differed from those of *R. acetosa* in number, size, and centromere position. These earlier observations and all experimental results described in the present paper apply to *Rumex hastatulus* as it occurs in North Carolina. A karyologically distinct race of *Rumex hastatulus* with four pairs of autosomes and XX versus XY sex chromosomes $(2n = 10$ in both sexes) occurs in Texas (SMITH 1963), but is not included in this study.

Alteration of chromosomal and genic balance relations provides a direct approach to the cytogenetic analysis of sex inheritance. Chromosome balance studies have been crucial in the development of our present understanding of sex determination in Drosophila, *Rumex acetosa*, Melandrium, Bombyx, and most recently, man. The method involves changing the numbers of whole autosome sets in relation to the number of sex chromosomes present in an individual, or it may involve modifying the balance effects of the genes in a particular chromosome by obtaining individuals which are hyposomic (e.g. monosomic, nullisomic) or hypersomic (e.g. trisomic, tetrasomic). The genic dosage effects of the various chromosome combinations, by affecting sex expression in different ways, reveal the nature and potency of the sex determiners carried in different elements of the chromosome complement.

The present paper describes the results of an experimental genic balance analysis of the mechanism of sex determination in the North Carolina population of *Rumex hastatulus*.

MATERIALS AND METHODS

Rumex hastatulus is an abundant winter annual throughout the southeastern Coastal Plain and adjacent Piedmont. The seeds germinate in the fall and the plants overwinter as leafy rosettes. They produced one to several flowering stalks in early spring, flower in April (in North Carolina), develop mature fruit in May, and die before the end of June. The plants occasionally become facultative biennials. Seedlings which start too late to flower during the first year (e.g. cultures planted in February or March) may be able to survive the summer in the rosette stage and flower in the following spring. Occasional rosette plants have been observed in nature during late spring or summer. Very rarely, an interesting experimental plant has been saved for a second season by severe pruning late in the flowering season followed by careful watering and sanitation throughout the summer.

Experimental cultures have been derived from the open-pollinated seed of

individual plants and from bulk seed lots collected from natural stands of *Rumex hastatulus* in various North Carolina localities. Inbred strains and a series of genetic marker stocks have been developed by a program of selection and sib-mating. The distorting effect of differential seedling survival is minimized by planting all seeds individually in small (two-inch) pots of sterilized soil. Root tips for determination of somatic chromosome number and morphology are collected from the pot ball, pretreated by the 8-hydroxyquinoline method of TJIO and LEVAN (1950) and stained in aceto-orcein. Plants desired for meiotic study and for controlled pollination are selected, repotted, and grown to maturity in the greenhouse. Buds for meiosis are collected in a modified Carnoy's solution (3 parts 100 percent alcohol:1part propionic acid:1part chloroform), passed through the alcohol series to 70 percent alcohol, and stained in propiono-carmine. The remaining seedlings are transplanted to the field in early spring and observed for sex expression and other characters.

Polyploid individuals were needed in order to initiate chromosome balance studies in *Rumex hastatulus*. Random-bred, field-collected seeds were germinated in sterilized soil. Treatments began as soon as the cotyledons were fully expanded. A small drop of a 1.0 percent aqueous solution of colchicine was placed on each epicotyl, each day for six, seven, eight, nine, or ten successive days. (This method was suggested by DR. ARNE HAGBERG of the Swedish Seed Association, Svalöf.) Two months later the surviving plants (195 of the 400 seedlings receiving the 6 to 7-day treatments, 83 of the 600 seedlings in the 8 to 10-day treatments) were transplanted to four-inch pots where they grew to maturity. Untreated samples were grown for comparison.

Colchicine retarded development in all the treated seedlings and produced characteristic temporary modifications in the vegetative structures: thickened, irregularly shaped leaf-blades, deep green color, mosaic growth patterns. Most of the surviving treated plants recovered from the primary effects of treatment by the time of flowering and grew vigorously. The chromosome numbers of staminate plants were determined by examining pollen mother cells, and were correlated with gross morphology and with sizes of guard cells and pollen grains. A series of pistillate plants selected because they showed similar changes in morphology was crossed with males known to be tetraploid. Tetraploid by diploid crosses were made in order to obtain triploid individuals.

The progenies of intercrosses among the colchicine treated plants constituted the C_1 generation. Somatic chromosome numbers of individual C_1 plants were determined, the sex expression of each plant was observed, and meiosis was studied in the pollen mother cells of the staminate plants and the intersexes. The tetraploids closely resembled the diploids. Although increased organ size and robustness occurred in certain 4n plants, the tetraploids did not exceed the range of the variable diploid population.

The following procedures have been adopted for determining the sex expression of individual plants: 1. The plants are observed to be pistillate or staminate by macroscopic examination. 2. The pistillate plants are examined with a reading glass to determine whether stamens are also present. If stamens are found in any

flowers, sample branches of the inflorescence are collected for more detailed examination. Sample branches are also collected from the inflorescences of each staminate plant, preserved in 70 percent alchohol, and examined in the laboratory. 3. Five flowers chosen at random are dissected under a 10× stereoscopic microscope. If any of the first sample is intersexual, a second sample of five flowers from the same plant is chosen and examined.

Results from independent samplings of the same series of plants are consistent. The numbers of strictly staminate plants are slightly overestimated because staminate plants which produce sporadic flowers containing tiny rudimentary pistils may be overlooked. The error is of little consequence because all samples are affected in the same way.

The term "intersex" is used in this paper to designate any plant which produces flowers with both staminate and pistillate structures. In the natural population such "intersexes" have the $2A + XY_1Y_2$ chromosome complement characteristic of strictly staminate plants and are "andromonoecious" (GOLDSCHMIDT 1955). As intersexual flower development also occurs in various experimentally induced situations, however, "intersex" is used as a general term for all cases of intermediate or overlapping sex expression. ALLEN (1940) provided the rationale for applying the terms *male* (= staminate) and *female* (= pistillate) to individuals and to specific organs of the sporophytic ("asexual") generation in angiosperms. Thus usage, accepted by most students of the genetics of sex, (*e.g.* GOLDSCHMIDT 1955; WARMKE 1946; WESTERGAARD 1958), is employed in the present paper.

The diploid karyotypes of *Rumex hastatulus* and the meiotic behavior of its sex chromosomes receive detailed treatment elsewhere (SMITH 1963). They are briefly characterized here in order to clarify the figures and interpretations which follow.

A complete haploid set of autosomes (= A) in the North Carolina race consists of three chromosomes: chromosome 1 is the longest with a submedian centromere; chromosome 2 is intermediate in length, submedian, but distinctly heterobrachial; chromosome 3 is half as long as 1, satellited, and heteromorphic (metacentric or acrocentric). The X resembles chromosome 1, but is more nearly metacentric. The two Y's resemble chromosome 2, but may be distinguished from 2 and from each other by centromere position (Figures 1 and 2).

Diakinesis and metaphase I configurations in diploid males ($2n = 9 = 2A + XY_1Y_2$) consist of three autosome pairs and a chain trivalent of sex chromosomes held together by two terminalized chiasmata. Each end of the central X is paired respectively with a terminal homologous segment in one arm of the Y_1 or of the Y_2 chromosome. The sex chromosome trivalent becomes co-oriented at metaphase I so that the centromere of the X chromosome lies toward one pole and both centromeres of the Y chromosomes toward the opposing pole. This leads to alternate disjunction of the X from the two Y's and the consistent production of two $A + X$ and two $A + Y_1Y_2$ microspores in each tetrad (Figures 3 to 5).

