AN ABSTRACT OF THE THESIS OF

Richard Charles Gethmann for the M. S. in Zoology
Name (Degree) (Major)

Date thesis is presented August 8, 1966

Title CROSSING OVER IN A REVERSED ACROCENTRIC ATTACHED-X CHROMOSOME OF DROSOPHILA MELANOGASTER

Abstract approved
(Major professor)

The frequency of exchanges was measured in a reversed acrocentric compound X chromosome that is deficient for interstitial heterochromatin. Previous studies on similar chromosomes containing interstitial heterochromatin have demonstrated a very low frequency of single exchanges, and high frequencies of the double and no exchange classes. It has been postulated that this abnormal distribution of exchanges is due to the interstitial heterochromatin that is part of the compound X's structure. The critical test of this explanation has not been done previously and is now provided by the present study.

Because the reversed acrocentric is lethal in the absence of the missing heterochromatin, duplications were used to cover the deficiency. Thus, the heterochromatin is present within the genome,

but as a separate chromosome. Three structurally different duplications were used. The results were as predicted by the explanation, the frequency of single exchanges is high, and the distribution of the exchanges is like that of free X chromosomes. There was no difference in the effect of the three duplications upon the exchange patterns.

CROSSING OVER IN A REVERSED ACROCENTRIC ATTACHED-X CHROMOSOME OF *DROSOPHILA MELANOGASTER*

by

RICHARD CHARLES GETHMANN

A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1967

APPROVED:

Redacted for Privacy

Associate Professor of Zoology
In Charge of Major

Redacted for Privacy

Chairman of Department of Zoology

Redacted for Privacy

Dean of Graduate School

Date thesis is presented August 8, 1966

Typed by Kay Smith

ACKNOWLEDGMENT

This thesis is dedicated to Dr. James D. Mohler, who suggested and directed this research, and without whose guidance and encouragement, the project could never have been completed.

I wish to extend my appreciation to Dr. George E. Brosseau, who provided information useful in interpreting some of the results, and to Dr. Steven R. Thompson and Mr. Patrick T. C. Wong, who provided criticism and advice, as well as supplying some of the stocks used in the study.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CROSSING OVER IN A REVERSED ACROCENTRIC ATTACHED-X CHROMOSOME OF *DROSOPHILA MELANOGASTER*

INTRODUCTION

A compound X chromosome is defined as two X chromosomes attached to the same centromere. There are six possible compound X chromosomes, all of which have been synthesized and described (Novitski, 1954). In Novitski's terminology, these chromosomes are named according to the order of the two X chromosomes (reversed or tandem), the centromere location (metacentric or acrocentric) and whether the structure is a ring or rod. For example, the attached-X chromosome of L. V. Morgan (1922) would be described as a reversed metacentric compound X chromosome. An extensive genetic analysis has been made of five of these six types (Sandler, 1954, 1957; Welsons, 1955; Sandler and Lindsley, 1963; Lindsley and Sandler, 1965), and the exchange patterns have been found to be comparable to that of free X chromosomes, with the exception of the reversed acrocentric (Sandler, 1954) and the reversed ring (Sandler, 1957). Sandler demonstrated that there is a very low frequency of single exchanges, while the double exchange class is near that of normal free X chromosomes. Furthermore, Sandler demonstrated that this cannot be due to any previously recognized factor in meiosis, such as chromatid interference

or strand preference. He also showed that a Y chromosome had the effect of doubling the frequency of homozygosis.

Although these two chromosomes are similar in structure, differing only in that the free end of the reversed acrocentric is attached to the centromere in the reversed ring, they are synthesized quite differently, and from different sources. Their common behavior, yet different origin, suggests that the abnormal exchange frequencies are due to some feature common to the structure of the two chromosomes. One such common feature is the presence of a block of interstitial heterochromatin, at the point of attachment of the two X chromosomes.

