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NON-RANDOM ASSORTMENT OF CHROMOSOME PAIRS AND

MEIOTIC DRIVE IN DROSOPHILA MELANOGASTER

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By

azuichi

Richard K. Sakai

Dissertation Committee:

Dr. Yuichiro Hiraizumi, Chairman
Dr. Chin S. Chung
Dr. Ming P. Mi
Dr. Newton E. Morton
Dr. D. Elmo Hardy

ABSTRACT

During meiosis different chromosome pairs are thought to assort quite randomly from each other; thus, an A/a; B/b (A and B refer to different chromosome pairs) chromosome type would be expected to produce four kinds of gemetes, AB, Ab, aB and ab in equal numbers. Several papers have been published reporting that in some cases the above principle was not always true (Novitski and Sandler, 1957; Grell, 1959). In all of these studies, however, the genotypes used were characterized by abnormal chromosome complements or involved chromosomes of more or less abnormal structure such as translocations or inversions. In the absence of such abnormalities it has been widely assumed that the principle of random assortment is generally true.

Recently, however, Hiraizumi and Nakazima (1967) reported that when the second chromosome of *D. melanogaster* carried segregation distorter but was free of any visible structural abnormality, it tended to assort more frequently with the Y chromosome. They also observed that the amount of non-random assortment was positively correlated with the frequency of recovery of the *SD*-bearing chromosome.

The above phenomenon was well established for the case of the *SD* system but it is possibly a unique phenomenon characteristic only of this sytem. If, however, the

phenomenon is generally true in nature, then it may be one of the important factors changing gene frequencies in populations.

The present investigation established the generality of non-random assortment between the second and sex chromosomes and confirmed the positive correlation between the segregation frequency of the second chromosome and the degree of non-random assortment. Preliminary mapping of the elements of the system was also done.

These findings are of interest because in many Drosophila experiments fitnesses of genotypes have been estimated by differential larval stage viabilities based upon the recovery frequencies among progeny flies under the assumption that the distorted segregation was due solely to the differential viabilities of the genotypes involved. With the establishment of the generality of the phenomenon of non-random assortment of chromosome pairs and the changes in segregation frequencies associated with it, many of these conclusions may have to be critically re-examined.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Background

During meiosis different chromosome pairs are thought to assort quite randomly from each other; thus, an A/a;B/b (A and B refer to different chromosome pairs) chromosome type would be expected to produce four kinds of gametes AB, Ab, aB, and ab in equal numbers. Several papers have been published reporting that in some cases the above principle was not always true (Novitski and Sandler, 1957; Grell, 1959). In all of these studies, however, the genotypes used were characterized by abnormal chromosome complements or involved chromosomes of more or less abnormal structure such as translocations or inversions. In the absence of such abnormalities it has been widely assumed that the principle of random assortment is generally true.

Recently, however, Hiraizumi and Nakazima (1967) reported that when the second chromosome of *D. melanogaster* carried segregation distorter (*SD*), the above principle did not hold: When the second chromosome carried *SD* but was free of any visible structural abnormality, it tended to assort more frequently with the Y chromosome. They also observed that the amount of non-random assortment was positively correlated with the *k* value (*k* is the frequency of recovery of the SD-bearing chromosome among the F_1 of the mating, cn bw ♀ x SD/cn bw ♂).

The above phenomenon was well established for the case of the SD system but it is possibly a unique phenomenon characteristic only of this system. If, however, the above phenomenon is generally true in nature, then it may be one of the important factors changing gene frequencies in population.

In many of the Drosophila experiments, fitnesses of genotypes have been estimated by differential larval stage viabilities based upon the recovery frequencies among progeny flies, but little attention has been paid to the possibility of non-random assortment and its effect upon segregation frequencies. Since many conclusions have been made based upon the relative segregation frequencies under the assumption that the distorted segregation was due solely to the differential viabilities of the genotypes involved and if the phenomenon of non-random assortment of chromosome pairs and the changes in segregation frequencies associated with it prove to be generally true, then many of these conclusions may have to be critically re-examined.

Purpose

The purpose of the present investigation is (1) to establish the formal genetics for the phenomenon of non-random assortment and meiotic drive (segregation frequency changes) associated with it for several typical cases and (2) to investigate whether or not the above phenomenon is observed in natural populations.

Materials: Stocks of D. melanogaster

1. Flies collected from a natural population of Odate, Japan. These samples were collected in the year 1963-1964 and since then have been kept in about 150 separate independent small culture vials.

2. cn bw. A well characterized stock carrying two recessive markers on chromosome 2: cn (cinnabar eye, 2R-57.5) and bw (brown eye, 2R-104.5). This is the standard stock used throughout the present investigation.

3. Attached-X, y f :-/Y; cn bw. A reversed acrocentric attached-X stock carrying two recessive markers, y (yellow body, 1-0.0) and f (forked bristles, 1-56.7). The background of this stock other than the X chromosome has been replaced by that of the standard cn bw stock.

4. L^2. A dominant marker (lobe eye, 2R-72.0).

5. stw^3. A recessive marker (straw body, 2R-55.1).

6. Wild-1 and wild-2.6. These are laboratory wild stocks which were originally collected from natural populations of Southern Japan more than five years ago and have been maintained in the laboratory in small culture vials.

The stw^3 stock was obtained from Dr. I. Oster of Bowling Green State University, Bowling Green, Ohio.

The remainder of the stocks utilized in the experiments are maintained in the laboratory of Dr. Y. Hiraizumi, Department of Genetics, University of Hawaii, Honolulu, Hawaii.

The formula of the culture madium used throughout the investigation was as follows:

1,000 c.c.	Water
66 g.	Sucrose
50 g.	Brewers' yeast
18 g.	Bacto-agar
6 c.c.	Propionic acid

Methods

The segregation frequency of the second chromosome was measured by *k*. The non-random assortment between the second and sex chromosome pairs was measured as follows: Let *a*, *b*, *c* and *d* be the observed numbers of *cn bw* females, *cn bw* males, *non-cn bw* females and *non-cn bw* males respectively among the F_1 progeny of the mating, *cn bw* ♀ x *+* /*cn bw* ♂. Let $\theta = E(ad/bc)$. If the segregating classes show the same sex ratio, the expected value of θ is 1. Any deviation of θ from 1 will measure the direction and amount of non-random assortment. Mi and Morton (1966) showed that the maximum likelihood score on the null hypothesis is $u_\theta = (ad - bc)/N$ where *N* is the total number of progeny flies, and that its variance is $k_\theta = (a+b)(c+d)(a+c)(b+d)/N^3$. U_θ and k_θ will be used for various statistical tests.

The values of u_θ, k_θ, $\underline{k}$ and the sex ratio for each individual culture were calculated and subjected to statistical analyses.

Some general comments

Since the phenomenon of non-random assortment was studied for the second and the sex chromosome pairs, only males are informative. Therefore in order to simplify the statements in the chapters following, in most cases unless otherwise indicated, the words "in the male" were omitted. For example the statement, "By changing the X chromosome the value of θ could be changed," should be read, "By changing the X chromosome in the male the value of θ could be changed."

The segregation frequency, $\underline{k}$, can be defined only for heterozygotes. In all cases, unless otherwise indicated, one of the second chromosomes was introduced into the male from the standard cn bw stock.

CHAPTER II

THE ANALYSIS OF stw^3

The stw^3 series

In an attempt to establish the formal genetic basis of the phenomenon of non-random assortment and the segregation frequency changes which may be associated with it, males of the following genotypes were constructed:

(1) X^{cb}/Y^{cb}; $stw^3/cn\ bw$

(2) X^{cb}/Y^{stw}; $stw^3/cn\ bw$

(3) X^{stw}/Y^{cb}; $stw^3/cn\ bw$

X^{stw}, Y^{stw}, X^{cb} and Y^{cb} are the X and Y chromosomes which came respectively from the stw^3 and the cn bw stocks (see Figure 1 for mating scheme).

One to three days old males were individually mated to two to three cn bw virgin females. Five days after the matings had been made, the parents were discarded. Fly counts were initiated on the second day of emergence and continued until all pupae had eclosed. The results of the matings involving the above three genotypes of males are summarized in Table I.

Non-random assortment between the stw^3 second and the sex chromosomes

Table II summarizes the values of u_θ and k_θ for each genotype in each generation.

FIGURE 1. MATING SCHEME FOR THE $\underline{stw}^3$ EXPERIMENT

$\underline{cn}\ \underline{bw}$ ♀♀ X $\underline{stw}^3$ ♂

$\downarrow$

$\underline{cn}\ \underline{bw}$ ♀♀ X $\frac{X^{cb}}{Y^{stw}};\frac{stw^3}{cn\ bw}$ X $\underline{yf}$:=; $\underline{cn}\ \underline{bw}$ ♀♀

$\downarrow$ $\qquad$ $\downarrow$

$\frac{X^{cb}}{Y^{stw}};\frac{stw^3}{cn\ bw}$ $\qquad$ $\frac{X^{cb}}{Y^{cb}};\frac{stw^3}{cn\ bw}$

$\frac{X^{cb}}{Y^{stw}};\frac{cn\ bw}{cn\ bw}$

$\underline{stw}^3$ ♀ X $\underline{cn}\ \underline{bw}$ ♂♂

$\downarrow$

$\underline{cn}\ \underline{bw}$ ♀♀ X $\frac{X^{stw}}{Y^{cb}};\frac{stw^3}{cn\ bw}$ X yf:=; $\underline{cn}\ \underline{bw}$ ♀♀

$\downarrow$ $\qquad$ $\downarrow$

$\frac{X^{cb}}{Y^{cb}};\frac{stw^3}{cn\ bw}$ $\qquad$ $\frac{X^{stw}}{Y^{cb}};\frac{stw^3}{cn\ bw}$

$\frac{X^{stw}}{Y^{cb}};\frac{cn\ bw}{cn\ bw}$

TABLE I. RESULTS OF MATINGS INVOLVING THE THREE GENOTYPES OF $\underline{stw}^3$ MALES

Genotype	Number of males tested	cn bw ♀	cn bw ♂	Total cn bw	non cn bw ♀	non cn bw ♂	Total non cn bw	Total
$\frac{X^{cb}}{Y^{cb}}; \frac{stw^3}{cn\ bw}$	261	6,317	6,345	12,662	6,848	6,856	13,704	26,366
$\frac{X^{cb}}{Y^{stw}}; \frac{stw^3}{cn\ bw}$	311	7,031	7,029	14,060	7,662	8,006	15,668	29,728
$\frac{X^{stw}}{Y^{cb}}; \frac{stw}{cn\ bw}$	319	6,768	6,282	13,050	7,591	7,681	15,272	28,322
TOTAL	891	20,116	19,656	39,772	22,101	22,543	44,644	84,416

TABLE II. SUMMARY OF U_θ AND K_θ FOR THE THREE GENOTYPES OF stw^3 MALES

	$\frac{X^{cb}}{Y^{cb}}; \frac{stw^3}{cn\ bw}$			$\frac{X^{cb}}{Y^{stw}}; \frac{stw^3}{cn\ bw}$			$\frac{X^{stw}}{Y^{cb}}; \frac{stw^3}{cn\ bw}$		
Generation*	U_θ	K_θ	n**	U_θ	K_θ	n	U_θ	K_θ	n
1	--	--	--	17.02	484.75	86	51.21	936.70	151
2	-42.53	863.21	164	56.58	516.51	99	48.56	331.10	87
3	41.27	752.92	97	-3.80	47.90	9	--	--	--
4	--	--	--	12.30	160.94	35	17.46	163.15	33
5	--	--	--	1.31	603.99	82	38.21	282.45	48
TOTAL	1.26	1616.13	261	83.41	1814.09	311	155.44	1713.40	319

*Number of generations of backcrosses to the cn bw or the attached X; y f: = /Y; cn bw stocks.

**Number of males tested.

About ten flies from the stw^3 stock were used to construct each of the above three genotypes, but since stw^3 is a relatively infertile stock and has been kept as a very small population, all of the samples would be expected to be reasonably homogeneous. Furthermore, in all three genotypes, the distribution of θ among lines within generation was homogeneous ($p > 0.20$ for all three genotypes).

For the genotype X^{cb}/Y^{cb}; stw^3/cn bw, θ was heterogeneous between the two backcrossed generations but the level of significance was low ($\chi^2 = 4.36$; d.f. = 1; $0.05 > p > 0.02$). The other two genotypes showed homogeneous distributions of θ among generations ($\chi^2 = 4.21$; d.f. = 4; $p > 0.30$ for the genotype X^{cb}/Y^{stw}; stw^3/cn bw and $\chi^2 = 2.86$; d.f. = 3; $p > 0.30$ for the genotype X^{stw}/Y^{cb}; stw^3/cn bw). Generally speaking it appears that the value of θ within each genotype remained constant throughout the backcrossed generations.