RESULTS

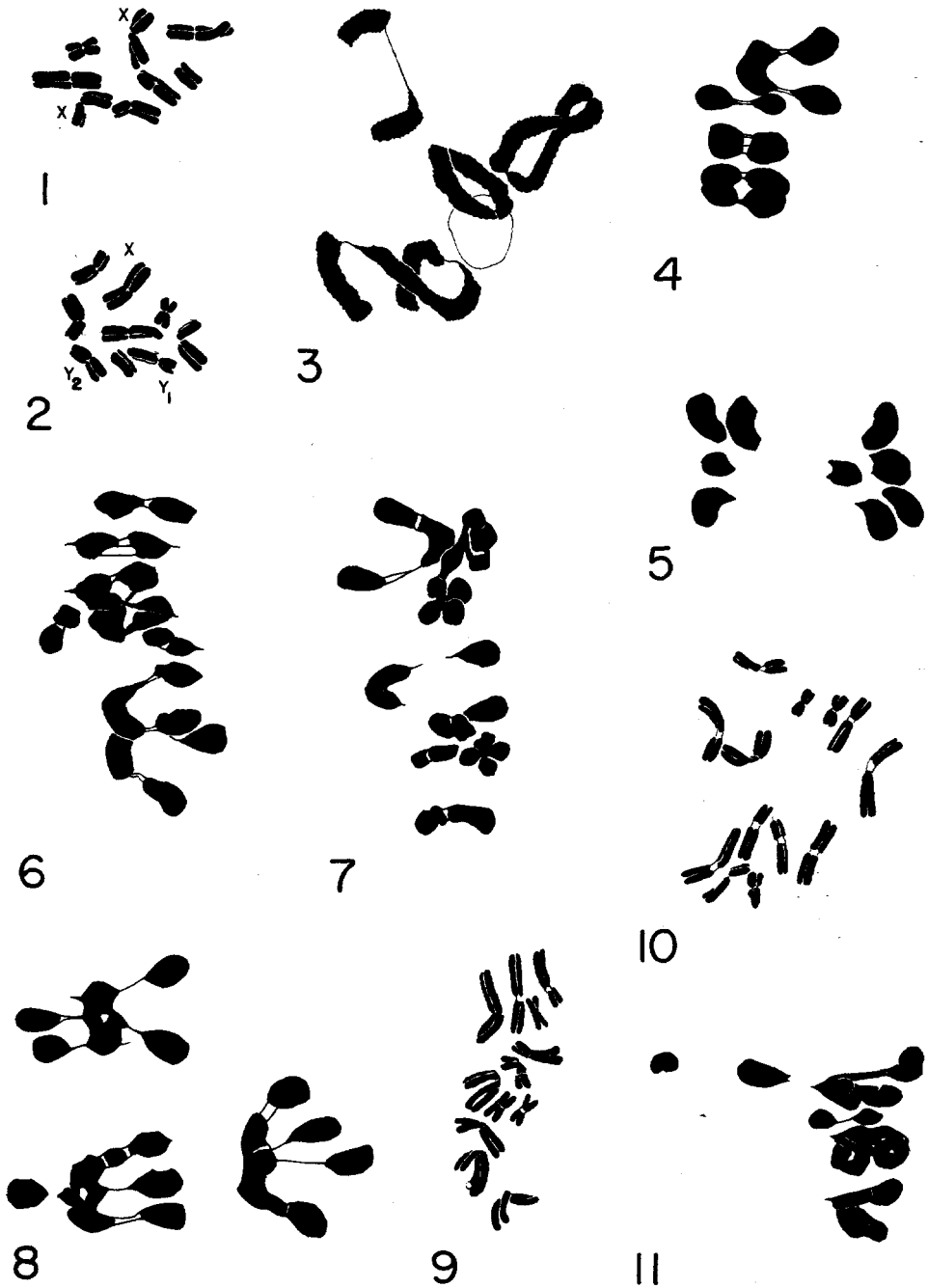*The natural sex ratio:* Independent determinations of the sex ratio among

FIGURE 1–11.—Chromosomes of *Rumex hastatulus*. FIGURE 1.—2n = 8, female. FIGURE 2.—2n = 9, male. FIGURE 3, 4, and 5.—Meiosis in diploid pollen mother cells: diakinesis and metaphase I ($1_{III} + 3_{II}$); anaphase I (n = 4, n = 5). FIGURE 6.—Metaphase I in 4n = 18 male, adjacent orientation of Y_1XY_2 trivalents. FIGURE 7.—Metaphase I in 4n = 18 male, alternate Y_1XY_2 orientation. FIGURE 8.—Three hexavalents of the $XXY_1Y_1Y_2Y_2$ chromosomes in tetraploid males. FIGURE 9.—3n = 12, female. FIGURE 10.—3n =13, male. FIGURE 11.—Late metaphase I in 3n = 13, male ($1_{IV} + 2_{III} + 1_{II} + 1_{I}$). Camera lucida drawings, 1650×.

mature plants of *Rumex hastatulus* in various North Carolina localities appear in Table 1A. Both sexes occurred in large numbers, but there was a deficiency of males in each locality. Plants grown in culture from field-collected, open-pollinated seed yielded further data on the natural sex ratio. Although plant to plant competition effects are minimal under these conditions, the deficiency of males was highly significant (Table 1B). The same trend toward smaller numbers of males occurred repeatedly in experimental cultures maintained under various controlled inbreeding and outbreeding systems. Determinations of chromosome number in early seedling stages (Table 1C) showed that the deficiency of male plants ($2n = 9$) occurs among young seedlings and does not arise from differential survival after emergence. The observed natural sex ratio is approximately 57 females:43 males instead of the expected 50:50. Since all megaspores have the $A + X$ constitution, there must be a differential survival or functioning of the $A + X$ and $A + Y_1Y_2$ male gametophytes or male gametes, or of the two classes of embryos which they produce.

TABLE 1

Sex ratios in Rumex hastatulus

Seed source	Females	Males	Total plants	Percent females
A. Natural population (field counts; N. C. counties)				
Wake (A)	201	129	330	60.9†
Wake (B)	239	155	394	60.7†
Harnett	317	255	572	55.4†
Sampson (A)	224	174	398	56.3*
Sampson (B)	197	158	355	55.5†
Wilson	521	461	982	53.1
Edgecombe	194	129	323	60.1†
Greene	330	238	568	58.1†
Lenoir	242	152	394	61.4†
Jones	105	72	177	59.3*
Craven	166	119	285	58.3†
Brunswick	45	35	80	56.3
Totals	2781	2077	4858	57.25†
B. Cultures: Open-pollinated seed				
Wake County, N. C. (1955)	167	166	333	50.2
11 localities (1956–59)	224	169	393	57.0†
53 localities (1962)	689	604	1293	53.3*
Progenies of individual females				
Pooled samples (1955–56)	252	246	498	50.6
Brunswick County, N. C. (69 half-sibships				
from a single population) (1959)	363	306	669	54.3*
Totals	1695	1491	3186	53.2†
C. Cultures: Sex ratio from chromosome numbers				
of seedlings. 53 random-bred samples;				
9.4 plants each (1962)	282	216	498	56.6†

* Significant chi-square value for 1:1 hypothesis.
† Highly significant.

Diploid intersexes: All of the more than 1400 diploid female plants examined have contained the expected $2n = 8$ $(2A + 2X)$ chromosome complement. The converse is also true (a rare exception will be described later); 8-chromosome plants produce strictly pistillate flowers and are female. Similarly, male plants at the natural diploid level always contain 9 chromosomes. Conversely, all plants with the $2n = 9$ $(2A + XY_1Y_2)$ constitution produce anthers and the majority of them are strictly staminate, but five to ten percent of these staminate plants also exhibit varying degrees of pistillate development. *Rumex hastatulus* is not *strictly* dioecious, as incorrectly stated in an earlier paper (SMITH 1955), for some of the apparently male plants are intersexes.

Table 2 presents data regarding the frequency of andromonoecious, diploid intersexes both in natural populations and in experimental strains produced by controlled breeding. The occurrence of these intersexual types in the natural population is recorded in the first line of the table. Two to five percent of all plants in the *Rumex hastatulus* population are intersexes; the remainder are unisexual. The data show that intersexuality is heritable and that its expression is quantitatively variable.