Sandler (1957, 1958) has speculated that the physical presence of this block of interstitial heterochromatin may be responsible for the observed exchange frequencies. In their model Sandler and Kastenbaum (1958) postulate that the physical basis of exchange starts at one end of the chromosome, and proceeds to the other end. At any given point along the chromosome, there exists a fixed probability, α, of being involved in an exchange. Once an exchange has occurred, the probability of a second exchange is now some lower value, β. However, in a reversed acrocentric and reversed ring, the block of interstitial heterochromatin has the effect of the first exchange, thereby reversing the probability of subsequent exchanges. Thus, the probability of the first exchange in these two compound

chromosomes is β. Sandler and Kastenbaum have estimated these two exchange probabilities, both from free X data and from the reversed compound chromosomes, and found that the calculated values of α and β were the same from the two different sources.

The heterochromatic regions of chromosomes are probably quite important to chromosomal behavior. For example, the phenomenon known as the interchromosomal effect is seen in the heterochromatic regions and crossing over in triploids is most pronounced in the heterochromatic regions. Sandler has postulated that it is the interstitial heterochromatin that is responsible for the exchange patterns and Y chromosome effect he has found in his reversed acrocentrics. It thus is of interest to study the exchange patterns of a reversed acrocentric deficient for any interstitial heterochromatin, not only as a test of Sandler's model, but also as a further study into the behavior of heterochromatin and its effect upon crossing over and disjunction.

MATERIALS AND METHODS

The reversed acrocentric (Figure 1) used in this study arose as a spontaneous chromosomal rearrangement in a triploid Drosophila melanogaster (Mohler, 1960). Although the cytology of this chromosome has yet to be examined, it is evident from its genetic behavior in triploids that it is a reversed acrocentric, in fact, its behavior cannot be readily explained by assuming any other type of a structure. In a triploid, with a sc^8 chromosome as the third X chromosome, the proximal region of the sc^8 chromosome crosses over regularly with the compound chromosome, but the distal region rarely crosses over with the reversed acrocentric (RA). The distally placed yellow locus has never been observed to crossover with the RA. If the third X chromosome is a normal chromosome, the distal region crosses over at a rate comparable to that of free X triploids (Neeley, 1963; Mohler, 1966) whereas exchange with the proximal region is nearly zero. These observations, plus the stability of the compound X, are all consistent with the interpretation that it is a reversed acrocentric.

The reversed acrocentric is lethal in diploids unless the deficiency is covered with duplications of X heterochromatin, including the suppressor-of-forked locus. The reversed acrocentric is maintained in diploid stocks by covering the deficiency with three

