On the Special Autosomes with Reference to the Sex-determination of *Rumex acetosa*, L.

By

Y. Takenaka

Botanical Inst., Preparatory Dept. of Keijo Imperial University

Introduction

Since the sex-chromosome was discovered in *Rumex acetosa* (Kihara and Ono, 1923a, b), *Elodea canadensis* (Santos, 1923), and *Melandrium* (Blackburn, 1923, Winge, 1923) the investigations of sex-chromosomes in angiosperms have been carried on very actively, and the latest compilation by Sinotô (1929) shows that 51 species of higher dioecious plants contain so-called sex-chromosomes. Nevertheless as far as the origin of sex-chromosome, or the phylogeny of the dioecious plants are concerned, there exists scarcely any explanation. The sex-chromosomes of *Rumex acetosa* have been, up to now, investigated by many observers. First Kihara and Ono (1923), and then Sinotô (1924) ascertained that the chromosome complements of *Rumex acetosa* were $14 = 12a + 2X$ in the female and $15 = 12a + X + 2Y$ in the male. Shimotomai and Ono (1928) discovered the triploid and tetraploid intersexual plants, and a little afterwards Ono (1928) the triploid female. It was also Ono (1930a, b) who published then his studies on many intersexual *acetosa* and discovered the male-intersexual plant carrying $12a + X + 2Y$ chromosomes. Takenaka (1931) observed intersexual and male-intersexual plants containing $12a + X + 2Y$ chromosomes and described new chromosomes as formed by translocation. Kihara and Yamamoto (1931) published their karyological investigations of abnormal forms of this species, while the latter successively reported the results of his karyological and physiological researches on the normal and abnormal plants (1932, '33, '34a, b, '35, and '36b). Ono (1935) concluded that the sex was determined by the ratio of the sex-chromosome (X) to the autosome. The writer (1936a) stated that the ratio of the number of the autosomes to that of the sex-chromosomes is generally in proportion to the degree of appearance of the sexual organs, and that exceptions to this were seldom found. Yamamoto (1936a) observed the new chromosome made by translocation. Satô and Sinotô (1935) discovered the triradial pairing and argued the occurrence of chiasmata of a tripartite sex-chromosome. Jensen (1936a, b) stated that the genus *Rumex* was compounded by hybridization and

that the so-called sex-chromosomes in *Rumex* were nothing more than an abnormality resulting from the previous hybridization of the species. It was explained by the writer (1935, '36b) that the sex-disturbing factors in the male- and female-intersexual plants carrying respectively $12a + X + 2Y$ and $12a + 2X$ were not dependent on X- and Y-chromosomes, but had some relation to a few autosomes. These plants were also produced by the writer through hybridization of normal wild plants collected from different districts. It was assumed by the writer from the results of both his karyological and his genetic studies, that the origin of the sex-chromosome in the dioecious plants which have not any remarkable phylogenetic relationship might be accounted for by aberrant chromosomes transformed owing to irregular nuclear divisions. The object of this paper is to give an account of some advanced researches on the sex-influencing chromosomes in the male- and female-intersexual plants.

Material and Methods

The materials in the present experiments were the progeny of a male-intersexual plant, family number XI, having a diploid chromosome complement. The karyological studies on somatic cells of these offspring (Takenaka, 1936b) have shown that both male and male-intersexual plants received almost always $12a + X + 2Y$ chromosomes, while both female and female-intersexual plants $12a + 2X$. For the research on the male- and female-intersexual chromosomes or factors, the following cross experiments were carried out:

1. Normal female $\times$ male-intersex,
2. Female-intersex $\times$ normal male,
3. Abnormal* female $\times$ male-intersex,
4. Female-intersex $\times$ abnormal male,
5. Female-intersex $\times$ male-intersex,
6. Abnormal female $\times$ abnormal male,
7. Abnormal female $\times$ normal male,
8. Normal female $\times$ abnormal male.

Before maturation, some branches of the plants were enclosed in paraffin-paper bags and after a week or more pollination was carefully carried out by pollen-grains of the paternal plants which were kept in the laboratory room so as to avoid contamination with foreign pollens.