There is a wide variation in flower structure among the plants classed as intersexes. Plants may produce inflorescences in which most flowers are strictly staminate, but a few flowers contain a small rudimentary pistil. Other plants occur in which many to all flowers of the inflorescence are structurally perfect with fully developed stamens and pistil. Some of these perfect-flowered plants can function as hermaphrodites. In one experimental family, the intersexual plants produced inflorescences in which the majority of the flowers were strictly pistillate, but a few of the flowers also contained one or more stamens. Even in these strongly pistillate intersexes, however, the chromosome constitution was that of a typical male, $2A + XY_1Y_2$.

TABLE 2

Frequencies of andromonoecious intersexes in diploid Rumex hastatulus

Breeding system	Females No.	Females Percent	Intersexes No.	Intersexes Percent	Males No.	Males Percent	Total no.
A. Natural population:							
44 field samples*	. . .	. . .	38	4.3	877	. . .	915
B. Progenies in culture:							
Naturally random-bred	801	54.2	20	1.4	657	44.5	1478
Random-bred in culture	268	57.1	15	3.2	186	39.7	469
Half-sib crosses	1403	58.7	97	4.1	890	37.2	2390
Full-sib crosses	1938	57.1	165	4.9	1294	38.1	3397
F's between local populations	372	57.3	11	1.7	266	41.0	649
C. Progenies from self-pollinated intersexes	241	48.1	116	23.2	144	28.7	501
D. Progenies from selfed-sibbed lines	753	53.0	269	18.9	399	28.1	1421

* Based on staminate individuals only.

Certain of the intersexual plants, both the perfect-flowered and the female intersexes, have produced seed following self-pollination. The yields and viabilities of selfed seed are low compared with yields and viabilities following cross-pollinations performed under similar conditions. In one group of progenies from selfing (Table 3), the chromosome numbers of all of the S_1 plants were determined. No plants of the theoretically expected $2A + Y_1Y_1Y_2Y_2$ class were obtained. With this class eliminated, the Mendelian expectation becomes 1 female:2 males plus male-intersexes, but the observed ratio was close to 1:1. Approximately half the expected $2A + XY_1Y_2$ plants failed to appear. This deficit of 9-chromosome plants was much greater than that observed in the crossbred material (Tables 1 and 2).

Inbreeding by means of self-pollination creates a very strong selective advantage for functional hermaphroditism. This is reflected in greatly increased frequencies of intersexes (Tables 2C and 3). Even a single generation from self-pollination produced a marked increase. Lines with this changed intersex frequency can be maintained by sib mating (Table 2D).

The failure of the expected $2A + Y_1Y_1Y_2Y_2$ to appear (Table 3) suggests that presence of the X chromosome in the complement is essential for viability. A chance observation provided evidence that the X chromosome carries genetic material which is required for development and survival. A single male plant, normal in all other respects, contained a large albino sector. This plant eventually produced an inflorescence within the albino region, which was used to pollinate the flowers of a normal female. At the same time, normal green branches of the male chimaera were used in similar crosses. The sex ratios of the resulting progenies were strikingly different (Table 4). When the albino branch was used as the male parent, no female plants occurred in the progeny, but the control cross of green × green produced a normal sex ratio. The deficiency factor which caused the albinism must have been located in the X chromosome. This factor also

TABLE 3

Progenies from self-pollination of diploid intersexes in Rumex hastatulus
Male intersex: $n = 6 + XY_1Y_2$; Gametes: $3 + X$ and $3 + Y_1Y_2$

	Expected classes			
	$6 + XX$	$2(6 + XY_1Y_2)$	$6 + Y_1Y_1Y_2Y_2$	Total
Observed (Chromosomes of each determined)	63 (Female)	24 41 (Intersex) (Male)	none	128

	Female		Intersex		Male		Total
	No.	Percent	No.	Percent	No.	Percent	no.
Selfed lines							
S_1	156	51.3	71	23.3	77	25.3	304
S_2	25	43.8	12	21.0	20	35.0	57
S_3	6	25.0	7	29.1	11	45.8	24
Selfed-sibbed-selfed	54	46.5	26	22.4	36	31.0	116
Total	241	48.1	116	23.2	144	28.7	501

TABLE 4

A sex-linked lethal in Rumex hastatulus

Parents	Progeny: All normal green			Stand (percent seed)
	Females	Intersexes	Males	
Green ♀ (XX) × white branch of ♂ (XY₁Y₂)	0	3	22	83.3
Green ♀ (XX) × green branch of ♂ (XY₁Y₂)	26	0	21	85.0

produced a lethal effect in the A + X pollen and thus prevented 2A + 2X females from appearing in the next generation.

Chromosome distribution and sex expression in experimental polyploids: 1. Tetraploids: Tetraploid individuals or races of *Rumex hastatulus* have not been found in the natural population. Induced tetraploidy, however, was readily obtained in both sexes of *Rumex hastatulus* (females, $4n = 16 = 4A +4X$; males, $4n = 18 = 4A + XXY_1Y_1Y_2Y_2$) by colchicine treatment. These plants were highly fertile permitting the development of stable polyploid strains by controlled breeding.

Most of the individuals in the progenies of autotetraploids have balanced 4n complements of chromosomes (Table 5). Either the chromosomes of the tetraploids pair and assort very much as in diploids, or most of the unbalanced types are eliminated in the gametophytic phase of the life cycle. Occasional aneuploids with $4n - 1$ or $4n + 1$ chromosomes do occur.

The most characteristic metaphase I configuration in 18-chromosome males $(12 + XXY_1Y_1Y_2Y_2)$ is $6_{II} +2_{III}$ (Figures 6 and 7). The autosomes pair instead of forming multivalents, or if quadrivalents are formed, these disjoin in pairs and produce regular distributions at anaphase I. The sex chromosomes usually associate in two Y_1XY_2 trivalents, sometimes in higher order multivalents. Preliminary analysis has failed to demonstrate a high frequency of Y_1Y_1, XX, or Y_2Y_2 bivalents as might have been expected and as WESTERGAARD (1940) did find in tetraploid Melandrium. On the other hand, rare sex-chromosome hexavalents occur in which it is evident that the two X chromosomes have paired and formed inter-

TABLE 5

Chromosome number frequencies in the first generation from seed following doubling by colchicine

	Chromosome numbers						Totals
	15	16	17	18	19	21	
Observed plants (1957)	1	44	107	54	5	1	212
(1960)	2	45	100	48	1		196
Totals observed	3	89	207	102	6	1	408
Expected frequencies*		102	204	102			408

* Hypothesis: Chromosomes assort as in diploids.

Females: $4n = 12 + XXXX$ Males: $4n = 12 + XXY_1Y_1Y_2Y_2$

Female gametes: $n = 6 + XX$ Male gametes: $n = \begin{cases} 6 + XX \\ 2(6 + XY_1Y_2) \\ 6 + Y_1Y_1Y_2Y_2 \end{cases}$

stitial chiasmata, while each of the four terminal segments of the two X chromosomes is associated with a different Y chromosome (Figure 8). The Y chromosomes in these multivalents, although fully homologous two by two, are not associated with each other. Such hexavalents and the high frequency of Y_1XY_2 trivalents suggest that X-Y pairing is preferential, even though in other cells Y-Y associations sometimes occur. Alternatively, these figures can be interpreted as evidence of extremely localized pairing in the Y chromosomes; once a terminal pairing segment finds a partner, no other pairing possibility exists in that chromosome.

The two Y_1XY_2 trivalents of the typical metaphase I configuration $(6_{II} + 2_{III})$ are oriented in two different ways with respect to eventual disjunction at anaphase: (1) adjacent, with both X's toward one pole and all four Y's toward the other (Figure 6), (2) alternate, with the X of one trivalent and the two Y's of the other oriented toward each pole (Figure 7). Since the position of one trivalent is random with respect to the other, XX versus $Y_1Y_2Y_1Y_2$ and XY_1Y_2 versus XY_1Y_2 assortments are equally frequent. The three combinations of sex chromosomes are produced in 1:2:1 proportions. Observations of actual chromosome distributions at anaphase I verified this interpretation, if the cells which could not be classified because of lagging chromosomes were neglected (Table 6). Most of the laggards eventually reach the poles, and irregularities in their distribution probably account for observed deviations from a completely balanced chromosome distribution.