The overall θ for the genotype X^{cb}/Y^{cb}; stw^3/cn bw did not deviate significantly from the one expected on the null hypothesis of $\theta = 1$ ($\chi^2 = 0.001$; d.f. = 1; $p > 0.95$), but it was significantly and highly significantly larger than one for the genotypes X^{cb}/Y^{stw}; stw^3/cn bw and X^{stw}/Y^{cb}; stw^3/cn bw respectively ($\chi^2 = 3.84$; d.f. = 1; $p = 0.005$ for the former genotype and $\chi^2 = 14.10$; d.f. = 1; $p < 0.005$ for the latter genotype).

The difference between the genotypes X^{cb}/Y^{cb}; stw^3/cn bw and X^{cb}/Y^{stw}; stw^3/cn bw was not significant (χ^2 =

1.30; d.f. = 1; $p > 0.20$) but it was highly significant between the genotypes X^{cb}/Y^{cb}; stw^3/cn bw and X^{stw}/Y^{cb}; stw^3/cn bw ($\chi^2 = 6.73$; d.f. = 1; $p < 0.01$). This suggests that the X^{stw} chromosome was of greater consequence than Y^{stw} in increasing θ.

Based upon these results, the following conclusions may be drawn:

1. The $\underline{stw}^3$ second chromosome tends to assort more often with the Y chromosome. By changing the X and probably the Y chromosomes the amount of non-randomness is also changed.

2. X^{stw} causes a greater increase in the value of θ than does Y^{stw}.

The segregation frequency of the $\underline{stw}^3$ second chromosome

Table III summarizes the segregation frequency of the $\underline{stw}^3$ second chromosome for each genotype in each generation. Hereafter the symbol, *k*, will be used to denote the proportion of $\underline{stw}^3$ second chromosomes recovered among the progeny of the mating, cn bw ♀ x $\underline{stw}^3$/cn bw ♂.

In the genotype X^{cb}/Y^{cb}; stw^3/cn bw the *k* value was different between the two backcrossed generations, but the level of significance was low ($F = 3.89$; $\nu_1 = 1$, $\nu_2 = 259$; $p = 0.05$). In the other two genotypes the *k* values were homogeneous among generations ($F = 1.92$, $\nu_1 = 4$, $\nu_2 = 306$, $p > 0.05$ for the genotype X^{cb}/Y^{stw}; stw^3/cn bw and $F = 1.23$,

TABLE III. SUMMARY OF THE SEGREGATION FREQUENCY (k) OF THE $\underline{stw}^3$ SECOND CHROMOSOME

	$\frac{X^{cb}}{Y^{cb}}$; $\frac{stw^3}{cn\ bw}$				$\frac{X^{cb}}{Y^{stw}}$; $\frac{stw^3}{cn\ bw}$				$\frac{X^{stw}}{Y^{cb}}$; $\frac{stw^3}{cn\ bw}$			
Generation	n	Σk	Σk^2	$\bar{k}$*	n	Σk	Σk^2	$\bar{k}$	n	Σk	Σk^2	$\bar{k}$
1	--	--	--	--	86	46.47	25.39	0.540	151	81.22	44.14	0.538
2	164	86.20	45.95	0.526	99	52.33	27.92	0.528	87	46.74	25.66	0.537
3	97	49.58	25.64	0.511	9	4.61	2.38	0.512	--	--	--	--
4	--	--	--	--	35	18.99	10.44	0.542	33	17.69	9.65	0.536
5	--	--	--	--	82	42.84	22.49	0.522	48	26.78	15.12	0.558
TOTAL	261	135.78	71.59	0.520	311	165.24	88.62	0.531	319	172.43	94.57	0.540

*Unweighted average k value.

$\nu_1 = 3$, $\nu_2 = 315$, $p > 0.05$ for the genotype X^{stw}/Y^{cb}; stw^3/ cn bw). There could be some difference in the *k* values among the backcrossed generations but such differences appear to be rather small; thus, it may not be too unreasonable to consider the *k* values to be homogeneous. (It should be noted here that the genotype X^{cb}/Y^{cb}; stw^3/ cn bw showed heterogeneity in both θ and *k* among generations, although the level of significance was low.)

The differences in the average *k* values among the three genotypes was highly significant ($F = 8.43$, $\nu_1 = 2$, $\nu_2 = 888$, $p < 0.001$). The differences of the average *k* values between the genotypes X^{cb}/Y^{cb}; stw^3/cn bw and X^{cb}/Y^{stw}; stw^3/cn bw and between X^{cb}/Y^{cb}; stw^3/cn bw and X^{stw}/Y^{cb}; stw^3/cn bw were both highly significant.

Based upon the results described above, the following conclusions may be drawn:

1. The segregation frequency of the $\underline{stw}^3$ second chromosome can be changed by changing the X and the Y chromosome.

2. The X^{stw} chromosome has a greater effect in increasing the *k* value than does the Y^{stw} chromosome.

Overall sex ratio

Table IV summarizes the overall sex ratio for each genotype in each generation. In this table two additional genotypes, X^{cb}/Y^{stw}; cn bw/cn bw and X^{stw}/Y^{cb}; cn bw/cn bw are included.

TABLE IV. SUMMARY OF THE OVERALL SEX RATIO (SR) FOR THE FIVE GENOTYPES OF stw^3 MALES

	$\frac{X^{cb}}{Y^{cb}}; \frac{stw^3}{cn\ bw}$				$\frac{X^{cb}}{Y^{stw}}; \frac{stw^3}{cn\ bw}$				$\frac{X^{stw}}{Y^{cb}}; \frac{stw^3}{cn\ bw}$			
Generation	n	ΣSR	$\Sigma (SR)^2$	$\overline{SR}$	n	ΣSR	$\Sigma (SR)^2$	$\overline{SR}$	n	ΣSR	$\Sigma (SR)^2$	$\overline{SR}$
1	--	--	--	--	86	43.82	22.59	0.510	151	75.42	38.07	0.499
2	164	82.17	41.75	0.501	99	49.26	24.81	0.498	87	41.92	20.56	0.482
3	97	48.57	24.58	0.501	9	4.26	2.03	0.473	--	--	--	--
4	--	--	--	--	35	17.50	8.86	0.500	33	15.72	7.60	0.476
5	--	--	--	--	82	41.96	21.68	0.512	48	23.59	11.73	0.492
TOTAL	261	130.74	66.33	0.501	311	156.80	79.97	0.504	319	156.65	77.96	0.491

TABLE IV. (Continued) SUMMARY OF THE OVERALL SEX RATIO (SR) FOR THE FIVE GENOTYPES OF $\underline{stw}^{3}$ MALES

	$\frac{X^{cb}}{Y^{stw}};\ \frac{cn\ bw}{cn\ bw}$				$\frac{X^{stw}}{Y^{cb}};\ \frac{cn\ bw}{cn\ bw}$			
Generation	n	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$	n	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$
1	--	--	--	--	--	--	--	--
2	49	23.37	11.23	0.477	105	49.53	23.77	0.472
3	26	12.77	6.31	0.491	--	--	--	--
4	--	--	--	--	--	--	--	--
5	--	--	--	--	52	23.43	10.76	0.450
TOTAL	75	36.14	17.54	0.482	157	72.96	34.53	0.465

The overall sex ratio among generations was significantly heterogeneous in the genotype X^{stw}/Y^{cb}; stw^3/cn bw ($F = 2.72$, $\nu_1 = 3$, $\nu_2 = 315$, $p < 0.05$). There was a general decrease in the overall sex ratio with increasing numbers of generations of backcrossing to the standard cn bw stock. Note that the biggest decrease is found between the first and the second backcross generations. No significant heterogeneity among generations was found in the genotypes X^{cb}/Y^{stw}; stw^3/cn bw and X^{cb}/Y^{cb}; stw^3/cn bw ($F = 1.72$, $\nu_1 = 4$, $\nu_2 = 306$, $p > 0.05$ for the former genotype and $F = 0.00$, $\nu_1 = 1$, $\nu_2 = 259$, $p > 0.05$ for the latter).

The genotypes X^{cb}/Y^{stw}; cn bw/cn bw and X^{stw}/Y^{cb}; cn bw/cn bw are of special interest since these provide information about the effects of X^{stw} and Y^{stw} alone upon the overall sex ratio. In the genotype X^{cb}/Y^{stw}; cn bw/ cn bw the sex ratio remained the same among generations ($F = 1.67$, $\nu_1 = 1$, $\nu_2 = 73$, $p > 0.05$) and was in good agreement with the standard sex ratio of 49.2.

On the other hand the genotype X^{stw}/Y^{cb}; cn bw/ cn bw showed a significantly heterogeneous distribution of sex ratios between generations ($F = 4.10$, $\nu_1 = 1$, $\nu_2 = 155$, $0.05 > p > 0.01$). There was a decrease in the overall sex ratio as backcrossing to the standard cn bw stock proceeded. Clearly this genotype gave a lower sex ratio than the standard of 49.2.

The sex ratio change among generations was significant for the genotypes X^{stw}/Y^{cb}; stw^3/cn bw and X^{stw}/Y^{cb};

cn bw/cn bw, but no change was detected for the other three types. It should be noted that both genotypes with significant sex ratio changes carried X^{stw}.

Since the genotypes X^{cb}/Y^{cb}; stw^3/cn bw and X^{cb}/Y^{stw}; stw^3/cn bw showed homogeneous sex ratio distributions among generations, the data were pooled for each genotype. This pooled data showed no significant difference in sex ratio between the two genotypes ($F = 0.00$, $\nu_1 = 1$, $\nu_2 = 570$, $p > 0.05$). This indicates that the Y^{stw} chromosome has little effect upon the overall sex ratio. If the above data are pooled and the sex ratio is compared with that of the standard <u>cn</u> <u>bw</u> stock, the difference was significant ($F = 3.57$, $\nu_1 = 1$, $\nu_2 = 705$, $p \simeq 0.05$). This suggests that the <u>stw</u>^{3} second chromosome increases the overall sex ratio in the next generation.

A plausible explanation of the decrease in the overall sex ratio of the genotypes X^{stw}/Y^{cb}; stw^3/cn bw and X^{stw}/Y^{cb}; cn bw/cn bw as backcrossing proceeds may be stated as follows: The X^{stw} chromosome by a meiotic mechanism causes a reduction in the overall sex ratio. There is an element on either the third or fourth chromosome in the <u>stw</u>^{3} stock which suppresses the action of the X^{stw} chromosome. As backcrossing proceeds the relative frequency of such an element will be decreased and concomitant with it the sex ratio will also be decreased. A constant sex ratio value will be attained when the genetic background has been completely replaced by that of the standard cn bw stock. Moreover, the larger decreases would be expected to occur during the earlier generations.

The reduction in the overall sex ratio in the genotypetypes with the X^{stw} chromosome could, of course, be explained by zygotic selection: Since the F_1 females from the mating X^{cb}/X^{cb} ♀ x X^{stw}/Y^{cb} ♂ would be heterozygous X^{stw}/X^{cb} while those for the mating X^{cb}/X^{cb} ♀ x X^{cb}/Y^{cb} ♂ would be homozygous X^{cb}/X^{cb}, the former mating may result in a greater proportion of females in the F_1 generation.

Zygotic selection alone, however, cannot explain the generation-dependent decrease in sex ratio. If we assume that the reduced sex ratio was in fact due to zygotic selection, then to explain the generation-dependent decrease there would have had to have been an autosomal element in the stw^3 stock which caused an increase in fitness among males only in the presence of the X^{stw} chromosome. Whatever the actual situation, there had to be an autosomal element which interacts specifically with the X^{stw} chromosome.

From the above it may be concluded that:

1. The stw^3 second chromosome tends to increase the overall sex ratio in the progeny generation.

2. The X^{stw} chromosome tends to reduce the overall sex ratio while the Y^{stw} chromosome is of little consequence.

3. There is in the stw^3 stock an element on the third or fourth chromosome which interacts specifically with the X^{stw} chromosome to change the overall sex ratio.

The relationship between θ and k

Hiraizumi and Nakazima (1967) observed that the segregation frequency (k) of the SD-bearing second chromosome was positively correlated with the amount of non-random assortment between the second and sex chromosomes. Therefore, it is interesting to examine this situation in the present investigation.

The average values of θ, k and the overall sex ratio for the three genotypes are listed in Table VA.

TABLE VA. SUMMARY OF THE AVERAGE VALUES OF θ, k AND SR FOR THE THREE stw^3 GENOTYPES

	$\frac{X^{cb}}{Y^{cb}}; \frac{stw^3}{cn\ bw}$	$\frac{X^{cb}}{Y^{stw}}; \frac{stw^3}{cn\ bw}$	$\frac{X^{stw}}{Y^{cb}}; \frac{stw^3}{cn\ bw}$
θ	1.001 ± 0.025	1.046 ± 0.024	1.091 ± 0.024
k	0.520 ± 0.004	0.531 ± 0.003	0.540 ± 0.004
SR	0.501 ± 0.003	0.504 ± 0.003	0.491 ± 0.003

Clearly there is a relationship between θ and the k values: When a genotype had a larger value of θ, it also showed a larger value of k. This is similar to the situation discovered in the SD system, suggesting that a similar mechanism may be operating in both systems.