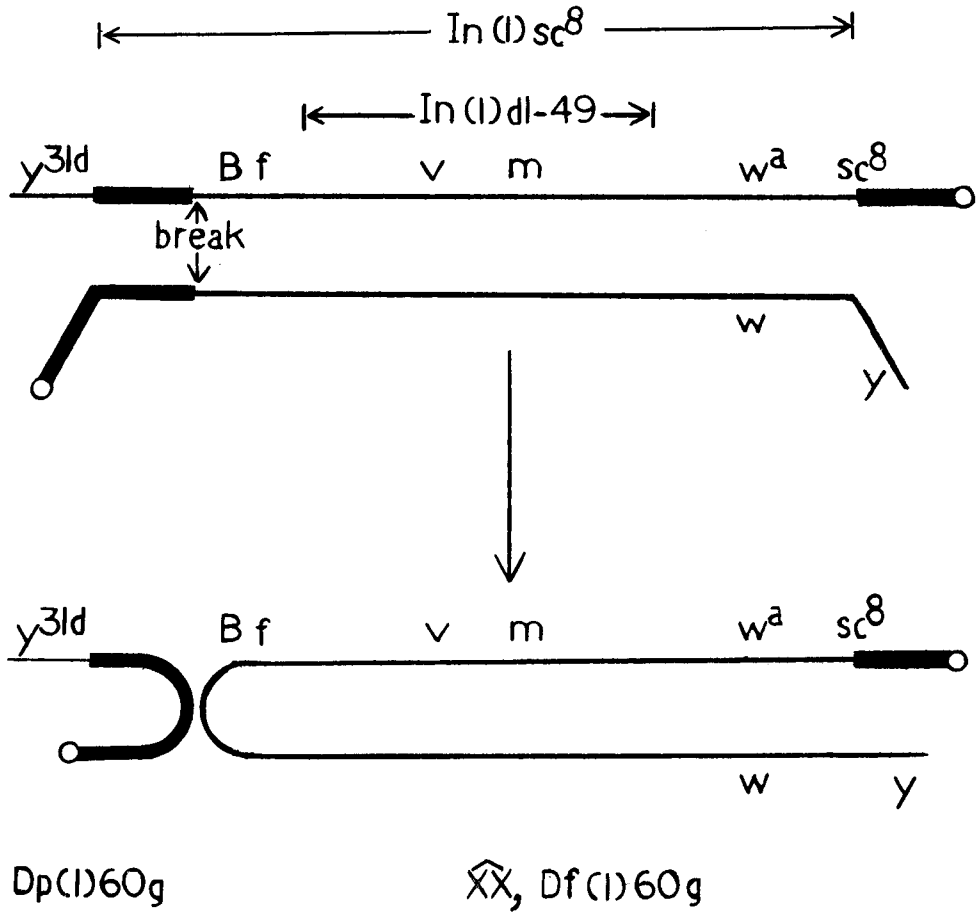

Figure 1. Diagram of proposed mechanisms by which the reversed acrocentric compound X chromosome was formed. A reverse exchange occurred between the scute-8, delta-49 chromosome and the structurally normal X chromosome. The point of breakage is indicated. The drawings represent the two strand stage for the sake of simplicity. The heterochromatin is represented by the thicker line. (Modified from Neeley, 1963)

different duplications. The first duplication is Dp(1;f)60 (Figure 1), which is the complement of the reversed acrocentric. The second duplication is Dp(1;f)65X^{c2}. This was produced by irradiation of the X^{c2} chromosome, and is marked by the normal alleles at the yellow, achaete, scute and suppressor-of-forked loci. The third duplication is Y, su^+-f (= Dp(1;Y), su^+-f) a male fertile Y chromosome marked by su^+-f from the X chromosome. It was produced by irradiation of T(XY^L. Y^S:4)B^S(16A1), X^D, $B^S$$Y^L$. Y^S males.

Two series of crosses were run, one series of flies heterozygous for the d149 inversion, and the other with inversion free flies. All three duplications were used in each series.

The procedure for selecting females heterozygous for the inversion is outlined in Table 2. Briefly, triploid females from the stock source were mated to yellow males. Heterozygous yB triploid daughters were selected and mated to males of the constitution $\overline{XY}$, y v f car/Dp65X^{c2} (see Table 1 for list of mutants used in this study). Diploid duaghters, heterozygous for d149, were selected and mated to $\overline{XY}$, y v/Dp60 males (Cross A-3). This constituted the first crossover test. Daughters of this cross were selected and mated to males of the constitution X, y/ Y, su^+-f (Cross A-4). This was the second test. The third test was made by selecting females over Y, su^+-f, and backcrossing these to Y, su^+-f males.

Because the stock sources of the RA all carried d149, the

Table 1. A list of mutants and chromosomal rearrangements used in this study (Bridges and Breheme, 1944, p. 97, 166, 238-240).