* The abnormal male and female plants are the offspring of family XI and of the female intersexes which are the progeny of family XI.

Experiments

Exp. I. Normal female $\times$ male-intersex

This breeding experiment was carried out to ascertain whether or not the male-intersexual chromosomes in the male-intersexual plants had any connection with the female-intersexuality. Pollination was tried in the springs of 1934 and 1935, and seeds were sown in the autumns of 1934 and 1935 respectively. Results were observed in the springs of 1935 and 1936.

One female-intersexual plant(?) grew up from the mating of "K-12 $\times$ 171-17". In this plant, one flower only, among several hundreds, was observed to have an anther-like element. Whether it is to be taken

Table 1. F_1 results from normal female $\times$ male-intersex

Maternal No.	Paternal No.	Classification of Progeny					
		σ	$+\sigma^*$	$\varnothing$	$-\varnothing^*$	$\varnothing$	$\varnothing$
143-6	121-14	5	7	—	—	27	
K-12	171-17	—	—	—	1 (?)	8	
K-12	17-134	21	1	—	—	43	
K-10	17-134	3	1	—	—	8	
K-5	17-134	2	3	—	—	24	
K-22	17-144	5	—	—	—	13	
K-26	17-147	2	—	—	—	3	
Total		38	12	—	1 (?)	126	

* $+\sigma$ and $-\varnothing$ indicate respectively male- and female-intersex.

as female-intersex or female is uncertain. From these results it may be supposed that the male-intersexual plants are not concerned in the female-intersexuality. And two paternal autosomes ($\alpha' + \beta'$) seem to be connected with the male-intersexuality when the certain of pollen-grains is neglected and the chromosome complements of paternal male-intersexes are all $15 = \alpha\alpha' + \beta\beta' + 8$ autosomes $+ X + 2Y$; because the ratio of σ to $+\sigma$ is 38:12, i.e. about 3:1, and the pollen-grains for the male-intersexual F_1 are a quarter of the whole. So the chromosome-formulae of the male-gametes are as follows:

$$\alpha + \beta + 4 \text{ autosomes} + 2Y$$

$$\alpha + \beta' + 4 \text{ autosomes} + 2Y$$

$$\alpha' + \beta + 4 \text{ autosomes} + 2Y$$

$$\alpha' + \beta' + 4 \text{ autosomes} + 2Y$$

Exp. II. Female-intersex $\times$ normal male

This hybridization was examined to ascertain whether the female-intersexual chromosomes were concerned in the male-intersexuality or not. Pollination was tried in the spring of 1935, and seeds thus attained were sown in the autumn of the same year. The results were observed in the spring of 1936.

The results showed the facts that the maternal female-intersexual plants involved the male-intersexual chromosomes and that, in

Table 2. F_1 results from female-intersex $\times$ normal male

Maternal No.	Paternal No.	Classification of Progeny				
		σ	$+\sigma$	♀	$-\text{♀}$	♀
163-18	K-11	5	—	—	—	5
17-96	K-11	8	—	—	—	21
211-6	K-11	6	—	—	2	5
162-18	K-10	2	1	—	—	2
	Total	21	1	—	2	33

the offspring, the female-intersexual individuals were much smaller in number than the female plants. From the latter fact the female intersex is supposed to be concerned with several autosomes. Accordingly all or most of the female plants (abnormal female) must carry some of the female intersex-influencing chromosomes. As the number of plants resulting from the experiment was too small to permit determination of the number of the male- and female-intersex-influencing chromosomes carried from the maternal female-intersexual plants, the writer will give an account of it at some future time.

Exp. III. Abnormal female $\times$ male-intersex

The results of Exp. II showed that the abnormal female generally involved some autosomes concerning the female-intersex and that the male-intersex depended on the several autosomes of the female-intersex. And also the results of Exp. I showed that the abnormal female was capable of containing some male-intersexual chromosomes. According to Exp. III, it may be possible to show several female-intersexual individuals, and the number of the male-intersexual plants may be greater than that in Exp. I.