Since at least 80 percent (Table 6) of the microspores, and of the gametes they produce, have balanced diploid chromosome complements, it is reasonable to assume that as many or more of the megaspores and egg cells are euploid (2A + 2X). The first generation offspring of induced autotetraploids should be produced with 16, 17, and 18 chromosomes in proportions of 1:2:1 plus a few exceptional individuals. This distribution of chromosome numbers did occur in the C_1 generation in two independent trials (Table 5). The plants with 16 chromosomes were female; those with 17 chromosomes were male, but nearly half of them bore some intersexual flowers; plants with 18 chromosomes were male. One exceptional

TABLE 6

Chromosome distribution at anaphase I in pollen mother cells of 3n and 4n Rumex hastatulus

Tetraploid $4n = 18 = 12a + XXY_1Y_1Y_2Y_2$

Number of cells	Chromosome distribution		
	9–9	10–8	Lagging chromosomes
Observed	24	25	9
Expected	24.5	24.5	. .

Triploid $3n = 13 = 9a + XXY_1Y_2$

Number of cells	Chromosome distribution			
	4–9	5–8	6–7	Lagging chromosomes
Observed	5	36	53	10
Expected	6.7	30.2	57.1	. .

male plant with only 16 chromosomes contained 12 autosomes plus XYYY instead of the four X chromosomes found in tetraploid females $(4A + 4X)$. The males and intersexes with 17 chromosomes contained 12 autosomes plus $XXXY_1Y_2$. The 18-chromosome males were like their male parents $(4A + XXY_1Y_1Y_2Y_2)$. In 1957 in both the 17- and the 18-chromosome classes three exceptional females occurred, and seven aneuploid plants also appeared: one female with 15 chromosomes, five males with 19 chromosomes, and a 21-chromosome male which produced some intersexual flowers (Table 5). Complete karyotypes of these anomalous plants are not available, but they are attributed to the irregular assortment of the lagging chromosomes observed in anaphase I.

2. *Triploids:* The original tetraploid by diploid crosses produced very few seed, but two triploid plants were obtained. One was a female with 12 chromosomes; the other was a male with 13 chromosomes. A second 12-chromosome female appeared spontaneously among the diploid members of an inbred strain.

The two triploid plants with $3n = 12$ chromosomes both proved to be strictly pistillate with the chromosome formula, $3A + 3X$ (Figure 9). The third triploid contained $3n = 13$ chromosomes and its flowers were strictly staminate (Figure 10). Mitotic and meiotic study demonstrated that the chromosome formula was $3A + XXY_1Y_2$. At metaphase I (Figure 11) the two X's and the two Y's usually formed a quadrivalent. The X chromosomes paired with each other, and each Y was associated with an end of an X. The two larger autosomes formed trivalents while the smallest formed a pair and a univalent. (At the top of Figure 11, the X chromosome on the left has already disjoined while the other X is still associated with the two Y's on the right. Since the metaphase I bivalent of chromosome 3 often has only a single chiasma in diploid meiosis, one of these chromosomes is usually unpaired in the triploid, extreme upper left.) If the extra chromosomes of the triploid were distributed at random, microspores with any number of chromosomes from four to nine would occur in predictable frequencies. Analysis of the anaphase I distributions in the $3n = 13$ triploid male showed this to be the case (Table 6).

TABLE 7

Sex expression in triploids

Sex	No. of chromosomes	Complement formula	No. of plants
Female			
	12	$3A + 3X$	11
	13*	$(3A + 1) + 3X$	1
Intersex			
	12†	$(3A - 1) + 2X + Y_1Y_2$	1
	12 + f	$3A + 3X + f$	2
	13	$3A + 3X + Y_1Y_2$	3
	13 + f	$3A + 2X + Y_1Y_2 + f$	1
Male			
	13	$3A + 2X + Y_1Y_2$	5

* Extra chromosome 1 or X-chromosome present.
† One chromosome 3 absent.

The sex expression and chromosome conditions of 24 triploids are summarized in Table 7. Eleven were strictly pistillate with $3n = 12$ and the chromosome balance formula, $3A + 3X$. One plant with 13 chromosomes was also strictly pistillate. This plant proved to be an aneuploid, tetrasomic instead of trisomic for one of the large chromosomes, with the formula $(3A + 1) + 3X$. Five of the plants with $3n = 13$ $(3A + 2X + Y_1Y_2)$ were strictly staminate; three others with the same chromosome constitution were intersexes. A second exceptional plant contained only 12 chromosomes but produced intersexual flowers. This plant was hyposomic, disomic instead of trisomic for chromosome 3, with the formula $(3A - 1) + 2X + Y_1Y_2$. The three remaining triploids with the constitution $3n + f$ will be considered after the discovery of the fragment and its behavior in diploids have been described.

A centric fragment which alters sex expression: A single male plant with 10 chromosomes was discovered among the open-pollinated progeny of a selected diploid 'line' which was examined in 1957. Subsequent study has shown that the extra chromosome is an apparently telocentric "fragment." The original fragment consisted of a single arm which was about half as long as chromosome 3 (Figure 12). Although a second chromosome arm was not detected, the centromere functioned normally. The mitotic behavior and transmission of this half-chromosome fragment have continued to be regular. The fragment appeared spontaneously in the second generation in culture following collection of the parent seed stock from the natural population.

The original male plant $(2n = 6 + XY_1Y_2 + f)$ was crossed to four different normal females. The resulting progenies contained $8 + f$ and $9 + f$ plants as well as normal diploids (Table 8). All $9 + f$ individuals were male and morphologically indistinguishable from the 9-chromosome males which lacked the centric fragment. All the $8 + f$ $(2A + XX + f)$ plants produced hermaphroditic flowers with six normal anthers and a well developed pistil. Their female sibs with the normal complement of eight chromosomes $(2A + 2X)$ were strictly pistillate. The $8 + f$ plants differed from diploid females only in the presence of the centric

TABLE 8

Segregation of a centric fragment which alters sex expression

		Progeny no.*				
2n	Sex	1	2	3	4	Total
8	female	9	7	4	5	25
8 + f	intersex	5	3	1	6	15
						40
9	male	6	6	2	0	14
9	intersex	3	4	2	0	9
9 + f	male	4	5	0	1	10
						33

* The four 1958 progenies are from ♀ $(2n = 8) \times$ ♂ $(2n = 9 + f)$.

fragment; therefore, the fragment contained a potent factor for the development of maleness.

This fragment produced similar effects at the triploid level. Three triploids containing the fragment (Table 7) were obtained following crosses between the original 9 + f plant and tetraploid females. Two plants with 12 + f chromosomes (3A + 3X + f) were intersexes. They differed from triploid females only in the presence of the fragment. The third plant contained 13 + f chromosomes (3A + 2X + Y_1Y_2 + f) and was also an intersex. There was no evidence in this individual that the presence of the fragment had strengthened the tendencies for maleness which were already present.

At meiosis in 8 + f plants, the fragment is an independent univalent which shows no tendency to pair with any of the autosomes or with the X chromosomes (Figures 13 and 14). The fragment is similarly independent in most diakinesis and metaphase I figures from 9 + f plants, but in some cells it is associated with the distal end of one of the Y chromosomes (Figures 15 and 16). This centric fragment (designated the "Y-fragment" in the remainder of this paper) contains a segment which is homologous to, and capable of chiasma formation with, a Y chromosome. The determiner for maleness within the fragment chromosome is probably located in that portion of the differential region of the Y-chromosome complex which is also carried by the fragment.