Mutants

Genetic symbol	Locus	Name of mutant	Character affected
ac	0.0-	achaete	bristle number
y, y^{31d}	0.0	yellow	body color
sc	0.0^{+}	scute	bristle number
w, w^{a}, sp-w	1.5	white, white-apricot spotted-white	eye color
cv	13.7	crossveinless	wing veination
v	33.0	vermilion	eye color
m	36.1	miniature	wing size
f	56.7	forked	bristle shape
B	57.0	Bar	eye shape
car	62.5	carnation	eye color
su-f	64.0	suppressor-of-forked	bristle shape

Chromosomal Rearrangements

In(1)sc^{8}	An X chromosome inversion, the left breakpoint is between ac and sc, right break point is between Block A of the heterochromatin and the spindle attachment. Shows a slight scute phenotype.
In(1)dl49	An X chromosome inversion, the left break point is between ruby and cv, the right break point is near garnet (44.4).

Table 2. Outline of the pedigree of females heterozygous for In(1)d149, used in the crossover tests. Females listed were selected from the previous cross.

Cross number	Phenotype of female	Genotype of female	Mated to males of the following genotype
A-1	3N, y^{31d} B v w^a	y w^a v · B f v m w^a/y^{31d} B f v m w^a	X, y
A-2	3N, y B	y w^a v · B f v m w^a/y	$\overline{XY}$, y v f car/$Dp65X^{c2}$
A-3	2N, B	y · B f v m w^a/$Dp65X^{c2}$	$\overline{XY}$, y v/Dp60
A-4	2N, y^{31d} B	y · B f v m w^a/Dp60	X, y/Y, su^+-f
A-5	2N, y B	y · B f v m w^a/Y, su^+-f	X, y/Y, su^+-f

inversion had to be removed by crossing over in triploids. The original plan was to make Cross B-1 (Table 3) until triploid females, homozygous for vermilion, crossveinless and spotted-white were recovered. However, triploid females homozygous for the region marked by crossveinless were sterile, so this approach had to be abandoned. The females for Cross B-2 (Table 3) were selected from sibling strains of the sterile homozygous females. The selection of inversion free RA triploid females was aided by the fact that crossovers involving the white locus could be recognized. In triploids, one dose of spotted-white (a pseudoallele of white), over two doses of white or white-apricot, can be distinguished from two doses of spotted-white over one dose of white. Therefore, crossovers distal to the white locus could be recognized. This was useful in selecting potential inversion free triploid females.

Diploid females for the crossover tests were collected in the same manner as were those with the inversion; $Dp65X^{c2}$ females were collected as progeny from triploid parents (Cross B-3), and Dp60 and Y, su^{+}-f females were selected as daughters from the previous crossover test (Crosses B-4 and B-5).

By conducting the crosses in this order, both the exchange patterns and the disjunctional patterns could be followed, except for females over Y, su^{+}-f. A separate test was set up to measure non-disjunction in these females. Y, su^{+}-f females were mated to

Table 3. Outline of the pedigree of females, lacking d149, used in the crossover tests. Females listed were selected from the previous cross.

Cross number	Phenotype of female	Genotype of female	Mated to males of the following genotype
B-1	3N, y^{31d} B v w^{a}	y w^{a} v · B f v m w^{a}/y^{31d} f v cv sp-w	y^{31d} f v cv sp-w sc^{8}
B-2	3N, y^{31d} B v sp-w	y w^{a} v · B f v cv sp-w/y^{31d} f v cv sp-w	X, y
B-3	3N, y B	y w^{a} v · B f v cv sp-w/y	$\overline{XY}$, y v f car/Dp65X^{c2}
B-4	2N, B	y · B f v cv sp-w/Dp65X^{c2}	$\overline{XY}$, y v/Dp60
B-5	2N, y^{31d} B	y · B f v cv sp-w/Dp60 y v · B f cv sp-w/Dp60 y cv v · B f sp-w/Dp60	X, y/Y, su^{+}-f
B-6	2N, y B	Same as Cross B-5, except over Y, su^{+}-f	X, y/Y, su^{+}-f

y cv v f car su-f males carrying a normal Y chromosome. Any females emerging from this cross would have to be non-disjunctional, since a normal Y chromosome does not cover the deficiency. Non-disjunction males would be recognized as those not receiving a normal allele of su-f.