Table 3. F_1 results from abnormal female $\times$ male-intersex

Maternal No.	Paternal No.	Classification of Progeny				
		σ	$+\sigma$	♀	$-\text{♀}$	♀
17-143	17-147	3	4	—	8	6
17-164	17-144	2	—	—	3	23
170-4	170-8	1	3	—	2	10
	Total	6	7	—	13	39

F_1 results of Exp. III were in accord with the writer's expectation.

Exp. IV. Female-intersex $\times$ abnormal male

It may be assumed that in the case of the abnormal male, which is the progeny of family XI, some of the female- and male-intersex-influencing chromosomes are involved among its chromosome-complement. Then many more female- and male-intersexual plants

Table 4. F_1 results from female-intersex $\times$ abnormal male

Maternal No.	Paternal No.	Classification of Progeny				
		σ	$+\sigma$	♀	$-\text{♀}$	♀
279-9	264-43	1	5	—	17	22
172-16	17-165	—	4	—	2	23
17-156	17-138	—	3	—	3	12
	Total	1	12	—	22	57

should be found here than are found in Exp. II.

As shown in Table 4, many male- and female-intersexual plants were counted.

Exp. V. Female-intersex $\times$ male-intersex

In this experiment more female- and male-intersexual plants may generally be expected than in Exp. IV. The results were contrary to the writer's expectation, but the members of the male- and female-intersexual plants were found in greater number than in any other experiments except Exp. IV.

In this experiment, one intersexual plant was found. It might have germinated from the seed resulting from the combination of the abnormal gametes, one of which at least was made by incomplete meiosis, such as non-disjunction.

Table 5. F_1 results from female-intersex $\times$ male-intersex

Maternal No.	Paternal No.	Classification of Progeny					
		σ	$+\sigma$	♀	$-\text{♀}$	♀	
163-18	17-144	—	—	—	1	3	
17-156	17-144	2	2	—	4	10	
122-36	276-5	3	1	—	2	2	
17-156	17-147	8	—	1	—	7	
170-11	170-8	—	—	—	1	2	
Total		13	3	1	8	24	

Table 6. F_1 results from abnormal female $\times$ abnormal male

Maternal No.	Paternal No.	Classification of Progeny					
		σ	$+\sigma$	♀	$-\text{♀}$	♀	
17-160	17-165	1	—	—	—	15	
17-143	17-138	5	2	—	6	32	
Total		6	2	—	6	47	

Exp. VI. Abnormal female $\times$ abnormal male

Results of this breeding experiment agreed with those expected, as shown by several male- and female-intersexual plants.

Exp. VII. Abnormal female $\times$ normal male

In this experiment, the writer anticipated the appearance of a few male-intersexual plants; and the result was satisfactory.

One intersexual plant found in this experiment might have germinated from the seed similar to that of the intersexual plant in Exp. V.

Table 7. F_1 results from abnormal female $\times$ normal male

Maternal No.	Paternal No.	Classification of Progeny					
		σ	$+\sigma$	♀	$-\text{♀}$	♀	
17-141	K-11	16	1	1	—	15	
17-103	K-11	5	3	—	—	1	
17-85	K-11	3	—	—	—	7	
17-98	K-10	—	—	—	—	22	
17-143	K-11	19	—	—	—	13	
Total		43	4	1	—	58	

Exp. VIII. Normal female $\times$ abnormal male

It was not necessary to carry out this experiment, because from the results of the preceding experiments no male- and female-inter-

Table 8. F_1 results from normal
female $\times$ abnormal male

Maternal No.	Paternal No.	Classification of Progeny				
		σ	+	σ	φ	— φ
K-24	17-138	5	—	—	—	21

sexual plants were expected to occur. This expectation was satisfactorily fulfilled, although the number of plants resulting from the experiment was too small.

Discussion and Conclusion

In the writer's previous investigation (1936b), it was explained that the intersexuality of male- and female-intersexual plants, in which the chromosome-complements were diploid, was caused neither by X-chromosome nor Y-chromosome, but by particular autosomes.

In the present work, Exp. I showed that paternal male-intersexual plants gave male-intersexuality to some male offspring. In the results of this experiment, the male-intersexuality was seen as determined by two autosomes; for the ratio of male to male-intersex was about 3:1: but these phenomena are not so simple, because it is doubtful whether the paternal materials have all the same chromosome-complements and whether they contain anything in addition to the chromosome-members necessary for the male-intersexuality.