The above relationship could also be examined within each genotype. The results are summarized in Table VB. In each genotype the tested males were grouped into several

TABLE VB. RELATIONSHIP BETWEEN k AND θ WITHIN GENOTYPE

	$\frac{X^{cb}}{Y^{cb}}; \frac{stw^3}{cn\ bw}$		$\frac{X^{cb}}{Y^{stw}}; \frac{stw^3}{cn\ bw}$		$\frac{X^{stw}}{Y^{cb}}; \frac{stw^3}{cn\ bw}$	
k interval	n	E(θ)	n	E(θ)	n	E(θ)
$k \geq 0.65$	7	0.984	8	1.066	15	1.154
$0.65 > k \geq 0.60$	21	1.045	34	1.024	42	1.210
$0.60 > k \geq 0.55$	42	1.016	78	1.018	95	1.140
$0.55 > k \geq 0.50$	107	1.020	119	1.065	96	1.026
$0.50 > k$	84	0.960	72	1.048	71	1.028

classes according to their _k_ values. A positive correlation was seen in the genotype X^{stw}/Y^{cb}; stw^3/cn bw (the regression coefficient was significantly larger than zero at the 5% level) with some indication, although not statistically significant, of a positive correlation in the genotype X^{cb}/Y^{stw}; stw^3/cn bw but none at all for the genotype X^{cb}/Y^{cb}; stw^3/cn bw.

The following conclusions may be drawn from the above analyses:

1. A genotype which is characterized by a larger value of θ also shows a larger value of _k_.

2. The effects of the elements determining the values of θ and _k_ are most pronounced when X^{stw} is present, somewhat weaker for Y^{stw} and are undetectable when both the X and Y chromosomes come from the standard _cn bw_ stock.

3. The similarity between the SD and the stw^3 systems in the relationship between θ and k suggests that a similar mechanism may underlie both systems.

The X^{stw}/Y^{stw} genotypes

Males of the genotype X^{cb}/Y^{stw}; cn bw were taken from the sixth generation of backcross to the standard cn bw stock and were mated with females from the original stw^3 stock. From the F1 generation of this mating two types of males, X^{stw}/Y^{stw}; stw^3/cn bw and X^{stw}/Y^{stw}; stw^3/stw^3, were collected and were individually mated with cn bw virgins. The genetic background other than the second and sex chromosomes is equivalent therefore to that of the first generation of backcrossing. The results are summarized in Table VI.

In the genotype X^{stw}/Y^{stw}; stw^3/cn bw, θ was homogeneous among cultures and the overall average θ was significantly larger than one (χ^2 = 15.25, d.f. = 1, 0.001 > p). The estimation of θ for the genotypes X^{cb}/Y^{stw}; stw^3/cn bw and X^{stw}/Y^{cb}; stw^3/cn bw were 1.046 and 1.091 respectively. The genotype X^{stw}/Y^{stw}; stw^3/cn bw gave the largest estimate of θ (1.212). The distribution of θ among the above three genotypes was significantly heterogeneous (χ^2 = 8.22, d.f. = 2, 0.02 > p > 0.01). Thus, the effects of the X^{stw} and the Y^{stw} chromosomes appear to be cumulative. The average k value for the genotype X^{stw}/Y^{stw}; stw^3/cn bw (0.536) was close to that of the genotype X^{stw}/Y^{cb}; stw^3/

TABLE VI. SUMMARY OF θ, k AND SR FOR THE $\frac{X^{stw}}{Y^{stw}}$ MALES

Genotype	n	U_θ	K_θ	Σk	Σk^2	$\bar{k}$	ΣSR	$\Sigma (SR)^2$	$\overline{SR}$
$\frac{X^{stw}}{Y^{stw}};\frac{stw^3}{cn\ bw}$	62	71.78	337.84	33.21	17.95	0.536	30.82	15.56	0.497
$\frac{X^{stw}}{Y^{stw}};\frac{stw^3}{stw^3}$	24	--	--	--	--	--	12.38	6.48	0.516

cn bw (0.540) and the difference between them was not significant. A larger body of data for the genotype X^{stw}/Y^{stw}; stw^3/cn bw appears necessary for a more critical study of the *k* value.

Since the overall sex ratio in males carrying the X^{stw} chromosome was found to be dependent upon the number of generations of backcrossing to the standard *cn bw* stock, only data from the first backcross generation for the genotypes X^{cb}/Y^{stw}; stw^3/cn bw and X^{stw}/Y^{cb}; stw^3/cn bw were taken for comparison with that of the genotype X^{stw}/Y^{stw}; stw^3/cn bw. The sex ratios for the three genotypes were respectively 0.510, 0.499 and 0.500. The differences in sex ratio among the three genotypes were not significant. It may be noted that the genotypes X^{stw}/Y^{cb}; stw^3/cn bw and X^{stw}/Y^{stw}; stw^3/cn bw showed nearly the same sex ratio in agreement with the conclusion that the Y^{stw} chromosome caused little change in the overall sex ratio.

The genotype X^{stw}/Y^{stw}; cn bw/cn bw is informative only for the overall sex ratio. The comparison of the overall sex ratio between the genotypes X^{stw}/Y^{stw}; stw^3/cn bw and X^{stw}/Y^{stw}; stw^3/stw^3 provides information on the effect of the *stw^3* second chromosome in the homozygous state. The overall sex ratio (0.514) of the genotype X^{stw}/Y^{stw}; stw^3/stw^3 is larger than that of the genotype X^{stw}/Y^{stw}; stw^3/cn bw but the difference is not significant ($F = 1.46$, $\nu_1 = 1$, $\nu_2 = 84$, $p > 0.05$). The sex ratio of 0.514 is very close to that of the *stw*^{3} males from the stock culture. The original *stw*^{3} male has the same sex and second chromosome pairs as those males of the genotype X^{stw}/Y^{stw}; stw^3/stw^3 and this suggests that the increase in the sex ratio observed in the latter genotype is real. Further accumulation of data seems necessary to clarify this point.

1. The effects of the X^{stw} and the Y^{stw} chromosomes with respect to θ appear to be cumulative.

2. It appears likely that a double dose (homozygosity) of the *stw*^{3} second chromosome causes a greater increase in the overall sex ratio than a single dose.

Conclusions from the *stw*^{3} experiment

1. The *stw*^{3} second chromosome tends to assort more frequently with the Y chromosome. The degree of this non-randomness can be changed by changing either the X or the Y chromosome.

2. By changing the X or the Y chromosome the segregation frequency of the *stw*^{3} second chromosome can be changed.

3. The amount of increase in θ (the measure of the amount of non-random assortment) is positively correlated with the increase in the *k* value (the segregation frequency of the *stw*^{3} second chromosome) not only among genotypes but also within genotypes.

4. The *stw*^{3} second chromosome increased the overall sex ratio in the progeny generation. The amount of this increase is probably greater for males homozygous for the *stw*^{3} second chromosome than for those heterozygous.

5. The X^{stw} chromosome caused a reduction in the overall sex ratio in the progeny generation while Y^{stw} had little effect.

6. The overall sex ratio is independent of the values of θ and *k*.

7. There appears to be an element on either the third or the fourth chromosome in the *stw*^{3} stock which suppresses the effect of the X^{stw} chromosome upon the overall sex ratio.

CHAPTER III

THE ANALYSIS OF $\underline{L}^2$

The $\underline{L}^2$ series

This series is essentially a repetition of the $\underline{stw}^3$ experiment and therefore only brief descriptions will be given below.

The principle genotypes used were as follows:

(1A) X^{cb}/Y^{cb}; L^2/cn bw

(1B) X^{cb}/Y^{cb}; L^2/cn bw

(2) X^{cb}/Y^{L}; L^2/cn bw

(3) X^{L}/Y^{cb}; L^2/cn bw

The (1B) genotype was obtained by more than fifty generations of backcrossing using the following scheme: cn bw ♀ x X^{cb}/Y^{cb}; L^2/cn bw ♂. The mating schemes used to obtain the other three genotypes were similar to those presented in Figure 1. A brief summary of results for the above genotypes are found in Table VII.

Non-random assortment between the $\underline{L}^2$ second and sex chromosomes

Table VIII summarizes the values of u_θ and k_θ for each genotype in the backcrossed generations.

In all three genotypes, θ was homogeneous within genotype within generation. As in the case of the $\underline{stw}^3$

TABLE VII. RESULTS OF MATINGS INVOLVING THE THREE GENOTYPES OF L^2 MALES

Genotype	n	cn bw ♀	cn bw ♂	Total cn bw	L^2 ♀	L^2 ♂	Total L^2	Total
$\frac{X^{cb}}{Y^{cb}};\frac{L^2}{cn\ bw}$	139	3,368	3,127	6,495	3,180	3,274	6,454	12,949
$\frac{X^{cb}}{Y^{L}};\frac{L^2}{cn\ bw}$	119	2,443	2,513	4,956	2,591	2,433	5,024	9,980
$\frac{X^{L}}{Y^{cb}};\frac{L^2}{cn\ bw}$	103	1,118	1,051	2,169	1,050	995	2,045	4,214
TOTAL	361	6,929	6,691	13,620	6,821	6,702	13,523	27,143

TABLE VIII. SUMMARY OF U_θ AND K_θ FOR THE THREE GENOTYPES OF L^2 MALES

	$\frac{X^{cb}}{Y^{cb}}; \frac{L^2}{cn\ bw}$			$\frac{X^{cb}}{Y^{L}}; \frac{L^2}{cn\ bw}$			$\frac{X^{L}}{Y^{cb}}; \frac{L^2}{cn\ bw}$		
Generation	n	U_θ	K_θ	n	U_θ	K_θ	n	U_θ	K_θ
1	--	--	--	31	-42.05	137.12	19	-2.98	84.32
2	26	19.78	200.75	42	-1.59	204.87	35	5.76	172.76
3	20	8.32	83.36	46	-16.54	281.29	--	--	--
∞*	93	65.23	506.50	--	--	--	--	--	--
TOTAL	139	93.33	790.61	119	-60.18	623.28	54	2.78	257.08

*Stock backcrossed through males to the standard cn bw females for more than 50 generations.

experiment, θ was nearly homogeneous among backcross generations, although the genotype X^{cb}/Y^{L}; L^2/cn bw showed significant, but not highly significant, heterogeneity. Furthermore since there was no difference in θ between the (1A) and the (1B) genotypes, they were pooled for the analysis. Hereafter this pooled body of data will be identified as X^{cb}/Y^{cb}; L^2/cn bw.

θ in the genotype X^{cb}/Y^{cb}; L^2/cn bw was highly significantly larger than one ($\chi^2 = 11.02$, d.f. = 1, $0.001 > p$) while that of the genotype X^{L}/Y^{cb}; L^2/cn bw showed no significant deviation from one ($\chi^2 = 0.030$, d.f. = 1, $0.90 > p > 0.75$). It is interesting that the genotype X^{cb}/Y^{L}; L^2/cn bw showed a significantly <u>smaller</u> value of θ than one (χ^2 = 5.81, d.f. = 1, $0.02 > p > 0.01$).

The distribution of θ among the three genotypes was heterogeneous (χ^2 = 16.09, d.f. = 2, $0.001 > p$). The difference in the value of θ between the genotypes X^{cb}/Y^{cb}; L^2/cn bw and X^L/Y^{cb}; L^2/cn bw was not significant (χ^2 = 2.23, d.f. = 1, $0.20 > p > 0.10$) but it was highly significant between the genotypes X^{cb}/Y^{cb}; L^2/cn bw and X^{cb}/Y^L; L^2/cn bw (χ^2 = 9.06, d.f. = 1, $0.01 > p > 0.001$). Thus the Y^L chromosome is able to cause a great change in the value of θ; indeed, by replacing the Y^{cb} with the Y^L chromosome, even the direction of non-random assortment can be changed. The X^L chromosome on the other hand appears to have little effect upon the value of θ.

The conclusions which may be drawn in this section are as follows:

1. The $\underline{L}^2$ second chromosome tends to assort more frequently with the Y^{cb} chromosome.

2. When the Y^{cb} chromosome is replaced with Y^L the above tendency is completely reversed: the $\underline{L}^2$ second chromosome tends to separate from the Y chromosome.

3. The X^L chromosome has little effect upon the value of θ.

<u>The segregation frequency of the $\underline{L}^2$ second chromosome</u>

Data for the *k* values are summarized in Table IX.