An exceptional intersex: The diploid number 2n = 8 has been observed in 1,461 plants of *Rumex hastatulus*. All except two have later produced strictly pistillate flowers; no stamens nor rudiments of stamens have developed.

In 1958 we determined the chromosome numbers of a large number of plants which belonged to collateral lines from the same half-sib family in which the plant with the spontaneous Y-fragment had appeared in the previous season. The chromosome counts were made during the seedling stages. All plants had normal diploid complements (2n = 8 or 2n = 9). As the plants flowered a few weeks later the sex of each plant was observed and recorded. A unique plant was found which was producing fertile hermaphroditic flowers (first line, Table 9) even though its chromosome number was 2n = 8. The chromosome constitution of this unusual individual was confirmed by examining meiosis in the pollen mother cells of the hermaphroditic flowers. Three pairs of autosomes and a pair of X chromosomes were present (Figures 17 and 18), precisely the situation that occurs in the megasporogenesis of a normal female. There was no evidence of alteration in morphology in either the mitotic or meiotic chromosomes to distinguish the chromosomes of this plant in any way from those of a normal 8-chromosome female.

This gynomonoecious individual continued to produce inflorescences during a period of several weeks. Additional flower samples were examined at biweekly intervals until the plant matured and died (Table 9). The earliest flowers examined were all intersexual, the latest produced were exclusively pistillate. Late inflorescences isolated for self-pollination failed to produce seed, but fruits and seeds developed on the exposed, open-pollinated branches and also following cross-pollination (without emasculation) by a male sib.
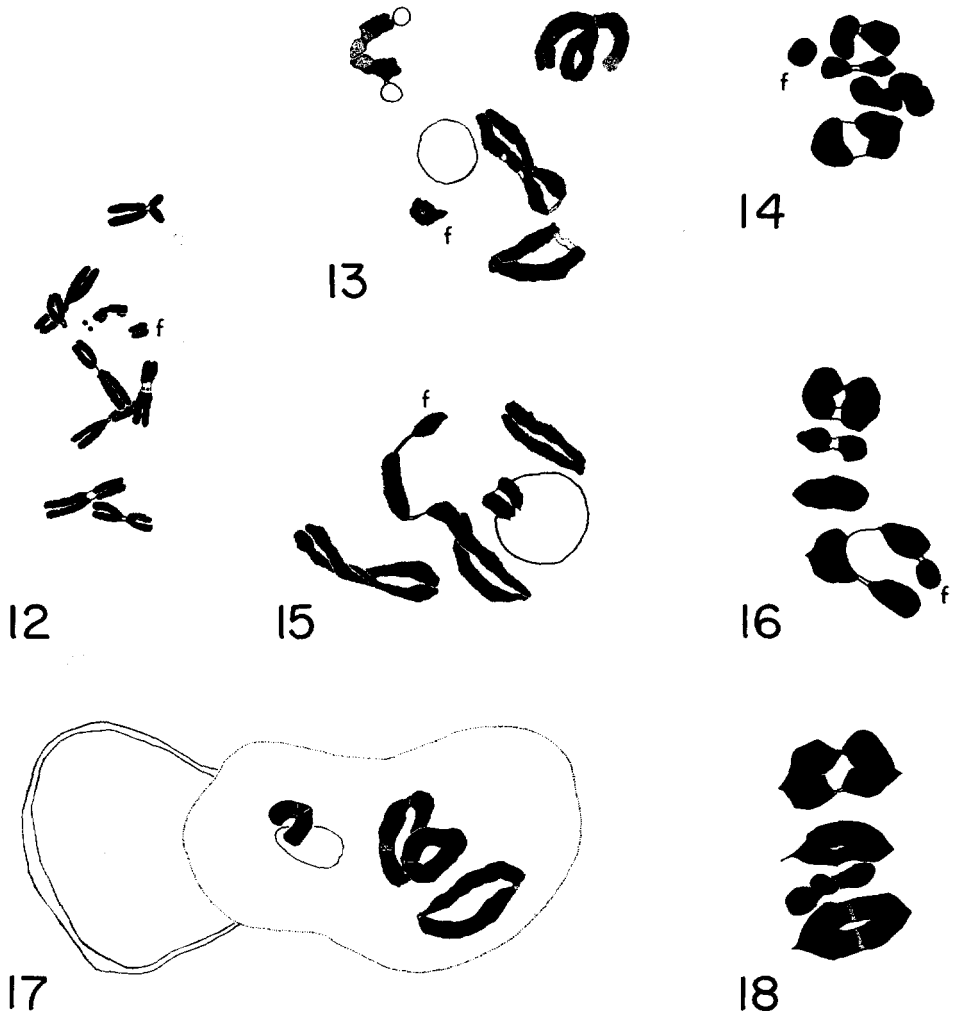
FIGURE 12–18.—*Rumex hastatulus*. FIGURE 12.—Somatic figure of the original $2n = 9 + f$ individual. FIGURES 13–14. $2n = 8 + f$ in diakinesis and metaphase I $(4_{II} + f_I)$. FIGURES 15–16. —$2n = 9 + f$ in diakinesis and metaphase I $(1_{III} + f + 3_{II})$. FIGURES 17–18.—Pollen mother cells of the $2n = 8 = 2A + XX$ intersex in diakinesis and metaphase I (4_{II}). Camera lucida drawings, 1650×.

The 94 offspring examined in the subsequent season included 67 strictly pistillate, 8-chromosome plants; 20 strictly staminate, 9-chromosome plants; and six andromonoecious intersexes with nine chromosomes. A single individual (from the sib-mating) contained an apparently normal $2A + 2X$ complement of eight chromosomes but was recorded as weakly intersexual: 21 pistillate flowers to four intersexual flowers, pistillate with one or more stamens. This second generation, gynomonoecious intersex was discovered too late in the season to

TABLE 9

An exceptional intersex in Rumex hastatulus

$2n = 8 = 2A + 2X$

	Numbers of flowers		
Date sample collected	Female	Intersexual	Male
April 15	0	30	0
April 30 (flowers)	16	14	0
April 30 (buds)	10	19	1
May 15	18	9	3
June 8	30	0	0

permit verification of its chromosome constitution by meiotic study. For this reason it has been omitted from the summary charts which follow.

Chromosome balance and sex: The chromosome balance relations and sex expression now known in *Rumex hastatulus* appear in Chart 1. Diploid, triploid, and tetraploid plants are strictly pistillate whenever the numbers of X chromosomes and the numbers of complete autosome sets are equal (X:A = 1:1). A single individual (omitting its offspring) among many failed to follow this rule. The "Y-fragment" added to the 2A + 2X or to the 3A + 3X complement exerts the same effect in both diploid and triploid and produces an intersex. The same fragment added to the $3A + 2XY_1Y_2$ triploid did not result in a strictly staminate plant, although all diploid $2A + XY_1Y_2 + f$ plants have been staminate. Tetraploid plants with a balanced complement of 18 chromosomes representing an exact doubling of the diploid male complement are also male (X:A = 1:2).

Tetraploids with X:A = 3:4 plus Y_1Y_2 are equally distributed between strict maleness and intersexuality. Results with a large number of plants of this 17-chromosome class have been consistent. On any sampling date approximately half the plants were producing flowers which exhibited varying degrees of intersexuality; the other half were strictly staminate. If the same series of plants was again sampled at a later stage in development, the overall result was the same; but some of the plants previously scored as male were showing some degree of intersexuality, while some of the intersexes were producing only staminate flowers. Triploids with three sets of autosomes, two X chromosomes, and a single set of Y chromosomes also may be male or intersexual (X:A = 2:3). Both the 13-chromosome triploids (X:A = 2:3 plus Y) and the 17-chromosome tetraploids (X:A = 3:4 plus Y) are near the threshold for the expression of bisexuality versus unisexual maleness (Chart 1). Even among diploids with the 9-chromosome constitution usually associated with maleness, X:A = 1:2 plus Y, intersexes sometimes occur.