All flies were raised on a standard agar, cornmeal and molasses media, supplemented with live yeast, and all crosses were made and raised in 25^{o}C. incubators. The crosses were made in vials, transferred two days later to half-pint bottles, and then subcultured for two days, three days, and three days for a total time of twelve days.

RESULTS

The consequences of exchanges within a reversed acrocentric have been worked out by Sandler (1954), and are given in Table 4 and Figure 2. From Table 4, it can be seen that one-fourth of the double exchanges about a mutant marker will result in homozygosis for the mutant. Also, one-fourth of all double exchanges and one-half of all single exchanges result in dicentric bridges. These bridges will occur at Anaphase II, and thus, will be lethal to the zygote. Therefore, the analysis of the frequency of exchanges is dependent upon two factors; 1) recovery of females homozygous for the mutant markers, and 2) an estimation of the size of the lethal class. The size of the lethal class can be estimated by the difference between the number of recovered females and males. If there are no lethals due to exchange or viability, and if the gametic products are produced equally, the number of male and female progeny should be the same. The frequency of the gametic types in the males used in the crosses was measured, and the results are listed in Table 1 of the Appendix. There is no significant deviation in the gametic types of Y, su^+-f males, however, Dp60 males showed a definite deviation after six days. Therefore, crosses using Dp60 males were counted for only the first six days, while crosses using Y, su^+-f males were counted over a 12 day period.

Table 4. The consequences of exchange of ranks 0, 1, and 2 in the reversed acrocentric compound X chromosome.

Rank and frequency	Crossovers involved	Products after Anaphase II separation
E_0	None	RA (v/+) and RA (v/+)
1/2 E_1	A or D	RA (v/+) and RA (v/+)
1/2 E_1	B or C	Bridge
1/4 E_2	A and C	RA (v/v) and RA (+/+)
1/4 E_2	A and D	RA (v/+) and RA (v/+)
1/4 E_2	B and C	RA (v/v) and RA (+/+)
1/4 E_2	B and D	Bridge

Those exchanges which produce products not different from the ones given in the table have been omitted when the omission does not alter the relative frequencies of the exchange products. E_0 = no exchange, E_1 = single exchange, E_2 = double exchange. The crossovers involved are shown in Figure 2 (From Sandler, 1954).

The frequency of double exchanges including any locus is directly estimated from the frequency of homozygosis. Since one-fourth of the double exchanges result in homozygosis for a mutant marker, the frequency of double exchanges would be four times the frequency of homozygosis. Once the frequency of double exchanges is known, the frequency of lethals resulting from double exchanges can be calculated, and subtracted from the overall lethal class. This will leave the frequency of lethal bridges due to single exchanges. Since one-half of the single exchanges result in lethals,

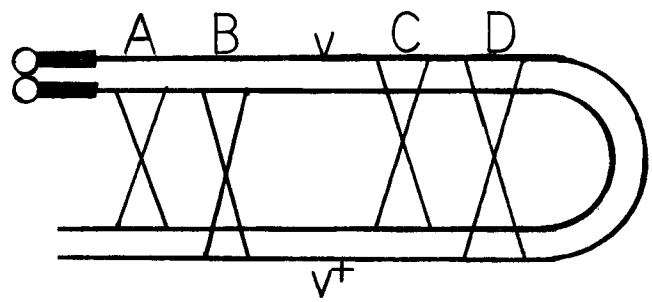

Figure 2. The genetically distinct types of exchange in the reversed acrocentric compound X chromosome. The consequences of all possible combinations of these exchanges are given in Table 4. (From Sandler, 1954)

the frequency of single exchanges is twice the frequency of single exchange bridges. The frequency of the no exchange class is calculated by subtracting the single and double exchange classes from the total.