TABLE IX. SUMMARY OF $\underline{k}$ VALUES FOR THE THREE GENOTYPES OF $\underline{L}^2$ MALES

	$\frac{X^{cb}}{Y^{cb}};\ \frac{L^2}{cn\ bw}$				$\frac{X^{cb}}{Y^{L}};\ \frac{L^2}{cn\ bw}$				$\frac{X^{L}}{Y^{cb}};\ \frac{L^2}{cn\ bw}$			
Generation	n	Σk	Σk^2	$\bar{k}$	n	Σk	Σk^2	$\bar{k}$	n	Σk	Σk^2	$\bar{k}$
1	--	--	--	--	31	16.49	9.00	0.532	19	9.64	4.95	0.507
2	26	12.59	6.17	0.484	42	20.95	10.57	0.499	35	16.72	8.09	0.477
3	20	10.92	6.07	0.546	46	23.31	11.96	0.507	--	--	--	--
∞	93	46.76	23.84	0.503	--	--	--	--	--	--	--	--
TOTAL	139	70.27	36.08	0.506	119	60.75	31.53	0.510	54	26.36	13.04	0.488

The distribution of k among generations was highly significant in the genotype X^{cb}/Y^{cb}; L^2/cn bw ($F = 5.79$, $\nu_1 = 2$, $\nu_2 = 136$, $0.01 > p$), and although not significant, the other two genotypes also suggested heterogeneity ($F = 2.32$, $\nu_1 = 2$, $\nu_2 = 116$, $p = 0.10$ for the genotype X^{cb}/Y^L; L^2/cn bw; $F = 3.23$, $\nu_1 = 1$, $\nu_2 = 52$, $0.10 > p > 0.05$ for the genotype X^L/Y^{cb}; L^2/cn bw).

The heterogeneity of the k values among generations is due principally to the second backcross generation which showed a generally lower value of k. Since all three genotypes were tested at the same time, some environmental condition such as temperature during that period may be the cause. Since the k values differed among generations, comparisons among genotypes were done within the same backcross generations.

The second and third generations are common to the genotypes X^{cb}/Y^{cb}; L^2/cn bw and X^L/Y^{cb}; L^2/cn bw. Upon comparison no significant difference was detected between these two genotypes. A similar comparison was also made between the genotypes X^{cb}/Y^{cb}; L^2/cn bw and X^{cb}/Y^L; L^2/cn bw for the first and the second generations and again there was no significant difference.

The second generation was common to all three genotypes and the k values were homogeneous among the genotypes within this generation ($F = 1.38$, $\nu_1 = 2$, $\nu_2 = 100$, $p > 0.25$).

The lobe eye phenotype causes a significantly large reduction in viability (L^2 is dominant) and is quite sensitive to environmental conditions. This may be the reason why k was heterogeneous among generations. Thus the information obtained about the value of k in the lobe experiment is inconclusive.

The overall sex ratio

The results are summarized in Table X.

There was no heterogeneity among backcross generations in the overall sex ratio for all of the three genotypes ($F = 0.00$, $\nu_1 = 1$, $\nu_2 = 44$, $p > 0.05$ for the genotype X^{cb}/Y^{cb}; L^2/cn bw; $F = 0.01$, $\nu_1 = 1$, $\nu_2 = 54$, $p > 0.05$ for the genotype X^{cb}/Y^L; L^2/cn bw; $F = 0.01$, $\nu_1 = 1$, $\nu_2 = 54$, $p > 0.05$ for the genotype X^L/Y^{cb}; L^2/cn bw), and no significant hetergeneity among genotypes ($F = 1.44$, $\nu_1 = 2$, $\nu_2 = 217$, $p > 0.05$). Therefore the three genotypes were pooled for the comparison with the sex ratio of the standard cn bw stock. The sex ratio for the pooled data (0.497) appeared to be slightly larger than that for the cn bw stock (0.492), but the difference was not statistically significant.

It may be mentioned here that the sex ratio of the genotype (1B), the backcrossed L^2 line, was nearly significantly lower than that of the comparable (1A) genotype ($F = 3.20$, $\nu_1 = 1$, $\nu_2 = 137$, $0.10 > p > 0.05$). Since the (1B) stock was established more than fifty generations ago and has been kept isolated from the original L^2 stock, the

TABLE X. SUMMARY OF THE OVERALL SEX RATIO FOR THE THREE GENOTYPES OF $\underline{L}^2$ MALES

	$\frac{X^{cb}}{Y^{cb}}; \frac{L^2}{cn\ bw}$				$\frac{X^{cb}}{Y^{L}}; \frac{L^2}{cn\ bw}$				$\frac{X^{L}}{Y^{cb}}; \frac{L^2}{cn\ bw}$			
Generation	n	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$	n	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$	n	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$
1	--	--	--	--	31	15.50	8.03	0.500	19	9.12	4.44	0.480
2	26	13.17	6.72	0.506	42	20.95	10.70	0.499	35	17.09	8.40	0.488
3	20	10.11	5.16	0.506	46	22.88	11.53	0.497	--	--	--	--
∞	93	45.59	22.60	0.490	--	--	--	--	--	--	--	--
TOTAL	139	68.87	34.48	0.495	119	59.33	30.26	0.498	54	26.21	12.84	0.485

difference in the sex ratio between them could be due to the difference in the $\underline{L}^2$ second chromosome itself, but because θ was homogeneous between them, it appears more likely that the $\underline{L}^2$ stock carried an autosomal element causing an increase in the overall sex ratio.

The conclusions which may be drawn from the above data are as follows:

1. The $\underline{L}^2$ second chromosome by itself does not cause an increase in the overall sex ratio.

2. The X^L and Y^L chromosomes have no apparent effect upon the overall sex ratio.

3. There is some evidence that the $\underline{L}^2$ stock contained an element on the third or fourth chromosome which causes an increase in the overall sex ratio.

<u>The X^L/Y^L genotypes</u>

Only a small number of males of the genotypes X^L/Y^L; L^2/cn bw and X^L/Y^L; L^2/L^2 were tested. The results are summarized in Table XI.

TABLE XI. SUMMARY OF U_θ, K_θ, $\underline{k}$ AND $\underline{SR}$ FOR THE TWO $\frac{X^L}{Y^L}$ GENOTYPES

Genotype	n	U_θ	K_θ	Σk	Σk^2	$\bar{k}$	ΣSR	$\Sigma (SR)^2$	$\overline{SR}$
$\frac{X^L}{Y^L}; \frac{L^2}{\text{cn bw}}$	8	1.68	42.24	3.79	1.86	0.474	3.81	1.86	0.476
$\frac{X^L}{Y^L}; \frac{L^2}{L^2}$	7	--	--	--	--	--	3.65	1.92	0.521

None of the comparisons similar to those done for the stw^3 series gave significant deviations nor significant heterogeneity. However, it is interesting to note that the genotype X^L/Y^L; L^2/L^2 showed a higher sex ratio than the genotype X^L/Y^L; L^2/cn bw. This suggests that males homozygous for the L^2 second chromosome give a higher sex ratio than do the heterozygous males (this is similar to the case of the stw^3 second chromosome). It may also be mentioned that the genotype X^L/Y^L; L^2/cn bw gave a value of θ greater than one, suggesting that in the presence of X^L the action of Y^L is suppressed. Additional data seem necessary for a more critical examination of the above phenomena.

It should be mentioned here that the effect of the Y^L chromosome rules out the possibility that the cn bw second chromosome is a special chromosome in that it always tends to separate from the Y chromosome for in the X^{cb}/Y^L; L^2/cn bw male this chromosome assorted more frequently with the Y^L chromosome.

Summary of the principle conclusions from the L^2 experiment

1. The L^2 second chromosome in the cn bw background tends to assort more often with the Y^{cb} chromosome.

2. The presence of the Y^L chromosome reverses the above situation.

3. There is some suggestive evidence that males homozygous for the L^2 second chromosome give a higher sex

ratio than either the $\underline{L}^2$ heterozygous males or the standard <u>cn</u> <u>bw</u> males.

4. The overall sex ratio is independent of θ.

CHAPTER IV

FACTORS AFFECTING NON-RANDOM ASSORTMENT

Background

Hiraizumi and Nakazima (1967) have shown that the element causing non-random assortment between the second and sex chromosome was the SD locus itself. They also showed that the two other elements in the SD system, Ac(SD) which is located at the centromeric region and St(SD) at the tip of 2R, caused an increase in the overall sex ratio but caused no detectable non-random assortment between the second and sex chromosomes.

Recently, Kataoka (1967) showed that a major suppressor for the SD action was located in the middle of the X chromosome. She also found indications of other modifying elements at the tip and base of the X chromosome. Her findings are valuable in that they suggest the possibility that the elements causing non-randomness could be distributed in several places along the X chromosome. On the second chromosome the only element causing non-randomness discovered thus far is SD. Therefore the location of the elements causing non-random assortment in the present material is of some interest.

In an attempt to map these elements females of the genotype X^{cb}/Y^{cb}; L^{2}/cn bw or X^{cb}/Y^{cb}; stw^{3}/cn bw, which were obtained from stocks that had been backcrossed for many generations, were mated with the standard cn bw males and a number of recombinant chromosomes were obtained. Each recombinant male was then mated individually to _cn bw_ virgins to multiply each of the recombinant chromosome. The results for θ are summarized in Table XII.

In this table the R (no c. o.) chromosomes are the chromosomes without apparent crossing over in the right arm of chromosome two. Since there were no markers on the left arm, some and perhaps all of the R (no c. o.) chromosomes might actually involve crossing over in the left arm.

The analysis of the $\underline{L}^2$ recombinant chromosomes

Six independent R (no c. o.) chromosomes were tested, many of which showed values of θ larger than one. Statistical tests showed that θ was heterogeneous among the six chromosome lines ($\chi^2 = 14.63$, d.f. = 5, $0.02 > p > 0.01$). The overall θ was highly significantly larger than one ($\chi^2 = 6.85$, d.f. = 1, $0.01 > p > 0.001$). The average θ for the R (no c. o.) chromosomes was compared with that for the genotype X^{cb}/Y^{cb}; L^2/cn bw: No significant difference was found ($\chi^2 = 0.27$, d.f. = 1, $0.70 > p > 0.05$). Thus it would appear that the element causing non-randomness is located on the right arm of the second chromosome although there still remained the possibility that this element could be located on the left arm close to the centromere. This possibility was ruled out, however, in the analysis of the other recombinant chromosomes.

TABLE XII. SUMMARY OF U_θ AND K_θ FOR THE RECOMBINANT LINES OBTAINED FROM $\frac{X^{cb}}{X^{cb}}$; $\frac{L^2}{cn\ bw}$ FEMALES

R (no cross over)*				R (cn L^2)				R (L^2 bw)			
Line	n	U_θ	K_θ	Line	n	U_θ	K_θ	Line	n	U_θ	K_θ
1	16	-8.67	57.00	1	15	-7.86	69.65	1	15	-2.92	59.30
2	11	-2.11	35.53	2	11	-0.73	45.92	2	12	0.83	45.26
3	12	2.45	45.62	3	18	1.93	69.22	3	11	-7.38	41.55
4	12	7.09	39.11	4	11	-4.64	40.95	4	14	1.52	55.37
5	12	18.12	38.59	5	16	4.49	60.22	5	20	-15.82	86.86
6	18	27.87	76.83	-	--	--	--	6	16	10.16	65.09
-	--	--	--	-	--	--	--	-	--	--	--
	81	44.75	292.68		71	-6.81	285.96		88	-13.61	353.43

*No cross overs in the right arm of chromosome 2.

TABLE XII. (Continued) SUMMARY OF U_θ AND K_θ FOR THE RECOMBINANT LINES OBTAINED FROM $\frac{X^{cb}}{X^{cb}}$; $\frac{L^2}{cn\ bw}$ FEMALES

R (bw)				R (cn)				R (cn L^2 bw)			
Line	n	U_θ	K_θ	Line	n	U_θ	K_θ	Line	n	U_θ	K_θ
1	20	1.12	124.29	1	15	7.02	101.49	1	86	91.69	507.70
2	12	15.18	78.65	2	20	7.18	116.75	-	--	--	--
3	11	-7.93	59.77	3	11	19.29	67.95	-	--	--	--
4	18	-5.80	99.13	4	14	2.84	80.78	-	--	--	--
5	17	1.85	97.03	5	20	-1.97	117.50	-	--	--	--
6	14	9.59	77.29	6	15	-3.72	108.48	-	--	--	--
7	15	-11.07	91.85	7	19	-3.15	136.19	-	--	--	--
	107	2.94	628.01		114	27.49	729.14		86	91.69	507.70

More precise information on the position of this element was obtained from the double cross over type, R (cn L^2 bw). This chromosome received the centromeric region and the tip of 2R from the standard cn bw chromosome and the interstitial region from the L^2 chromosome. The value of θ for this line was highly significantly larger than one (χ^2 = 16.56, d.f. = 1, 0.001 > p). There was no heterogeneity in the value of θ between this recombinant and the original L^2 chromosome (χ^2 = 1.2, d.f. = 1, 0.30 > p > 0.20). Thus the element responsible for the non-random assortment appears to be located in the vicinity of the L^2 locus. Hereafter this element will be called "Non-random segregator" (symbol Nrs).