DISCUSSION

Sex determination in Rumex hastatulus: The well documented investigations of ONO (1935) and YAMAMOTO (1938) demonstrated that an X/A Y-neutral

	DIPLOIDS	TRIPLOIDS	TETRAPLOIDS
♀	2A+2X	3A+3X	4A+4X
♂♀	2A+2X (I plant) 2A+2X+$\underline{f}$ 2A+XY$_1$Y$_2$ (7.7%)	3A+3X+$\underline{f}$ (2 plants) 3A+XXY$_1$Y$_2$ (3 plants) 3A+XXY$_1$Y$_2$+$\underline{f}$ (I plant)	4A+XXXY$_1$Y$_2$ (50.0%)
♂	2A+XY$_1$Y$_2$ (92.3%) 2A+XY$_1$Y$_2$+$\underline{f}$	3A+XXY$_1$Y$_2$ (5 plants)	4A+XXXY$_1$Y$_2$ (50.0%) 4A+XXY$_1$Y$_1$Y$_2$Y$_2$

CHART 1.—Chromosomes and sex expression in *Rumex hastatulus*. When numbers of plants or percentages are not specified, the sex expression has been consistent in a large number of plants.

mechanism of sex determination operates in *Rumex acetosa* and its close relatives in the subgenus Acetosa. The detailed studies of LÖVE (1944) suggest that an X/Y mechanism similar to that of Melandrium determines sex expression among the species of the subgenus Acetosella. The present results with *R. hastatulus* show that the control of sex expression in this plant differs significantly from either of the systems previously discovered in Rumex. Since no one has demonstrated a close relationship between *R. hastatulus* and the other dioecious members of its genus (LÖVE and SAKAR 1956), the present analysis of sex determination in this species must be based upon independent evidence.

1. *Diploid conditions:* The data from the North Carolina race of *Rumex hastatulus* satisfy WESTERGAARD's (1958) criteria for demonstrating the presence of heteromorphic sex chromosomes in a dioecious organism. The *R. hastatatus* population is prevailingly dioecious. Heteromorphic sex-chromosome associations are observed in the meiosis of male individuals but do not occur in females. The XX pair is easily identified in the somatic complement of female plants, and the mitotic X, Y$_1$, and Y$_2$ chromosomes can be distinguished in the male complement.

It is obvious from females that the Y chromosomes carry no essential genes for development which are not present either in the autosomes or the X chromosomes. The diploid female genotype (2A + 2X) normally produces strictly pistillate phenotypes but is capable (twice in about 1500 observed cases) of producing intersexual flowers with functional stamens. The possibility exists that the

gynomonoecious intersexes were not true $2A + 2X$ individuals, but resulted from a cryptic translocation (or a rare unequal crossover between X and Y) which transferred a Y chromosome segment and provided a factor for maleness not usually present in the female complement. The collateral relationship of the original intersex with the plant which contained the spontaneous Y-fragment is suggestive. The progeny of the intersex, however, contained a disproportionate number (73 percent) of 8-chromosome females. Some of these may have arisen from seed produced by self-pollination of the perfect flowers produced early in development. All the hybrid progeny would have a 1:1 chance of receiving the postulated altered chromosome; the odds would be 3:1 in any plants derived from self-pollination. If a cryptically changed chromosome had actually been present, its effect should have been manifest in more than one of the 68 offspring with eight chromosomes.

The author interprets the $2A + 2X$ gynomonoecious intersex as a cytogenetically normal female which demonstrates the bisexual potentiality of the autosome plus X chromosome complement. The original plant and the single 8-chromosome intersex observed in its progeny demonstrate that neither of the Y chromosomes is absolutely necessary for maleness.

The diploid male genotype $(2A + XY_1Y_2)$ normally produces staminate flowers but is capable of producing intersexual flowers (1 to 5 percent of plants in the cross-bred population) with functional pistils. In rare instances the $2A + XY_1Y_2$ genotype produces an intersexual phenotype which resembles a strictly pistillate plant except for the presence of one or more functional stamens in a few of the flowers. The bisexual potentiality of the diploid male genotype is thus evident. In these intersexual cases the presence of the Y chromosomes not only fails to inhibit the development of pistillate structures, but also fails on occasion to promote the full development of the androecium which normally consists of six stamens.

2. *Genetics of X and Y, and the sex ratio:* The X chromosomes of *Rumex hastatulus* contain genetic material which is essential for normal vegetative development and survival, but which is not present in the Y chromosomes. The white branch of the chlorophyll deficient chimaera resulted from a genetic deficiency in the X chromosome. The deficient condition of the X also prevented the formation of functional $A + X$ pollen, but the $A + Y_1Y_2$ pollen performed normally. Although present in the cells of the albino sector, however, these normal Y chromosomes did not mask the effect of the deficiency in the X chromosome; the Y's also lacked the genes for normal chlorophyll production.

Conversely, the Y chromosomes do contain genetic material which is important in vegetative development. Several Mendelian recessive characters have been isolated from inbred progenies of *Rumex hastatulus*. One of these, which will be presented in a separate paper, has proved to be both sex-linked and sex-limited. Hemizygous males are normal because the Y chromosomes contain genetic material which masks the effect of the X-linked recessive.

The absence of the $2A + Y_1Y_1Y_2Y_2$ class from the progeny of self-pollinated andromonoecious males $(2A + XY_1Y_2)$ may be explained in one of two ways:

(a) The $A + Y_1Y_2$ class fails to occur or fails to function in megagametogenesis. If true, this would explain both the absence of the $2A + Y_1Y_1Y_2Y_2$ class and the 1:1 (females: males + intersexes) sex ratio following self-pollination. If the intersexes fail to produce functional $A + Y_1Y_2$ eggs, all female gametes would be $A + X$ and the expectation would be the same as in the normally cross-bred population. This hypothesis can be tested by observing megasporogenesis and megagametogenesis in perfect-flowered intersexes.

(b) The Y chromosomes lack genes which are essential for diploid development. Male gametophytes regularly transmit the haploid Y chromosomes, but diploid genotypes homozygous for Y_1 and Y_2 and lacking X could be deficient in genes essential for sporophyte viability.

The genetic inefficiency of the Y chromosomes also suggests an explanation for the disparate, male-deficient, sex ratios which commonly occur in *Rumex hastatulus*. Male determining $A + Y_1Y_2$ gametophytes develop and function but the $A + X$ gametophytes may be more vigorous and have a selective advantage. Abundant pollination (usually the case in *Rumex hastatulus*) would lead to competition between the two kinds of male gametophytes, and would produce an excess of females. Certation effects of this kind have been demonstrated in several dioecious plant species: Melandrium, *Rumex acetosa*, Cannabis, and Humulus (reviewed by ALLEN 1940).

3. *Chromosome balance:* The one to one relationship between the number of X chromosomes and the number of autosome sets is associated with strict femaleness not only in *Rumex hastatulus* (Chart 1) but also in *Melandrium album*, in *Rumex acetosa*, and in *Drosophila melanogaster*. Differences between these species in the mode of sex determination became apparent when the chromosome conditions associated with maleness or intersexuality were compared. Males, and in special circumstances intersexes (WARMKE 1946; WESTERGAARD 1948) occurred in Melandrium only when Y was present. In euploids, this result was not affected by the X/A balance conditions. Conversely in euploids of Drosophila (BRIDGES 1932) and of *Rumex acetosa* (YAMAMOTO 1938), males or intersexes developed whenever the X/A balance was less than one. This result was not affected by the presence or absence of the Y chromosome.

The relations between chromosome constitution and sex in *Rumex hastatulus* are compared in Chart 2 with the effects of similar chromosome constitutions in Melandrium, *Rumex acetosa*, and Drosophila. The effects of various other chromosome combinations are known in the latter three species; only those which correspond closely to the situations found in *R. hastatulus* are listed in the table. The items in Chart 2 are arranged: (1) in order of decreasing values of the X/A ratio, (2) in order of increasing chromosome number.