The total number of homozygous recessive females recovered depends upon the linkage configuration of the reversed acrocentric. If the markers are linked cis (i.e., all linked on the same arm), the number of homozygotes recovered will be less than if the markers are linked non-cis. The effect is as follows. If two markers are in a cis linkage, and a double exchange includes both these markers, one chromatid would be homozygous for both mutants, while the other would be homozygous for the wild markers. If, however, the markers are on different arms, the double exchange will result in one chromatid homozygous for one mutant, and the other homozygous for the other mutant. Both of these would be counted as crossovers, instead of only one of them.

Progeny from cis linked females give the best estimate of the double exchange class, and all of Sandler's calculations are based upon progeny from females linked in cis. This was possible since his RA had a high no exchange class, and therefore, he would have many non crossover progeny from which to select his females for the crossover tests. However, in our case, it was impossible to collect enough data from cis linked females. Therefore, by counting

progeny from non-cis females, the calculated frequency of the E_2 class becomes an overestimation of the actual frequency of double exchanges, while the single and no exchange classes are underestimated.

Alternatively, if the frequency of homozygosis is calculated on the frequency of a single mutant, then the double exchange class will be underestimated, and the other two classes will be overestimated. Table 5 lists the frequency of homozygosis for the total number of crossovers, and for vermilion, which was the mutant most often recovered. Listed also is the female to male ratio.

Table 5. The frequency of total crossover females, the frequency of females homozygous for vermilion, and the female to male ratio. The homozygosis frequencies are calculated on the basis of total males recovered.

Cross	Frequency of total homozygotes	Frequency of vermilion	Female to male ratio
RA/Dp60 1st 6 days	4.84	3.23	0.48
RA/Dp60 2nd 6 days	5.28	3.34	0.49
RA/Y, su^+-f 1st 6 days	5.58	4.03	0.53
RA/Y, su^+-f 2nd 6 days	7.01	4.84	0.56
RA/Dp65X^{c2}	8.62	7.21	0.74
RA/Dp65X^{c2} Repeat	7.84	5.88	0.72

Using the data for the first six days of the Dp60 females (the types of progeny are given in Table 2 of the Appendix), the exchange frequencies can be calculated as follows. Since one-fourth of all double exchanges result in homozygosis for mutant markers, the frequency of double exchanges would be (4) (4.84) or 19.36%. The reduction in females is one minus the female to male ratio, or (1-0.48), which is 51.92%. Since one-fourth of all double exchanges result in lethal bridges, the frequency of single exchange bridges must be 51.92-4.84, or 47.08%. The frequency of single exchanges would be twice this value, or 94.16%. This leaves a minus 13.54% for the no exchange class. A similar analysis of the data, with the frequency of vermilion substituted to make the estimate of the double exchange class, gives the following exchange frequencies: E_0= -10.32, E_1 = 97.40, and E_2 = 12.92.

The calculated exchange frequencies for all the crossover tests are listed in Table 6, along with values taken from Sandler (1954). It is immediately evident that the reduction of single exchanges Sandler found in his reversed acrocentrics is not present in this one. Furthermore, it is evident from comparing the exchange frequencies calculated from the two different estimates of the frequency of double exchanges, that both methods give similar frequencies for the three exchange classes.

The exchange frequencies for Dp60 and Y, su^+-f females are

Table 6. The frequencies of exchange from the inversion free crossover tests, based on the frequency of recovered homozygous females, and on the frequency of vermilion. Frequencies are given as percentages. Included is data from Sandler (1954).