The other four types of recombinants are R (L^2 bw), R (cn), R (cn L^2) and R (bw).

Six R (L^2 bw) and seven R (cn) independent recombinant chromosome lines were tested: Both types showed a homogeneous distribution of θ among chromosomes within genotype (χ^2 = 5.46, d.f. = 5, 0.50 > p > 0.30 for the genotype R (L^2 bw) and χ^2 = 5.70, d.f. = 6, 0.50 > p > 0.30 for the genotype R (cn)). The overall θ for each genotype did not deviate from one (χ^2 = 0.53, d.f. = 1, 0.50 > p > 0.30 for the former genotype and χ^2 = 1.04, d.f. = 1, 0.05 > p > 0.30 for the latter). There was no heterogeneity between the two genotypes (χ^2 = 1.38, d.f. = 1, 0.30 > p > 0.20).

Five R (cn L^2) and seven R (bw) independent chromosome lines were also tested and the same situation as described above was found: θ was homogeneous among chromosomes within lines ($\chi^2 = 1.65$, d.f. = 4, $p > 0.80$ for the genotype R (cn L^2) and $\chi^2 = 6.88$, d.f. = 6, $0.50 > p > 0.30$ for the genotype R (bw)). The values of θ for each line also did not deviate from one ($\chi^2 = 0.16$, d.f. = 1, $0.70 > p > 0.50$ for the former genotype and $\chi^2 = 0.01$, d.f. = 1, $0.95 > p > 0.90$ for the latter). If the data for all four recombinant types are pooled, there is still no significant heterogeneity in the value of θ among genotypes ($\chi^2 = 1.69$, d.f. = 3, $0.70 > p > 0.50$) nor is the overall θ significantly different from one ($\chi^2 = 0.50$, d.f. = 1, $0.50 > p > 0.30$).

It is interesting to note that the R (cn L^2) and the R (L^2 bw) lines gave values of θ smaller than one while the R (bw) and the R (cn) lines gave values of θ slightly larger than one (none of these differences were significant). If the values of θ for the first two genotypes are pooled and compared with those of the latter two genotypes, the difference is not significant ($\chi^2 = 1.34$, d.f. = 1, $0.30 > p > 0.20$). The first group is characterized by the presence of <u>Nrs</u>, while it is absent from the second group. Thus there is a suggestion that under these conditions <u>Nrs</u> is suppressed. The results above also suggest the presence of other elements which may affect the value of θ, one located between the centromere and $\underline{L}^2$ and the other between $\underline{L}^2$ and the tip of 2R. Hereafter these elements will be designated as E-1 (centromeric region) and E-2 (tip of 2R). With these elements it is possible to construct the following model to explain the results:

1. Individually E-1 or E-2 suppresses or even reverses the effect of Nrs, but if both E-1 and E-2 are present, the suppression disappears.

2. The original L^2 chromosome carried E-1, Nrs and E-2 while the cn bw chromosome carried none of these elements.

E-1, Nrs and E-2 may also affect the overall sex ratio. Table XIII summarizes the overall sex ratios for the five genotypes.

The R (no c. o.) genotype showed a nearly homogeneous distribution of the overall sex ratio among the six lines. The average sex ratio was 0.491 which is very close to the standard of 0.492 and also to that of the original L^2 stock (sex ratio = 0.495). These results suggest that the left arm of the second chromosome is of little consequence in the determination of the overall sex ratio although the possibility of elements located on 2L close to the centromere cannot be ruled out.

The overall sex ratio also showed a homogeneous distribution among genotypes and among lines for the four recombinant types (see on following page).

Although the differences were not statistically significant, the genotypes R (L^2 bw) and R (cn L^2) showed

TABLE XIII. SUMMARY OF SR FOR THE RECOMBINANT LINES OBTAINED FROM $\frac{X^{cb}}{X^{cb}}$; $\frac{L^2}{cn\ bw}$ FEMALES

	R (no cross over)				R (cn L^2)				R (L^2 bw)			
Line	n	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$	n	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$	n	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$
1	16	7.74	3.77	0.484	15	7.06	3.39	0.471	15	7.25	3.56	0.483
2	11	5.35	2.63	0.486	11	5.20	2.51	0.473	12	5.77	2.80	0.481
3	12	6.05	3.07	0.504	18	8.81	4.38	0.489	11	5.39	2.68	0.490
4	12	5.84	2.88	0.487	11	5.37	2.66	0.488	14	6.34	2.93	0.453
5	12	6.34	3.37	0.528	16	7.88	3.93	0.492	20	9.76	4.86	0.488
6	18	8.43	3.99	0.468	--	--	--	--	16	8.39	4.46	0.524
7	--	--	--	--	--	--	--	--	--	--	--	--
	81	39.75	19.71	0.491	71	34.32	16.87	0.483	88	42.90	21.29	0.488

TABLE XIII. (Continued) SUMMARY OF SR FOR THE RECOMBINANT LINES OBTAINED FROM $\frac{X^{cb}}{X^{cb}}$; $\frac{L^{2}}{cn\ bw}$ FEMALES

	R (bw)				R (cn)				R (cn L^2 bw)			
Line	n	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$	n	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$	n	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$
1	20	9.83	4.88	0.492	15	7.56	3.85	0.504	86	43.38	22.25	0.504
2	12	5.91	2.94	0.492	20	10.18	5.24	0.509	--	--	--	--
3	11	5.64	2.93	0.513	11	5.49	2.76	0.499	--	--	--	--
4	18	8.70	4.24	0.483	14	6.44	3.22	0.460	--	--	--	--
5	17	8.49	4.30	0.499	20	10.05	5.12	0.502	--	--	--	--
6	14	6.93	3.45	0.495	15	7.30	3.58	0.487	--	--	--	--
7	15	7.84	4.11	0.523	19	9.29	4.56	0.489	--	--	--	--
	107	53.34	26.85	0.498	114	56.31	28.33	0.494	86	43.38	22.25	0.504

Source of Variance	S.S.	d.f.	M.S.	F.
Among genotypes	0.0122	3	0.0041	1.00
Among lines within genotype	0.0861	21	0.0041	1.11
Within line	1.3300	355	0.0037	--

a sex ratio which was lower than that of the standard while the genotypes R (cn) and R (bw) showed ratios which were higher. It may be recalled that the former two genotypes carried Nrs while the latter two lacked this element and instead carried either E-1 or E-2. This suggests that E-1 and E-2 increase the overall sex ratio while Nrs reduces it.

The original L^2 second chromosome from the backcrossed stock carried E-1, E-2 and Nrs and gave an overall sex ratio of 0.490. If either E-1 or E-2 are removed, the sex ratio was reduced (0.486) while the absence of Nrs caused an increase in the overall sex ratio (0.496). If both E-1 and E-2 are removed, genotype R (cn L^2 bw), the sex ratio was increased (0.504) to a value larger than that of the comparable original backcrossed L^2 stock (0.490). However, perhaps because of the small numbers involved, the difference was not statistically significant ($F = 2.69$, $\nu_1 = 1$, $\nu_2 = 177$, $0.01 > p > 0.005$). Thus no definite conclusions could be drawn concerning the overall sex ratio but there was a suggestion of the possible function of E-1 and E-2.

From the above it may be concluded that:

1. The element, Non-random segregator (Nrs), which is responsible for a value of θ greater than one for its carrier chromosome is located close to the $\underline{L}^2$ locus on the second chromosome.

2. There appears to be two elements modifying the action of Nrs: E-1 which is located between the centromere and the $\underline{L}^2$ locus and E-2 located between $\underline{L}^2$ and tip of 2R. Individually E-1 or E-2 suppresses the effect of Nrs but if both E-1 and E-2 are present, their suppressing action disappears.

3. The suppression of Nrs by either E-1 or E-2 may be reflected in the reduction in the overall sex ratio.

4. Both E-1 and E-2 in the absence of Nrs tend to increase the overall sex ratio.

The analysis of the $\underline{stw}^3$ recombinant chromosomes

For the purpose of mapping, the $\underline{stw}^3$ chromosome has the disadvantage of not having any markers in the interstitial region between cn and bw. It was of course possible to construct chromosomes with markers but to avoid the complications which could have arisen due to the unknown nature of the marker stocks, only the recombinant chromosomes obtained from females of the genotype X^{cb}/Y^{cb}; stw^3/cn bw were used. Three types of recombinants, R (no c. o.), R (cn) and R (bw) were collected.

As in the $\underline{L}^2$ experiment, the R (no c. o.) chromosomes were those which had no apparent cross overs on the right arm of the second chromosome, but which might have had recombinations on the left arm. However, since $\underline{stw}^3$ has no marker in the interstitial region, double crossing over between cn and bw (about fifty map units apart) could have occurred and yet have remained undetected.

A summary of the values of θ are given in Table XIV.

TABLE XIV. SUMMARY OF U_θ AND K_θ FOR THE RECOMBINANT CHROMOSOMES OBTAINED FROM $\frac{X^{cb}}{X^{cb}}; \frac{stw^3}{cn\ bw}$ FEMALES

	R (no cross over)			R (cn)			R (bw)		
Line	n	U_θ	K_θ	n	U_θ	K_θ	n	U_θ	K_θ
1	14	2.46	70.58	16	14.73	71.61	11	-12.00	59.40
2	10	3.34	56.08	16	8.62	74.82	16	-4.42	77.26
3	10	3.46	39.09	14	-17.20	61.10	9	8.19	43.57
4	16	2.15	55.05	15	3.75	71.70	14	3.51	73.61
5	10	1.36	40.95	15	8.92	66.63	17	-7.09	73.11
6	14	5.96	76.53	14	10.42	62.03	10	-21.10	35.79
	74	18.73	338.28	90	29.24	407.89	77	-32.91	362.74

Six R (no c. o.) chromosome lines were tested among which θ was found to be homogeneous ($\chi^2 = 0.15$, d.f. = 5, $p > 0.99$). The overall θ was greater than one but not significantly so ($\chi^2 = 1.04$, d.f. = 1, $0.50 > p > 0.30$).

The difference in the values of θ between the R (no c. o.) and the original backcrossed <u>stw</u>^{3} chromosome line was not significant ($\chi^2 = 0.20$, d.f. = 1, $0.70 > p > 0.50$). Thus at least with the present data, the left arm seems to have little effect upon the value of θ. Homogeneity among the six R (no c. o.) lines might mean either no double cross over types were included among these lines or no element which can change the value of θ was located on the <u>stw</u>^{3} second chromosome. Analysis of the other recombinant chromosomes indicated that the former was the case.

Six R (cn) chromosomes were tested and the overall θ was larger than one but no significantly so ($\chi^2 = 2.10$, d.f. = 1, $0.20 > p > 0.10$). There was also a suggestion that θ was heterogeneous among lines ($\chi^2 = 9.91$, d.f. = 5, $0.10 > p > 0.05$). It is interesting to note that of these six lines one showed an almost significantly higher value of θ than one ($\chi^2 = 3.03$, d.f. = 1, $0.10 > p > 0.05$) while another had a significantly lower value ($\chi^2 = 4.84$, d.f. = 1, $0.05 > p > 0.02$).

The results of the analysis for θ for the six R (bw) chromosomes suggest that the value of θ may be smaller than one ($\chi^2 = 2.99$, d.f. = 1, $0.10 > p > 0.05$). There was significant heterogeneity among lines ($\chi^2 = 14.52$, d.f. = 5, $0.02 > p > 0.01$).

Although more data is necessary, the above results suggest the presence of two elements, <u>Nrs</u> and <u>E-1</u>, on the <u>stw</u>^{3} second chromosome. <u>Nrs</u> is located in an intermediate position between the cn and bw loci (closer to bw) and therefore, some of the R (cn) carried Nrs giving a value of θ larger than one. E-1 is located to the right of cn on 2R and some of the R (cn) (in a relatively low frequency) will carry both E-1 and Nrs, thus giving a value of θ smaller than one. Some of the R (cn) will carry neither Nrs nor E-1 and these will give θ equal to one. Thus the average θ will be greater than one for the R (cn) lines and there will be heterogeneity among lines.

Some of the R (bw) chromosomes, on the other hand, will carry both E-1 and Nrs, while some will carry only E-1. The former will give a value of θ smaller than one while the latter will give a value equal to one. Only a few of these recombinants will carry neither Nrs nor E-1 and these will also show a value of θ equal to one. Thus the overall θ for the R (bw) lines will be smaller than one and there will be heterogeneity of θ among lines.

Since E-1 causes an increase in the overall sex ratio, one may expect to have a higher average overall sex ratio in the R (bw) lines as compared with the R (cn) lines. The data on the overall sex ratio are summarized in Table XVA and the analysis of variance in Table XVB.