Rumex hastatulus in Chart 2 is intermediate between Melandrium and Drosophila. If Y is present in Melandrium the plants are male; if no Y is present the plants are female. Both statements are true regardless of the numbers of autosomes and X chromosomes. No intersexes occur in these euploid combinations. In Drosophila (and in *R. acetosa*), if the X/A ratio is 0.50 the individuals are male, if X/A falls between 0.50 and 1.00 the individuals are intersexes. The Y

chromosome has no effect. In *Rumex hastatulus* maleness appears when Y is present and is not expressed when Y is absent (similar to Melandrium), but also intersexes occur when Y is present and the X/A balance is intermediate, >0.50, <1.00, (similar to Drosophila). Intersexes in low frequency regularly occur even when X/A equals 0.50 in normal diploids.

The most critical classes for distinguishing between the effects of X/A balance and the male determining effect of the Y chromosome (2A + X, 3A + XX, 4A + XX, 4A + XXX), are lacking in the data from *Rumex hastatulus*. No plants either with 7, 2A + X, or with 11, 3A + XX, chromosomes have been found; the few plants with 14 or 15 chromosomes have resulted from aneuploidy among the autosomes. Meiotic regularity (in tetraploids) and selective elimination of hypoploid combinations following sporogenesis have prevented these classes from appearing.

The three male-intersex classes in *Rumex hastatulus*, however, provide evidence of an underlying X/A balance in the sex determination of this species. The increasing frequencies of intersexes among the 2A + XY, 3A + XXY, and 4A + XXXY plants are positively correlated with increasing magnitudes of the X/A ratio. The occurrence of the gynomonoecious intersex (2A + 2X) strengthens this interpretation, because it shows that the capability for bisexual development does not depend upon the presence of the Y chromosomes.

Chromosome formula	X/A	Melandrium	Rumex[a] hastatulus	Rumex[a] acetosa	Drosophila
2A+XX	1.00	♀	♀ (+1♂)	♀ (+1♂)[b]	♀
3A+XXX	1.00	♀	♀	♀	♀
4A+XXXX	1.00	♀	♀	♀	♀
4A+XXX	0.75	♀	—	♂	♀♂
4A+XXXY	0.75	♂	♂ or ♀(1:1)	♂	—
3A+XX	0.67	♀	—	♂	♀♂
3A+XXY	0.67	♂	♂ or ♀(5:3)	♂	♀♂
2A+XY	0.50	♂	♂ or ♀(12:1)	♂(+7♀)[b]	♂
4A+XX	0.50	♀	—	—	♂
4A+XXYY	0.50	♂	♂	♂	—

[a] $Y = Y_1 + Y_2$ for both species of Rumex

[b] Noted by Ono as "unexpected sex types"

CHART 2.—Chromosome constitution and sex in Melandrium, Rumex and Drosophila. The exceptional intersexes observed by ONO in *R. acetosa* were found among 158 + plants with the 2A + 2X, and 93 + plants with the 2A + XY constitution, respectively.

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The Y chromosomes of *Rumex hastatulus* are not neutral in sex determination, however, but contain genetic material which promotes the expression of maleness. The presence of the Y chromosomes in all male and intersex classes of *R. hastatulus* in Chart 2 is confounded in each case with an X:A constitution which would also be expected to favor maleness. The behavior of the spontaneous Y-fragment and the effect of this fragment upon sex in various chromosome combinations, however, have provided direct evidence of the sex-determining role of the Y chromosomes in *Rumex hastatulus*.

This Y-fragment, or half chromosome, could have arisen by spontaneous fragmentation of one of the Y's near the centromere, or from a translocation involving a Y chromosome. The other segment or chromosome involved in the original aberration has been lost, but the present Y-fragment assorts at random in meiosis and persists in the population. The sex and chromosome constitution of *Rumex hastatulus* plants with and without the added Y-fragment are compared in Chart 3 with known types from Melandrium, *Rumex acetosa* and Drosophila with and without extra Y chromosomes. The addition of the Y-fragment to a chromosome complement which normally produces only female individuals converts the complement to one which produces intersexes. The Y-fragment and therefore the Y chromosome from which it arose contain genetic material which promotes or enhances the development of functional stamens in *Rumex hastatulus*. This effect

Chromosome formula	X/A	Melandrium	Rumex[a] hastatulus	Rumex[a] acetosa	Drosophila
2A+XX	1.00	♀	♀(+1♂̣)	♀(+1♂̣)[b]	♀
2A+XX+f	1.00	—	♂̣	—	—
2A+XXY	1.00	♂	—	♀	♀
3A+XXX	1.00	♀	♀	♀	♀
3A+XXX+f	1.00	—	♂̣	—	—
3A+XXXY	1.00	♂	—	♀	♀
3A+XXY	0.67	♂	♂ or ♂̣(5:3)	♂̣	♂̣
3A+XXY+f	0.67	—	♂̣	—	—
2A+XY	0.50	♂	♂ or ♂̣(12:1)	♂(+7♂̣)[b]	♂
2A+XY+f	0.50	—	♂	—	—
2A+XYY	0.50	♂	—	4♂(+3♂̣)[b]	♂

[a] Y=Y₁+Y₂ for both species of Rumex

^{a}Y=Y$_1$+Y$_2$ for both species of Rumex

[b] Noted by Ono as "unexpected sex types"

CHART 3.—The effect of the Y-fragment in *Rumex hastatulus* compared with the effect of an added Y chromosome in Melandrium, *R. acetosa* or Drosophila.

resembles the action of the Y chromosome in Melandrium, but in *R. hastatulus* there is no evidence that the Y chromosomes also inhibit pistillate development. Conversely, the extra Y chromosomes in Drosophila and in *Rumex acetosa* (Chart 3) have no effect upon sex expression.

4. *Theories of sex determination:* WARMKE (1946) and WESTERGAARD (1946) independently discovered the male-promoting and female-inhibiting effects of the Y chromosome in *Melandrium album.* WARMKE suggested an X/Y balance mechanism of sex determination in which the autosomes had little or no influence upon sex expression, but WESTERGAARD (1948) found that the extra autosomes present in aneuploids did alter sex expression. He concluded that the basic potentialities for both maleness and femaleness are determined by genes located in all the chromosomes and present in both sexes, but that actual sex expression is controlled by a multigenic trigger mechanism located in the differential region of the Y chromosome. The Y chromosome suppresses femaleness and promotes maleness by controlling essential steps in anther development when it is present; its absence permits female development, but blocks essential steps in androecium development. TAZIMA (KIHARA 1953) reported an X/Y mechanism in the silkworm, Bombyx, but in this form with female heterogamety, the strong Y determiner promotes femaleness. Sex determination in Bombyx closely resembles that in Melandrium, but in reverse; whenever Y is present the individual is female regardless of the X/A balance relation. Recent studies of trisomics in man have shown that the Y chromosome contains male sex determiners (see GOWEN 1961) but these do not exert the strong influence found in Melandrium and Bombyx.

GOLDSCHMIDT (1955) described these X/Y mechanisms as special cases of the X/A balance system found in Drosophila. He reviewed the evidence and presented a detailed reinterpretation of Melandrium sex determination as resulting from the balance between autosomal male determiners and X-chromosomal female determiners which is modified by the female suppressor-male enhancer effect of the Y chromosome.

GOLDSCHMIDT's formulation of the basic concepts in the genetic theory of sex determination is explicit: "(1) the old assumption that each sex contains the potentiality for the other sex was proved to be the result of the presence of both kinds of genetic determiners in either sex; (2) the existence of a quantitative relation, later termed "balance" (though it is actually an imbalance), between the two types of sex determiners decides sexuality, that is femaleness, maleness, or any grade of intersexuality; (3) one of the two types of sex determiners (male ones in female heterogamety, female ones in male heterogamety) is located within the X-chromosomes, the other one, outside of them; (4) as a consequence of this, the same determiners of one sex are faced by either one or two portions of those of the other sex in the X-chromosomes; (5) the balance system works so that two doses in the X-chromosomes are epistatic to the determiners outside the X, but one dose is hypostatic; (6) intermediate dosage (or potency) conditions in favor of one or the other of the two sets of determiners result, according to their amount, in females, males, intersexes, or sex-reversal individuals in either direction; ..."