	Exchange frequencies calculated on the frequency of total homozygotes recovered			Exchange frequencies calculated on the frequency of vermilion		
	E_0	E_1	E_2	E_0	E_1	E_2
RA/Dp60 1st 6 days	-13.52	94.16	19.36	-10.32	97.40	12.92
RA/Dp60 2nd 6 days	-13.10	91.98	21.12	- 9.22	95.86	13.36
RA/Y, su^+-f 1st 6 days	- 4.24	81.88	22.36	- 1.12	85.00	16.12
RA/Y, su^+-f 2nd 6 days	- 2.36	74.64	28.08	2.00	78.64	19.36
RA/Dp65X^{c2}	30.40	35.12	24.48	33.22	37.94	28.84
RA/Dp65X^{c2} Repeat	27.84	40.80	31.36	31.76	44.72	23.52
ND34 (from Sandler)	78.0	5.4	16.4			
ND34/FR2 (from Sandler)	44.0	3.6	52.4			

similar, but there is a marked reduction of single exchanges in the $Dp65X^{c2}$ females, with no apparent change in double exchanges. Since the $Dp65X^{c2}$ females were collected from triploids, whereas the other two were selected from diploids, a second crossover experiment of $Dp65X^{c2}$ was run, to exclude the possibility of this effect being due to the triploid ancestry. The experiment was repeated as follows. Progeny from the Y, su^{+}-f series were crossed to $Dp65X^{c2}$ males, and the daughters from this cross were used for the repeat crossover test. This cross is labeled as $Dp65X^{c2}$-repeat, and again, the apparent reduction of the single exchange class is found. Thus the reduction of single exchanges does not appear to be due to the triploid ancestry.

Since the single exchange class is calculated from the reduction in females, the female:male ratio also reflects this apparent reduction of single exchanges. The female:male ratio for Dp60 and Y, su^{+}-f crosses is about 0.5, while the ratio for $Dp65X^{c2}$ is 0.73. This difference in the sex ratio is also seen in the dl49 series of crosses (Tables 5, 6, and 7 of the Appendix). The female:male ratio for $Dp65X^{c2}$ is 1.0, and for Dp60 and Y, su^{+}-f, the ratio is 0.80.

There are two possible explanations for the sex ratio difference between $Dp65X^{c2}$ and the others. The first explanation is that $Dp65X^{c2}$ actually reduces single exchanges. The alternate is that there is no reduction of single exchanges, but that $Dp65X^{c2}$ is lethal

to a fraction of the zygotes. In this case, the zygotes affected would be the males, and fewer would be recovered than expected. This would lead to a higher female:male ratio, and would lead to an incorrect estimate of the single exchange class.

The following test was set up to test the second of these two possibilities. Reversed metacentric (RM) females over $Dp65X^{c2}$ were mated to both types of males used in the crossover tests. Since there are no lethal exchanges in a reversed metacentric, a reduced number of males would indicate a reduced viability associated with the ring duplication. The data is given in the first two lines of Table 7, and there is an obvious discrepancy depending on which male source was used. There is a reduction of males when the RM is crossed to Dp60, but not when crossed to Y, su^{+}-f. Furthermore, if the RM over Dp60 is mated to Y, su^{+}-f males, there is an excess of males.

Table 7. Progeny and sex ratio of crosses of the reversed metacentric.

Cross			Female progeny	Male progeny	Female to male ratio
Female		Male			
RM/$Dp65X^{c2}$	X	Dp60	439	333	1.32
RM/$Dp65X^{c2}$	X	Y, su^{+}-f	756	731	1.03
RM/Dp60	X	Y, su^{+}-f	1243	1923	0.65

These findings, along with the sex ratios of all the crosses,

are summarized in Table 8. First, in the RA series, the different sex ratio is found when the RA is over $Dp65X^{c2}$, and is independent of the male source. In the RM over $Dp65X^{c2}$, there is an apparent reduction when Dp60 males are used, but not when Y, su^{+}-f males are used. There is a reduction of females in the RM/Dp60 crosses.

Table 8. Sex ratios of all crosses of compound X chromosomes.