From Table XVB it can be seen that the overall sex ratio was homogeneous among genotypes and among lines within genotype. It should be pointed out that the R (bw) lines showed a higher average sex ratio than the R (cn) lines

TABLE XVA. SUMMARY OF THE SEX RATIO FOR THE RECOMBINANT CHROMOSOMES OBTAINED FROM $\frac{X^{cb}}{X^{cb}}$; $\frac{stw^3}{cn\ bw}$ FEMALES

	R (no cross over)				R (cn)				R (bw)			
Line	n	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$	n	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$	n	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$
1	14	6.53	3.10	0.466	16	7.72	3.76	0.482	11	5.58	2.85	0.507
2	10	4.70	2.24	0.470	16	7.87	3.92	0.492	16	8.16	4.20	0.510
3	10	5.19	2.72	0.519	14	6.19	3.21	0.478	9	4.58	2.36	0.509
4	16	8.05	4.12	0.503	15	7.38	3.65	0.492	14	7.10	3.64	0.507
5	10	4.79	2.33	0.479	15	7.22	3.51	0.481	17	8.48	4.26	0.499
6	14	6.81	3.35	0.486	14	7.04	3.61	0.503	10	4.87	2.40	0.487
TOTAL	74	36.07	17.86	0.487	90	43.92	21.66	0.488	77	38.77	19.71	0.504

Source of Variance	Sum of Squares	d.f.	Mean Square	F
Among genotypes	0.0134	2	0.0067	2.23
Among lines within genotypes	0.0370	15	0.0025	0.80
Within lines	0.6720	223	0.0030	--

($F = 3.88$, $\nu_1 = 1$, $\nu_2 = 165$, $p \simeq 0.05$). Since the original $\underline{stw}^3$ second chromosome under the X^{cb}/Y^{cb} background showed a sex ratio of 0.501, the reduction in the overall sex ratio in the above three recombinant types ($F = 6.94$, $\nu_1 = 1$, $\nu_2 = 500$, $0.01 > p > 0.001$) must be due to recombinations which occurred in the left arm.

These results suggest the presence of a fourth element, E-3, which is located on 2L not close to the centromere. This element causes an increase in the overall sex ratio but does not affect the value of θ.

From the above the following conclusions may be drawn:

1. The $\underline{stw}^3$ second chromosome carried Nrs and E-1.

2. This chromosome also carried an element, E-3, on the left arm not close to the centromere. E-3 increases the overall sex ratio but has little affect upon the value of θ.

3. Since the value of θ for the $\underline{stw}^3$ second chromosome was close to the expected value of one, there does not appear to be any interaction between E-1 and E-3.

Zygotic selection versus prezygotic selection

Throughout the previous discussion no attention had been paid to zygotic selection. Although the phenomenon of non-random assortment was quite consistent, the amount was not very large; therefore, one might suspect that such a small amount of deviation could be due simply to larval viability differences among genotypes. This possibility was excluded through the following experiments.

Hiraizumi and Watanabe (1967) showed that when males heterozygous for the cn bw chromosome were aged (stw^3/cn bw males under various combinations of the sex chromosomes), the segregation frequency of the second chromosome was changed and parallel to it the value of θ was also changed. These results eliminated the possibility of zygotic selection and to a large extent, although not completely, the possibility of sperm competition.

The following experiment was carried out to investigate the latter possibility. Standard cn bw females were mated with males of the genotypes X^{stw}/Y^{cb}; stw^3/cn bw and X^{cb}/Y^{stw}; stw^3/cn bw. In each mating four virgin females and one male were kept together for three days and then the male was discarded. Thereafter on every third day the inseminated females were transferred into fresh vials until most of the sperms were exhausted.

If the sperm competition hypothesis is true, there must be some general increase or decrease in the values of

θ and $\underline{k}$ concomitant with the increase in the age of the females, whereas if no sperm competition occurs, these values would remain more or less the same. If for example the sperm carrying the Y and the $\underline{stw}^3$ second chromosomes has a higher competitive ability than the other three types (assuming the same competitive ability among these three), then one would expect θ and $\underline{k}$ to be the highest in the first vial with a decline in these values in subsequent transfers. If, on the other hand, the above type of sperm has a higher survival rate in the female as compared with the three remaining types, then the values of θ and $\underline{k}$ would be expected to increase with increasing age of the females. The results for these experiments are summarized in Table XVI.

There is no indication of a change in θ or $\underline{k}$ with increasing age of the females: Not only was the regression not significant, but there was also no general tendency demonstrated. Thus the phenomenon of non-random assortment had to be due to some mechanism before sperm formation--probably a meiotic mechanism.

It is interesting to note that the overall sex ratio consistently decreased as the inseminated females aged. This suggests that the survival rate of the X-bearing sperm (independent of the constitution of the second chromosome) in the female body is higher than that of the Y-bearing sperm.

TABLE XVI. FEMALE AGING EFFECTS UPON THE VALUES OF θ, k AND SR

		$\frac{X^{stw}}{Y^{cb}}$; $\frac{stw^3}{cn\ bw}$						$\frac{X^{cb}}{Y^{stw}}$; $\frac{stw^3}{cn\ bw}$			
Transfer	n	θ	k	SR	No. of flies counted	Transfer	n	θ	k	SR	No. of flies counted
1	37	1.04	0.509	0.480	4745	1	51	1.39	0.515	0.521	4560
2	43	1.22	0.526	0.504	2847	2	51	1.13	0.504	0.519	2852
3	43	1.12	0.511	0.487	3487	3	52	1.68	0.521	0.501	2916
4	43	1.44	0.524	0.475	2937	4	51	1.24	0.516	0.506	2621
5	41	1.30	0.498	0.472	1449	5	48	1.30	0.516	0.496	1387
6	34	1.20	0.557	0.478	751	6	28	1.24	0.493	0.541	444
7-9	19	1.16	0.534	0.458	304	7-9	6	1.23	0.510	0.537	74

Summary of the $\underline{stw}^3$ series

1. The $\underline{stw}^3$ second chromosome tends to assort more frequently with the Y chromosome. The degree of this non-randomness can be changed by changing either the X or the Y chromosome.

2. By changing the X or the Y chromosome the segregation frequency of the $\underline{stw}^3$ second chromosome can be changed.

3. The amount of increase in θ (the measure of the amount of non-random assortment) is positively correlated with the increase in the $\underline{k}$ value (the segregation frequency of the $\underline{stw}^3$ second chromosome) not only among genotypes but also within generations.

4. The $\underline{stw}^3$ second chromosome increased the overall sex ratio in the progeny generation. The amount of this increase is probably greater for males homozygous for the $\underline{stw}^3$ second chromosome than for those heterozygous.

5. The X^{stw} chromosome caused a reduction in the overall sex ratio in the progeny generation while the Y^{stw} chromosome had little effect.

6. The overall sex ratio is independent of the values of θ and $\underline{k}$.

7. There appears to be an element on either the third or the fourth chromosome in the $\underline{stw}^3$ stock which suppresses the effect of the X^{stw} chromosome upon the overall sex ratio.

Summary of the $\underline{L}^2$ series

1. The $\underline{L}^2$ second chromosome tends to assort more frequently with the Y^{cb} chromosome. When Y^{cb} is replaced by Y^L, the $\underline{L}^2$ second chromosome tends to separate from Y^L. The X^L chromosome has little effect upon the value of θ.

2. The $\underline{L}^2$ second chromosome does not cause an increase in the overall sex ratio. The X^L and Y^L chromosomes also have little effect upon the overall sex ratio.

3. There is some evidence that the $\underline{L}^2$ stock carried an autosomal element which causes an increase in the overall sex ratio.

4. The overall sex ratio is independent of θ.

Location of the elements on chromosome two

1. An element, Nrs, located near the middle of the right arm of the second chromosome is responsible for the non-random assortment.

2. Three other elements, E-1 located close to the centromere and E-2 located near the tip of 2R and E-3 located on 2L, were found.

3. Individually E-1 or E-2 suppresses the action of Nrs, but if both are present together with Nrs, suppression disappears. E-3 has little effect upon the value of θ.

4. E-1, E-2 and E-3 in the absence of Nrs increase the overall sex ratio while Nrs decreases it.

Discussion of the stw^3 and L^2 experiments

It has been established in the above experiments that non-random assortment between the second and sex chromosomes occurs consistently for two laboratory stocks when they were tested under the standard cn bw background. There were at least two kinds of X and two kinds of Y chromosomes which could modify the amount and direction of non-randomness. It was also demonstrated that in the stw^3 stock the phenomenon of non-random assortment is related to the segregation frequency changes in the second chromosome and that the segregation frequency of the second chromosome could be changed by changing the X or the Y chromosome. This is a fairly important finding since in many studies undertaken to estimate the viability of the second chromosome, similar mating schemes were utilized. One such study is that of Hiraizumi and Crow (1959). In their study, for most of the chromosome lines, the Y chromosome came from natural populations but in others Y chromosomes from laboratory stocks were used. The X chromosome came from laboratory stocks. Thus the genotype of the sex chromosomes differed from that of the natural population and thereby the estimates of viability which were obtained might be biased and might not measure the actual amount of viability differences.

In the present study one element causing non-randomness was located in the middle of chromosome two and at least two other elements were found which could modify the action of <u>Nrs</u>. All of these findings suggest that a number of elements on the autosomes as well as the sex chromosomes can affect the assortment of chromosome pairs and perhaps more importantly they can affect the segregation frequency of structurally normal chromosome pairs.

CHAPTER V

THE ANALYSIS OF A NATURAL POPULATION

Non-random assortment between the Odate second and sex chromosomes

In the previous chapter, the formal basis of the phenomenon of non-random assortment was established. However, one may still suspect that this phenomenon is true only for these "special" laboratory stocks but is not general. Therefore an analysis of a natural population is of interest in determining the generality of this phenomenon.

The population analyzed is one which was collected from Odate, Akita Prefecture in the northern part of the mainland of Japan. The mating schemes are presented in Figure 2.

If non-random assortment actually occurs in natural populations, the chromosomes involved in this phenomenon will show different frequency distributions between the two sexes. The results for θ in the male and female series are summarized in Tables XVII and XVIII respectively.

The four genotypes tested were as follows:

	Male series	Female series
Genotype	(1) X^{cb}/Y^{cb}; $\underline{+}/\underline{cn}\ \underline{bw}$	(3) X^{cb}/Y^{cb}; $\underline{+}/\underline{cn}\ \underline{bw}$
	(2) $X^{cb}/\underline{Y}$; $\underline{+}/\underline{cn}\ \underline{bw}$	(4) $\underline{X}\ /Y^{cb}$; $\underline{+}/\underline{cn}\ \underline{bw}$

FIGURE 2. MATING SCHEME FOR THE MALE AND FEMALE SERIES

Male Series

$\underline{cn}\ \underline{bw}$ ♀♀ X Odate ♂

$\downarrow$

$\underline{cn}\ \underline{bw}$ ♀♀ X $\frac{X^{cb}}{\underline{Y}};\ \frac{\underline{+}}{cn\ bw}$ X $\underline{y}f{:}{=};\ \underline{cn}\ \underline{bw}$ ♀♀

$\downarrow$ $\downarrow$

$\frac{\underline{X}^{cb}}{\underline{Y}};\ \frac{\underline{+}}{cn\ bw}$ $\frac{X^{cb}}{Y^{cb}};\ \frac{\underline{+}}{cn\ bw}$

Female Series

Odate ♀ X $\underline{cn}\ \underline{bw}$ ♂♂

$\downarrow$

$\underline{cn}\ \underline{bw}$ ♀♀ X $\frac{\underline{X}}{Y^{cb}};\ \frac{\underline{+}}{cn\ bw}$ X $\underline{y}f{:}{=};\ \underline{cn}\ \underline{bw}$ ♀♀

$\downarrow$ $\downarrow$

$\frac{\underline{X}^{cb}}{Y^{cb}};\ \frac{\underline{+}}{cn\ bw}$ $\frac{\underline{X}}{Y^{cb}};\ \frac{\underline{+}}{cn\ bw}$

$\frac{\underline{X}}{Y};\ \frac{cn\ bw}{cn\ bw}$

TABLE XVII. SUMMARY OF U_θ AND K_θ FOR THE MALE SERIES

	(1) $\frac{X^{cb}}{Y^{cb}}; \frac{+}{cn\ bw}$			(2) $\frac{X^{cb}}{\underline{Y}}; \frac{+}{cn\ bw}$		
Lines	n*	U_θ	K_θ	n	U_θ	K_θ
a-1	13	-4.49	84.28	13	7.87	82.68
B-2	13	-2.49	78.78	13	-1.12	91.32
B-4	14	22.83	92.22	14	1.53	113.59
c-4	14	6.05	98.44	14	3.03	109.70
d-1	16	11.85	101.72	16	-3.06	105.22
e-2	13	-8.40	77.96	13	12.11	83.66
f-2	14	-12.76	95.62	14	-5.59	83.58
f-9	18	18.24	122.76	18	12.22	120.89
k-5	11	18.84	74.67	11	-7.30	79.86
m-1	11	-2.04	67.24	11	-13.12	83.31
TOTAL	137	47.63	893.68	137	6.56	953.81

*number of males tested.