These principles variously stated form the basis of the present and most past genetic interpretations of sex determination. The number and nature of the actual determiners and their mode of action in development have been controversial. BRIDGES (1932) stated the essential similarities and differences between the "genic balance" concept and GOLDSCHMIDT's view. Drosophila workers emphasized "the cooperation of all genes which are themselves qualitatively different" and which "act together in a quantitative relation," while GOLDSCHMIDT placed "primary emphasis upon the quantitative aspect of individual genes." The controversial final point in GOLDSCHMIDT's formulation postulates a "turning point" in development which produces "true intersexuality." This occurs in flowering plants only when the androecium transforms into a gynoecium or vice versa. The concept is not pertinent in the present discussion which deals with a mechanism of sex determination that imposes dioecism upon a previously established monoecious pattern of development.

5. *Conclusions:* The facts regarding sex determination in the North Carolina race of *Rumex hastatulus* lead to the following interpretations and conclusions: (1) The potentiality for the production of both gynoecia and androecia is present in all individuals and must be inherent in the autosome plus X chromosome complement which is also present in all individuals. The Y chromosomes have a positive but secondary role in sex determination. (2) The $1A:1X$ ratio is extremely stable in determining a strictly female development, but even this is subject to modification, either by the internal or external environment (the $2A + 2X$ intersexes), or by the specific genotype (addition of the centric "Y-fragment"). (3) The effect of the "Y-fragment" indicates that the normal Y chromosomes promote the development of the androecium, but from the same experiments there is no evidence that the Y's inhibit gynoecium development. Strongly female intersexes can appear even in diploids despite the presence of both Y chromosomes. (4) The $2A:1X$ balance favors the development of the androecium and must also inhibit gynoecium development. (Critical experimental evidence is lacking because monosomic $2A + X$, hypoploid $4A + 2X$, or $4A + 3X$ classes which lack Y chromosomes have not been observed, but the variability in sex phenotype among diploids which contain Y chromosomes supports this conclusion. If the Y's do not inhibit pistil development, the $2A:1$ X balance must do so.) (5) The ultimate sex expression in a $2A + XY_1Y_2$ plant is subject not only to the $2A:1X$ balance and the male-enhancing effect of the Y chromosomes, but also to the random assortment of other genes in the constantly segregating background genotype. Andromonoecious intersexes occur in a consistent low frequency and at random in the unselected cross-bred population, and in a wide variety of both natural and cultural environments. The frequency of these intersexes must be genotypically controlled because it is easily altered by selection. This modification in the expression of intersexuality by the chance result of segregation and assortment in the background genotype can occur at any level of ploidy.

The Y chromosomes and the "Y-fragment" in *R. hastatulus* carry at least one factor for the enhancement of maleness, but it is not comparable in strength to the effect of the Y chromosome in Melandrium. In the existence of this Y chromo-

some effect, sex determination in *R. hastatulus* also differs from the X/A balance, Y-neutral mechanism which occurs both in *Rumex acetosa* and in Drosophila. Sex is determined in *R. hastatulus* by a fundamental X/A balance mechanism which is modified in the male direction by the Y chromosomes, with the expressed sex phenotypes also subject to variation induced either by the environment or the genotype. Sex determination in *R. hastatulus* resembles the condition found in Melandrium, not an X/Y balance nor a Y-trigger mechanism as WARMKE and WESTERGAARD have proposed but as a Y-modified, X/A balance system similar to the interpretation of the Melandrium case suggested by GOLDSCHMIDT.

SUMMARY

The North Carolina race of *Rumex hastatulus* is dioecious: females, $2n = 8$ ($2A + XX$); males, $2n = 9$ ($2A + XY_1Y_2$). Sex ratios deviate significantly from the expected, approximately 57 females to 43 males instead of 50:50. This is attributed to certation. Dioecism is not absolute, 1.0 to 5.0 percent of the plants produce intersexual flowers. These diploid intersexes have the male constitution ($2A + XY_1Y_2$), are functionally staminate, sometimes functionally hermaphroditic. The X chromosome is essential for normal development. The Y chromosomes, neither essential nor genetically inert, contain a region which promotes maleness.

Meiotic chromosome behavior in induced tetraploids was regular; most plants in progenies of autotetraploids had balanced 4n complements. In males, $4n = 18$ ($4A + XXY_1Y_1Y_2Y_2$), the sex chromosomes frequently formed Y_1XY_2 trivalents instead of the expected XX, Y_1Y_1, and Y_2Y_2 pairs. The relation of chromosome constitution to sex expression in diploids, triploids, and tetraploids provides evidence of an underlying X/A balance in sex determination which is modified toward maleness by the Y chromosomes. This X/A plus Y mechanism of *Rumex hastatulus* is intermediate between the X/A system of Drosophila and the X/Y mechanism of Melandrium.

LITERATURE CITED

ALLEN, C. E., 1940   The genotypic basis of sex-expression in angiosperms. Botan. Rev. **6**: 227–300.

BRIDGES, C. B., 1932   The genetics of sex in Drosophila. pp. 55–93. *Sex and Internal Secretions*, 1st edition. Edited by E. ALLEN. Williams and Wilkins Co., Baltimore.

GOLDSCHMIDT, R. B., 1955   *Theoretical genetics*. University of California Press. Berkeley.

GOWEN, J. W., 1961   Genetic and cytologic foundations for sex. pp. 3–75. *Sex and Internal Secretions*, 3rd edition, Edited by W. C. YOUNG. Williams and Wilkins Co., Baltimore.

KIHARA, H., 1953   Genetics of Bombyx and Drosophila: a comparison of materials, methods and results. Seiken Zihô, Rep. Kihara Inst. Biol. Res. **6**: 15–29.

LÖVE, A., 1944   Cytogenetic studies on Rumex subgenus Acetosella. Hereditas **30**: 1–136.

LÖVE, A., and N. SARKER, 1956   Cytotaxonomy and sex determination of *Rumex paucifolius*. Can. J. Botany **34**: 261–268.

ONO, T., 1935   Chromosomen und Sexualität von *Rumex Acetosa*. Tôhoku Imperial Univ., Science Reports, Ser. 4, **10**: 41–210.

SMITH, BEN W., 1955   Sex chromosomes and natural polyploidy in dioecious Rumex. J. Heredity **46**: 226–232.
  1963   The evolving karyotype of *Rumex hastatulus*. Evolution (in press).

TJIO, J. H., and A. LEVAN, 1950   The use of oxyquinoline in chromosome analysis. Ann. Estac. Exptl. Aula Dei **2**: 21–64.

WARMKE, H. E., 1946   Sex determination and sex balance in Melandrium. Am. J. Botany **33**: 648–660.

WESTERGAARD, M., 1940   Studies on polyploidy and sex determination in polyploid forms of *Melandrium album*. Dansk Bot. Arkiv. **10(5)**: 1–131.
  1946   Aberrant Y chromosomes and sex expression in *Melandrium album*. Hereditas **32**: 419–443.
  1948   The relation between chromosome constitution and sex in the offspring of triploid Melandrium. Hereditas **34**: 257–279.
  1958   The mechanism of sex determination in dioecious flowering plants. Advan. Genet. **9**: 217–281.

YAMAMOTO, Y., 1938   Karyogenetische Untersuchungen bei der Gattung Rumex. VI. Geschechtsbestimmung bei eu- und aneuploiden Pflanzen von *Rumex Acetosa* L.   Kyoto Imperial Univ., Mem. Coll. Agr. **43**: 1–59, Taf. I–IV.