In Exp. II, it was pointed out that the female-intersexual plants gave female-intersexuality to some of their female offspring as was seen in the case of the male-intersexual offspring in the male-intersex. And now it is noteworthy that the male-intersexual chromosomes are included in the female-intersexual plants.

The results obtained by Exps. I and II were still more clearly confirmed by Exps. III, IV, V, VI, and VII, and it was found that both abnormal males and abnormal females carried some of the male- and female-intersexual chromosomes.

As suggested in the writer's previous papers (1931, '36b), these male- and female-intersexual chromosomes are supposed to be transformed from normal chromosomes by irregular divisions, such as deficiency, inversion, translocation, interchange, and attachment. Yamamoto (1935) also has described a case of simple translocation of a certain autosome of *Rumex acetosa*. Jensen (1936b) stated that sex-chromosomes in *Rumex* were nothing more than abnormality resulting from the previous hybridization of the species. Nevertheless, Ono (1935) and the writer (1936b) came to a definite conclusion in the studies of *Rumex acetosa* that an X-chromosome involves a female-factor or gene and autosomes have connection with a male-factor or gene. As pointed out by Jensen (1936a), some of the general phenomena seen in hybrids were also observed karyologically by the writer (1931, 1936b).

Accordingly it is suggested that various forms of *Rumex acetosa* exist and are co-mixed in the fields. Winge (1932) stated that sex-chromosomes could be experimentally changed into autosomes. Marsden-Jones and Turrill (1935) observed six sexual types of *Ranunculus acris*, and stated that these variations were not due to the differences in the number of chromosomes. Hirata (1927) investigated female- and male-intersexual plants of hemp and stated that dioecious forms had been evolved from hermaphrodite ones. Many investigators have studied the sex of maize. For example, Jones (1934) announced that the chromosomes of normal maize always included many male- and female-genes, and that in the dioecious plants specific genes promoted one sex and suppressed the other. Akemine (1935) made a study of various sex expressions of *Coptis japonica* and observed no heteromorphic pair in them. Von Ubisch (1936) tried to breed the hermaphroditic plants in *Antennaria dioica*, although no karyological investigation was carried out, and found a certain relationship among male, intersex and female.

From what has been mentioned about *Rumex acetosa*, the writer has come to the conclusion that some of the autosomes of the male- and female-intersexual plants carried respectively the male- and female-genes; that the male-intersexual chromosomes weaken the function of autosomes (maleness) and also the female-intersexual chromosomes suppress the function of X-chromosomes (femaleness); and that the female-intersexual factors or genes are concerned with more numerous chromosomes than the male-intersexual factors or genes are. The origin of these sex-influencing chromosomes seems to be due to various irregular divisions of some autosomes. From this point of view, reversible phenomena are assumed between all hermaphroditic and dioecious plants in the vegetable kingdom, through irregular chromosome divisions or abnormal gene distributions.

Literature Cited

- Akemine, T. 1935. On the sex expression of *Coptis japonica*, Makino. Jour. Facul. Sci. Hokkaido Imp. Univ. V. 5, 1-7.
 Blackburn, K. B. 1923. Sex chromosomes in plants. Nature 112, 687-688.
 Hirata, K. 1927. Sex determination in hemp. Jour. Genet. 19, 65-79.
 Jensen, H. W. 1936a. Meiosis in *Rumex*. I. Polyploidy and the origin of new species. Cytologia 7, 1-22.
 — 1936b. Meiosis in *Rumex*. II. The origin and behaviour of the so-called sex-chromosomes in *Rumex*. Ib. 7, 23-34.
 Jones, D. F. 1934. Unisexual maize plants and their bearing on sex differentiation in other plants and animals. Genetics 19, 552-567.
 Kihara, H. and Ono, T. 1923a. Cytological studies on *Rumex*. I. Chromosomes of *Rumex acetosa*, L. Bot. Mag. (Tokyo) 37, 84-90.
 — and — 1923b. Cytological studies on *Rumex*. II. On the relation of chromosome number and sexes in *Rumex acetosa*, L. Ib. 37, 147-149.