The genotypes (1) and (3) are analogous and differ only in whether the genotype was constructed from male or female flies.

Genotype (1) showed a homogeneous distribution of θ among the ten tested lines ($\chi^2 = 15.32$, d.f. = 9, $0.10 > p > 0.05$). The overall θ was larger than one but this was not significant ($\chi^2 = 2.54$, d.f. = 1, $0.20 > p > 0.10$).

TABLE XVIII. SUMMARY OF U_θ AND K_θ FOR THE FEMALE SERIES

	(3) $\frac{X^{cb}}{Y^{cb}}; \frac{+}{cn\ bw}$			(4) $\frac{X}{Y^{cb}}; \frac{+}{cn\ bw}$		
Line	n*	U_θ	K_θ	n	U_θ	K_θ
a-1	9	11.07	55.34	9	9.99	63.67
B-2	14	-14.78	101.32	14	-5.68	122.04
B-4	7	8.62	48.46	7	20.92	55.64
c-4	16	2.95	112.34	16	22.87	128.85
d-1	16	13.89	144.52	16	21.38	111.82
e-2	10	21.85	75.71	10	8.29	54.38
f-2	16	6.01	137.49	16	-14.11	142.09
f-9	11	-0.24	76.78	11	8.11	98.42
k-5	10	12.51	81.73	10	29.29	96.08
m-1	17	-6.81	137.86	17	3.20	139.33
TOTAL	126	55.06	971.55	126	104.26	1012.32

*number of males tested.

Genotype (3) also showed a homogeneous distribution of θ among the ten lines tested (χ^2 = 13.02, d.f. = 9, 0.20 > p > 0.10) and the overall θ was larger than one but again not significantly so (χ^2 = 3.12, d.f. = 1, 0.10 > p > 0.05).

The difference in θ between the analogous genotypes (1) and (3) was not significant (χ^2 = 0.01, d.f. = 1, 0.95 > p > 0.90) but the overall θ of the pooled data for the two genotypes was significantly larger than one (χ^2 = 5.65, d.f. = 1, $p \approx 0.02$).

Thus the second chromosome showed, on the average, a significantly larger value of θ than unity, but no difference in θ was detectable between the male and female series.

The genotype $X^{cb}/\underline{Y}$; $\underline{+}$/cn bw showed an overall θ close to 1 (χ^2 = 0.045, d.f. = 1, $0.90 > p > 0.80$) and the value of θ was homogeneous among lines (χ^2 = 7.01, d.f. = 9, $0.70 > p > 0.50$). Although it was not significant, it is interesting to note that the overall θ of this genotype appeared to be smaller than that of the genotypes (1) and (3).

The genotype $\underline{X}/Y^{cb}$; $\underline{+}$/cn bw showed a highly significantly larger value of θ than one (χ^2 = 10.74, d.f. = 1, $0.01 > p > 0.001$) but θ was also heterogeneous among lines (χ^2 = 19.44, d.f. = 9, $0.05 > p > 0.02$). The difference in θ between the genotype $\underline{X}/Y^{cb}$; $\underline{+}$/cn bw and the pooled genotypes X^{cb}/Y^{cb}; $\underline{+}$/cn bw was not significant (χ^2 = 1.4, d.f. = 1, $0.30 > p > 0.20$). If the above four genotypes are pooled, θ was homogeneous among genotypes (χ^2 = 4.55, d.f. = 3, $0.30 > p > 0.20$) and the overall θ was signifcantly larger than one (χ^2 = 11.90, d.f. = 1, $0.001 > p$).

Since there was some suggestion of heterogeneity in θ among the ten lines, a more refined analysis of the data could be done on the basis of each line separately. Accordingly in order to test the effect of the Y chromosome, a test for heterogeneity in the values of θ between the genotypes X^{cb}/Y^{cb}; $\underline{+}$/cn bw and $X^{cb}/\underline{Y}$; $\underline{+}$/cn bw was made for each line separately. Only two lines, B-4 and k-5, gave nearly significant and significant differences in θ between the two genotypes ($\chi^2 = 2.79$, d.f. = 1, $0.10 > p > 0.05$ for B-4 and $\chi^2 = 4.56$, d.f. = 1, $0.05 > p > 0.02$ for k-5). In both cases the value of θ decreased when Y was substituted. Since θ in these two classes was homogeneous within genotype ($\chi^2 = 0.000$, d.f. = 1, $p > 0.95$ for the genotype X^{cb}/Y^{cb}; $\underline{+}$/cn bw and $\chi^2 = 0.52$, d.f. = 1, $0.50 > p > 0.30$ for the genotype $X^{cb}/\underline{Y}$; $\underline{+}$/cn bw), they were pooled within genotype. The difference in θ between genotypes now became highly significant ($\chi^2 = 6.99$, d.f. = 1, $0.01 > p > 0.001$).

In order to test the effect of the X chromosome, a similar comparison was made for the female series between the genotypes X^{cb}/Y^{cb}; $\underline{+}$/cn bw and $\underline{X}/Y^{cb}$; $\underline{+}$/cn bw. Again only B-4 and k-5 showed an appreciable amount of difference in the values of θ between the two genotypes ($\chi^2 = 1.01$, d.f. = 1, $p = 0.30$ for B-4 and $\chi^2 = 1.02$, d.f. = 1, $p \simeq 0.30$ for k-5). If the two lines are pooled within genotype, and the two genotypes compared, the difference is still not significant ($\chi^2 = 1.99$, d.f. = 1, $0.20 > p > 0.10$) but there is a suggestion that by substituting the X, the value of θ was increased. Let us call these two lines Group 1, and the remaining eight lines Group 2. In the male series Group 1 showed a highly significantly larger value of θ than Group 2 for the genotype X^{cb}/Y^{cb}; $\underline{+}$/cn bw ($\chi^2 = 6.91$, d.f. = 1, $p \simeq 0.01$) but there was no significant difference in the value of θ for the genotype $X^{cb}/\underline{Y}$; $\underline{+}$/cn bw ($\chi^2 = 0.32$, d.f. = 1, $0.70 > p > 0.50$).

In the female series the value of θ in Group 1 was highly significantly greater than that of Group 2 for the genotype $\underline{X}/Y^{cb}$; $\underline{+}$/cn bw ($\chi^2 = 9.27$, d.f. = 1, $0.01 > p > 0.001$) and also larger, though not significantly, for the genotype X^{cb}/Y^{cb}; $\underline{+}$/cn bw ($\chi^2 = 1.68$, d.f. = 1, $0.20 > p > 0.10$). In Group 1 the genotypes (1) and (3) [X^{cb}/Y^{cb}; $\underline{+}$/cn bw] and (4) [$\underline{X}/Y^{cb}$; $\underline{+}$/cn bw] showed a significantly higher value of θ than one ($\chi^2 = 10.40$, d.f. = 1, $p \simeq 0.001$ for genotype (1), $\chi^2 = 3.43$, d.f. = 1, $p \simeq 0.05$ for genotype (3) and $\chi^2 = 16.61$, d.f. = 1, $0.001 > p$ for genotype (4)).

The conclusions which may be obtained from the above data are as follows:

1. In the Group 1 lines (B-4 and k-5) many if not all of the second chromosomes carried an element which cause an increase in the value of θ.

2. The Group 1 lines contained in high frequency a $\underline{Y}$ chromosome which suppressed the action of the above element.

3. The Group 1 lines contained an $\underline{X}$ chromosome which enhanced the action of the above element.

Similar kinds of X, Y and second chromosomes might be included in other lines in Group 2, but perhaps in lower frequencies or with smaller effects. If such elements do occur in the Group 2 lines, they have remained undetected due to the smallness of the data. It is clear, however, that in the Odate population some of the sex and the second chromosome pairs interact to change the value of θ--the non-random assortment between them.

The overall sex ratio

A summary of the results for the overall sex ratio for the male and female series are given in Tables XIX and XX. The two additional genotypes which were included are as follows:

Genotype (5) $X^{cb}/\underline{Y}$; cn bw/cn bw
(6) $\underline{X}$ $/Y^{cb}$; cn bw/cn bw

The genotypes (1) and (3) [X^{cb}/Y^{cb}; $\underline{+}$/cn bw] showed a higher sex ratio than that of the standard of 0.492 ($F = 3.76$, $\nu_1 = 1$, $\nu_2 = 396$, $p \approx 0.05$), but it was not clear whether this was due to the effect of the second chromosome or to either the third or fourth chromosome from the Odate population. Information on this point can be obtained from the other four genotypes because among these the residual background other than the second and sex chromosomes had been randomized. These comparisons are summarized in Table XXI.

TABLE XIX. SUMMARY OF SR FOR THE MALE SERIES

		(1) $\frac{X^{cb}}{Y^{cb}}; \frac{\pm}{cn\ bw}$			(2) $\frac{X^{cb}}{\underline{Y}}; \frac{\pm}{cn\ bw}$			(5) $\frac{X^{cb}}{\underline{Y}}; \frac{cn\ bw}{cn\ bw}$		
Line	n	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$
a-1	13	6.29	3.08	0.484	6.64	3.41	0.511	6.63	3.41	0.510
B-2	13	6.47	3.25	0.498	6.62	3.39	0.509	6.73	3.51	0.518
B-4	14	6.66	3.20	0.476	6.84	3.37	0.488	7.36	3.92	0.526
c-4	14	7.09	3.62	0.506	7.21	3.73	0.519	7.30	3.85	0.521
d-1	16	8.43	4.48	0.527	8.15	4.16	0.509	7.73	3.78	0.483
e-2	13	6.68	3.46	0.514	6.32	3.10	0.486	6.18	3.00	0.475
f-2	14	6.67	3.20	0.476	7.09	3.64	0.506	6.55	3.09	0.468
f-9	18	9.22	4.76	0.512	8.85	4.39	0.492	8.87	4.39	0.493
k-5	11	5.98	3.29	0.544	5.63	2.90	0.512	5.38	2.65	0.489
m-1	11	5.51	2.77	0.501	5.51	2.78	0.501	5.36	2.62	0.487
TOTAL	137	69.00	35.11	0.504	68.86	34.87	0.503	68.09	34.22	0.497

TABLE XX. SUMMARY OF SR FOR THE FEMALE SERIES

		(3) $\frac{X^{cb}}{Y^{cb}}; \frac{\pm}{cn\ bw}$			(4) $\frac{X}{Y^{cb}}; \frac{\pm}{cn\ bw}$			(6) $\frac{X}{Y^{cb}}; \frac{cn\ bw}{cn\ bw}$		
Line	n	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$	ΣSR	$\Sigma(SR)^2$	$\overline{SR}$
a-1	9	4.36	2.12	0.484	4.25	2.02	0.472	4.30	2.06	0.478
B-2	14	7.12	3.64	0.508	6.72	3.24	0.480	6.65	3.19	0.475
B-4	7	3.40	1.66	0.486	3.41	1.67	0.487	3.37	1.64	0.481
c-4	16	8.03	4.08	0.502	7.78	3.81	0.486	8.02	4.05	0.501
d-1	16	8.14	4.18	0.509	7.95	3.97	0.497	7.30	3.37	0.456
e-2	10	5.15	2.67	0.515	5.08	2.62	0.508	5.00	2.51	0.500
f-2	16	7.75	3.79	0.484	7.73	3.74	0.483	7.61	3.66	0.476
f-9	11	5.70	3.00	0.518	5.27	2.53	0.479	5.30	2.59	0.482
k-5	10	5.27	2.79	0.527	4.75	2.28	0.475	4.93	2.45	0.493
m-1	17	8.40	4.19	0.494	8.61	4.39	0.506	8.56	4.36	0.504
TOTAL	126	63.32	32.12	0.502	61.55	30.27	0.488	61.04	29.88	0.484

TABLE XXI. ANALYSIS OF VARIANCE TO TEST THE EFFECT OF THE $\underline{+}$ SECOND CHROMOSOME UPON THE OVERALL SEX RATIO

Sex chromosomes	Second chromosomes	
	$\frac{+}{cn\ bw}$	$\frac{cn\ bw}{cn\ bw}$
$\frac{X^{cb}}{\underline{Y}}$	$\frac{X^{cb}}{\underline{Y}}; \frac{+}{cn\ bw}$	$\frac{X^{cb}}{\underline{Y}}; \frac{cn\ bw}{cn\ bw}$
$\frac{\underline{X}}{Y^{cb}}$	$\frac{\underline{X}}{Y^{cb}}; \frac{+}{cn\ bw}$	$\frac{\underline{X}}{Y^{cb}}; \frac{cn\ bw}{cn\ bw}$

Source of Variance	Sum of squares	d.f.	Mean square	F
Between $\underline{+}$ and cn bw	0.0029	1	0.0029	0.10
Between $\frac{X}{\underline{Y}}$ and $\frac{X}{\overline{Y}}$	0.0231	1	0.0231	7.70*
Second x sex	0.0000	1	0.0000	0.00
Among lines within genotype	0.1090	36	0.0030	1.43**
Within lines	1.0010	487	0.0021	

*Significant at the 1% level.

**Significant at the 5% level.

There was no difference between the $\underline{+}$/cn bw and the cn bw/cn bw classes. This indicates that the second chromosome from the Odate population causes little increase in the overall sex ratio. Thus the higher sex ratio of the genotypes (1) and (3) [X^{cb}/Y^{cb}; $\underline{+}$/cn bw] had to be due to either the third or the fourth chromosome from the Odate population.

The effect of the X chromosomes can be detected by comparing the genotypes X^{cb}/Y^{cb}; $\underline{+}$/cn bw and $\underline{X}/Y^{cb}$; $\underline{+}$/cn bw. The results of this comparison are given in Table XXII.

TABLE XXII. ANALYSIS OF VARIANCE TO TEST THE EFFECT OF THE X CHROMOSOME UPON THE OVERALL SEX RATIO

Source of Variance	Sum of squares	d.f.	Mean square	F
Between genotypes	0.0129	1	0.0129	4.77*
Among lines within genotype	0.0480	18	0.0027	1.42
Within line	0.4470	232	0.0019	

*Significant at the 1% level.

It appears that the X chromosome on the average reduced the overall sex ratio significantly ($p \simeq 0.01$). If the data for the genotypes X^{cb}/Y^{cb}; $\underline{+}$/cn bw (♂ and ♀ series) are pooled and compared with the pooled data for the genotypes $\underline{X}/Y^{cb}$; cn bw/cn bw and $\underline{X}/Y^{cb}$; $\underline{+}$/cn bw (since the Odate second chromosome has little effect upon the overall sex ratio), the probability becomes even smaller.

Zygotic selection in favor of the $\underline{X}/X^{cb}$ heterozygous females as compared with the X^{cb}/X^{cb} females could explain the excess of females for the genotypes $\underline{X}/Y^{cb}$; $\underline{+}$/cn bw and $\underline{X}/Y^{cb}$; cn bw/cn bw. This possibility was excluded by Kataoka (1967), who found that when the $\underline{X}$ chromosomes are transmitted through the females, the sex ratio was the same as that of the control X^{cb} chromosomes. If zygotic selection actually occurs, the reciprocal matings should have shown the same amount of female excess.

A comparison between the genotypes X^{cb}/Y^{cb}; $\underline{+}$/cn bw and $X^{cb}/\underline{Y}$; $\underline{+}$/cn bw provides information on the effect of the $\underline{Y}$ chromosome. Results are summarized in Table XXIII.

TABLE XXIII. ANALYSIS OF VARIANCE TO TEST THE EFFECT OF THE $\underline{Y}$ CHROMOSOME UPON THE OVERALL SEX RATIO

Source of Variance	Sum of squares	d.f.	Mean square	F
Between genotypes	0.0000	1	0.0000	0.00
Among lines within genotype	0.0683	18	0.0038	1.81*
Within lines	0.5330	254	0.0021	

*Significant at the 5% level.

Clearly there was no significant difference between the two genotypes. Thus, the $\underline{Y}$ chromosome either has little effect upon the overall sex ratio or it increases the sex ratio but its action is suppressed by the $\underline{+}$ second chromosome.

Further tests were carried out to distinguish the above two possibilities. Males of the genotype $X^{cb}/\underline{Y}$; cn bw/cn bw backcrossed for five generations to cn bw females were tested for the overall sex ratio. The results are summarized in Table XXIV.

TABLE XXIV. SUMMARY OF SR FOR TWO BACKCROSS GENERATIONS OF $\frac{X^{cb}}{\underline{Y}}$; $\frac{cn\ bw}{cn\ bw}$ MALES

Backcross generation	n*	ΣSR	Σ(SR)^2	$\overline{SR}$
2	137	68.09	34.21	0.497
5	131	65.62	33.18	0.501
Standard**	135	66.50	33.11	0.492

*Number of males tested.

**Standard cn bw males.

There was no difference in the sex ratio between the second and fifth generation ($F = 0.28$, $\nu_1 = n$, $\nu_2 = 266$, $p >> 0.05$). The data from the two generations were then pooled and the sex ratio compared with that of the standard of 0.492: The difference was not significant ($F = 1.28$, $\nu_1 = 1$, $\nu_2 = 401$, $p >> 0.05$). This result indicates that the Y chromosome has little effect upon the overall sex ratio.

The conclusions which may be drawn from this section may be summarized as follows:

1. The *X* chromosome tends to reduce the overall sex ratio. This is in agreement with the observations of Kataoka (1967).

2. Either the third or the fourth chromosome of the Odate population carries an element which causes an increase in the overall sex ratio.

3. The *Y* chromosome has little effect upon the overall sex ratio.

The segregation frequency of the Odate second chromosome

All of the Odate stocks used in this experiment were established three or four years ago and since then have been kept in separate culture vials. Thus, the average effect of the second chromosomes upon larval stage viability would be expected to be quite variable among lines. Since the recovery frequency of the second chromosomes in the progeny is dependent upon the viability differences among the genotypes, comparisons of the *k* values among lines, in relation to the value of θ, are less meaningful, but the relationships among the four genotypes X^{cb}/Y^{cb}; $\underline{+}$/cn bw, $X^{cb}/\underline{Y}$; $\underline{+}$/cn bw, X^{cb}/Y^{cb}; $\underline{+}$/cn bw and $\underline{X}/Y^{cb}$; $\underline{+}$/cn bw within a line may still provide some information.

In order to eliminate or at least to minimize the effect of viability differences among lines, an attempt was made in the following way: An average $\bar{\theta}$ and $\bar{k}$ of the four

TABLE XXV. SUMMARY OF $\Delta\theta$ AND Δk FOR THE FOUR GENOTYPES

	$\frac{X^{cb}}{Y^{cb}}; \frac{+}{cn\ bw}$		$\frac{X^{cb}}{Y}; \frac{+}{cn\ bw}$		$\frac{X^{cb}}{Y^{cb}}; \frac{+}{cn\ bw}$		$\frac{X}{Y^{cb}}; \frac{+}{cn\ bw}$	
Line	$\Delta\theta$	Δk	$\Delta\theta$	Δk	$\Delta\theta$	Δk	$\Delta\theta$	Δk
a-1	-0.153	-0.025	-0.005	0.017	0.100	0.005	0.057	0.001
B-2	0.027	0.012	0.047	0.012	-0.087	-0.018	0.012	-0.015
B-4	0.044	-0.003	-0.190	-0.025	-0.026	0.008	0.172	0.019
c-4	-0.012	-0.008	-0.045	-0.004	-0.047	0.002	0.104	0.009
d-1	0.022	0.006	-0.123	-0.020	0.002	0.019	0.097	-0.006
e-2	-0.228	-0.013	0.025	-0.019	0.169	0.027	0.033	0.004
f-2	-0.072	-0.015	0.004	0.015	0.105	-0.007	-0.038	0.006
f-9	0.067	-0.016	0.019	-0.005	-0.085	-0.002	0.000	0.024
k-5	0.097	-0.021	-0.246	0.024	-0.002	0.014	0.150	-0.016
m-1	0.023	0.008	-0.105	0.001	0.004	0.003	0.076	-0.013

genotypes were computed for each of the ten lines; then the two quantities $\Delta\theta i = \overline{\theta} - \theta i$ and $\Delta ki = \overline{k} - ki$ were computed for each genotype in each line. Thus averages of the $\Delta\theta i$ and Δki are zero for each line. Table XXV lists the values of Δki and $\Delta\theta i$ for each genotype in each line.

If these values are arranged according to the value of Δki regardless of the line and the genotype involved, we obtain the results shown in Table XXVI.

TABLE XXVI. RELATIONSHIP BETWEEN Δk AND $\Delta\theta$

Δk interval	$\Delta\theta$	n*
$\Delta k \geq 0.010$	0.0168	10
$0.010 > \Delta k \geq 0.000$	0.0115	11
$0.000 > \Delta k \geq -0.015$	-0.0081	11
$-0.015 > \Delta k \geq -0.025$	-0.0268	8

*n = number of lines.

Since some lines would not contain elements which caused deviations of θ from one, Table XXVI would be expected to be rather conservative, yet, there is a clear relationship between Δki and $\Delta\theta i$: Δki is positively correlated with $\Delta\theta i$. This is similar to the results obtained in the $\underline{stw}^3$ experiment.

Discussion for Chapter V

The analysis of samples from the Odate population indicated that an element causing non-random assortment between the second and the sex chromosomes was present in nature. The exact location of this element has not yet been determined. It is noteworthy that the Odate population contained a _Y_ chromosome which suppresses the action of the above element as well as an _X_ chromosome which enhances its action. These findings are quite similar to those discovered in the _stw_^{3} and _L_^{2} experiments and extends the generality of the phenomenon of non-random assortment.

It is interesting to note that in two of the ten lines tested in which the second chromosome caused non-random assortment, the enhancer _X_ and the suppressor _Y_ seemed to co-exist in rather high frequency (they may even be fixed). This finding suggests that in natural populations, the elements causing non-random assortment as well as elements which enhance or suppress this action can co-exist to regulate the non-random assortment of chromosome pairs.

The analysis of the overall sex ratio indicated that an _X_ chromosome which causes a low frequency of males in the next generation (a driven X) as well as elements located on either the third or the fourth chromosome which causes an increase in the frequency of males can co-exist in nature to regulate the overall sex ratio in a population.

There could be other elements in the Odate population regulating non-random assortment and the overall sex ratio, but they could not be detected in the present investigation either because of their small effects or because of their low frequency. The St(SD)-like element discussed by Kataoka (1967) could be one such element. The discovery of such elements in other populations would provide valuable information toward the understanding of how the phenomena discovered in the present investigation are operating in natural populations.

APPENDIX A

Two additional stocks were investigated and the results for θ are summarized in Table XXVII.

TABLE XXVII. SUMMARY OF U_θ AND K_θ FOR w-1 AND w-2.6

Genotype	n	U_θ	K_θ
$\frac{X^{cb}}{Y'}; \frac{+'}{cn\ bw}$	35	35.02	200.42
$\frac{X'}{Y^{cb}}; \frac{+'}{cn\ bw}$	35	-1.15	178.25
$\frac{X^{cb}}{Y^{2.6}}; \frac{+^{2.6}}{cn\ bw}$	29	-14.94	148.89
$\frac{X^{2.6}}{Y^{cb}}; \frac{+^{2.6}}{cn\ bw}$	35	10.34	190.07

W-1 and W-2.6 were originally isolated from a natural population in Japan many years ago and have been maintained in laboratory cultures since that time. Although the number of flies tested is very small, the genotype X^{cb}/Y^1; W-1/cn bw gave a value of θ significantly greater than one ($\chi^2 = 6.12$, d.f. = 1, $0.02 > p > 0.01$). This may extend the generality of the phenomenon.

APPENDIX B

The genetic mechanism causing non-random assortment and meiotic drive is not yet known. Hiraizumi and Nakazima (1967) proposed a hypothesis to explain this phenomenon for the _SD_ system: _SD_ and the X chromosome compete to reach the functional pole (Peacock and Erickson, 1963). Thus, when _SD_ reached the functional pole, the probability of the X chromosome also reaching this pole is reduced and hence _SD_ tends to assort with the Y chromosome. They also suggested that a part of the X chromosome and _SD_ are homologous and tend to "pair" in low frequency. This might be the cause of the competition. They further speculated that an element with meiotic drive which was originally located on the X chromosome was inserted into the centromeric region of the second chromosome; the inserted element then behaving as _SD_. Thus an _SD_-bearing second chromosome and an X chromosome which had retained the driven element would have homology. Under these conditions such an _X_ chromosome would be able to suppress the action of _SD_.

Kataoka (1967) investigated the _X_ chromosomes of the Odate population and found that 60-80% of the X chromosomes suppressed the action of _SD_. She then demonstrated that these suppressor X chromosomes showed, as would be expected on the competitive hypothesis, higher segregation frequencies.

A mechanism analogous to this hypothesis could also be invoked to explain the results of the present investigation: Nrs competes with the X chromosome to reach the functional pole. The X^{stw} chromosome is a weak competitor and the X^{cb} is a suppressor X for Nrs. Thus X^{cb}/Y^{cb}; $stw^3/cn\ bw$ will give $\theta = 1$ with a relatively low value of *k* while X^{stw}/Y^{cb}; $stw^3/cn\ bw$ will give a value of θ greater than one and since X^{stw} is a weak competitor, the *k* value will increase.

Whatever the actual mechanism, the present investigation demonstrated that the phenomenon of non-random assortment of chromosome pairs and the segregation frequency changes associated with it actually occurs.

It should be pointed out that the above phenomena were well established for the second and sex chromosomes, but that this does not preclude the possibility that other chromosome pairs may also exhibit non-random assortment.

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