- Kihara, H. und Yamamoto, Y. 1931. Karyomorphologische Untersuchungen an *Rumex acetosa*, und *Rumex montanus*, Desf. Cytologia 3: 48-118.
- Marsden-Jones, E. M. and Turrill, W. B. 1935. Studies in *Ranunculus*. III. Further experiments concerning sex in *Ranunculus acris*. Jour. Gent. 31, 363-378.
- Ono, T. 1928. Further investigations on the cytology of *Rumex*. I-V. Bot. Mag. (Tokyo) 42, 524-532.
- 1930a. Further investigations on the cytology of *Rumex*. IV-VIII. Ib. 44, 168-176.
- 1930b. Chromosomenmorphologie von *Rumex acetosa*. Sci. Rep. Tohoku Imp. Univ. IV. 5, 415-422.
- 1935. Chromosomen und Sexualität von *Rumex acetosa*. Ib. V. 10, 41-210.
- Santos, J. 1923. Differentiation among chromosomes in *Elodea*. Bot. Gaz. 75, 42-59.
- Satô, D. and Sinotô, Y. 1935. Chiasma studies in plants. III. Chromosome pairing and chiasma behaviour in the male *Rumex acetosa* with special reference to the tripartite sex-chromosome. Jap. Jour. Genet. 11, 219-226.
- Shimotomai, N. and Ono, T. 1923. Triploid and tetraploid intersex of *Rumex acetosa*, L. Bot. Mag. (Tokyo) 42, 266-270.
- Sinotô, Y. 1924. On chromosome behaviour and sex determination in *Rumex acetosa*, L. Bot. Mag., (Tokyo) 43, 153-162.
- 1929. Chromosome studies in some dioecious plants, with special reference to the allosomes. Cytologia 1, 109-191.
- Takenaka, Y. 1931. Further reports of cytological and genetic investigations of *Rumex acetosa*, L. I. New chromosome and chromosome-fragments. Bot. Mag. (Tokyo) 45, 475-486.
- 1935. A new remark on the sex of *Rumex acetosa*, L. Bot. & Zool. (Tokyo) 3, 2178-2179.
- 1936a. Further reports of cytological and genetic investigations of *Rumex acetosa*, L. II. Polyploid plants and those offsprings. Ib. 4, 1193-1204.
- 1936b. Further reports of cytological and genetic investigations of *Rumex acetosa*, L. III. On male- and female intersex plants. Jour. Chosen Nat. Hist. Soc. 21, 58-76.
- Von Uebisch, G. 1936. Genetic studies on the nature of hermaphroditic plants in *Antennaria dioica* (L). Gärtn. Genetics 21, 282-294.
- Winge, Ö. 1923. On sex chromosomes, sex determination and preponderance of females in some dioecious plants. C. R. Trav. Lab. Carlsberg 15, 1-25.
- 1932. The nature of sex chromosomes. Proceed. Sixth International Cong. Genetics 1, 343-355.
- Yamamoto, Y. 1932. A cyto-genetical study on *Rumex acetosa* with 3 Y-chromosomes. Kwagaku (Science) 2, 5-6.
- 1933. An autohexaploid plant of *Rumex acetosa*. Jap. Jour. Genet. 8, 2-3.
- 1934a. Karyogenetische Untersuchungen bei der Gattung *Rumex* I. Hetero- und Euploidie bei *Rumex acetosa*, L. Cytologia 5, 317-336.
- 1934b. Reifungsteilungen bei einer asynaptischen Pflanze von *Rumex acetosa*, L. Bot. & Zool. (Tokyo) 2, 1160-1168.
- 1935. Karyogenetische Untersuchungen bei der Gattung *Rumex*. II-III. Jap. Jour. Genet. 11, 6-17.
- 1936a. Karyogenetische Untersuchungen bei der Gattung *Rumex*. IV. Ein Fall von einfacher Translocation bei *Rumex acetosa* L. Cytologia 6, 407-412.
- 1936b. Über den Einfluß von Kaliumchlorat auf getrenntgeschlechtige Pflanzen. Jap. Jour. Genet. 12, 73-80.