Cross	Female genotype	Male genotype	Female to male ratio
1	RAd149/$Dp65X^{c2}$	$\overline{XY}$/Dp60	1.05
2	RAd149/$Dp65X^{c2}$	X/Y, su^{+}-f	1.00
3	RAd149/Dp60	X/Y, su^{+}-f	0.78
4	RAd149/Y, su^{+}-f	X/Y, su^{+}-f	0.82
5	RA/$Dp65X^{c2}$	$\overline{XY}$/Dp60	0.74
6	RA/$Dp65X^{c2}$	X/Y, su^{+}-f	0.72
7	RA/Dp60	X/Y, su^{+}-f	0.49
8	RA/Y, su^{+}-f	X/Y, su^{+}-f	0.54
9	RM/$Dp65X^{c2}$	$\overline{XY}$/Dp60	1.32
10	RM/$Dp65X^{c2}$	X/Y, su^{+}-f	1.03
11	RM/Dp60	X/Y, su^{+}-f	0.65

The apparent ambiguity of the reversed metacentric data can be cleared up once it is recognized the Y, su^{+}-f chromosome has a reduced viability effect upon reversed metacentric zygotes. This

is shown in the sex ratio of Cross 11 of Table 8. When the viability effect of the Y chromosome is taken into account in Cross 10, the corrected sex ratio shows a reduction of males, similar to that of Cross 9. Thus, Crosses 9 and 10 demonstrate a reduced viability of the ring duplication. The behavior of the Y duplication is similar to that of a class of Y chromosomes known as "killer Y's" (Brosseau, 1966). These Y chromosomes have a lethal effect in sensitive strains of Drosophila. Sensitivity to this killing effect is common among laboratory stocks, and it appears that the reversed metacentric has a sensitive genotype, but the reversed acrocentric does not.

This conclusion of the viability effect of $Dp65X^{c2}$ was demonstrated by the following event. Triploid females containing compound X chromosomes often will pick up an extra Y chromosome. Diploid RA females, containing both $Dp65X^{c2}$ and a Y chromosome were recovered. In crosses of these females, the duplication segregated at random with the RA. This resulted in a high rate of nondisjunction of the duplication. Since the duplication was distributed equally to both female and male progeny, the single exchange class should not have the lower single exchange frequency, because the viability effect should operate in both female and male progeny, thus its effect would not be seen. Table 9 lists the progeny from these females, along with the calculated exchange frequencies. It can be seen that the duplication did segregate at random, and the reduction

of the single exchange class is not seen.

Table 9. Progeny from RA/Y/Dp65X^{c2} females, mated to $\overline{XY}$, yv/Dp60 males. All females were heterozygous for cv, v, and f.

Regular heterozygous females	304	$E_0 = -2.36$
Exceptional heterozygous females	276	$E_1 = 76.08$
Regular homozygous females	37	$E_2 = 26.28$
Exeptional homozygous females	41	
Total females	658	
Regular males	469	
Exceptional males	719	
Total males	1188	

Sandler demonstrated that a Y chromosome has the effect of doubling the frequency of homozygosis in his reversed acrocentrics. Since there is some ambiguity in measuring the frequency of homozygosis, due to the non-cis linked females used in the crossover tests, the Y chromosome effect can best be compared by comparing the frequencies of homozygosis of the individual mutants. Table 10 lists the frequencies of homozygosis for the mutants used in the crossover tests. Included are two lines from Sandler (1954). The Dp60 series is equivalent to Sandler's ND34, since neither contain any Y chromosome material. Y, su^{+}-f is equivalent to his ND34/FR2,

Table 10. The frequency of homozygosis of mutants used in the crossover tests. Calculations are based on the number of recovered females, and are given as percentages. Included is data from Sandler (1954, Table 11).

Cross	f	v	cv	sp-w
RA/Dp65X^{c2}	4.46	9.77	3.40	0.21
RA/Dp65X^{c2} Repeat	4.19	8.20	2.73	0.00
RA/Y, su^{+}-f 1st 6 days	3.56	7.54	3.33	0.36
RA/Y, su^{+}-f 2nd 6 days	4.38	8.68	2.95	0.25
RA/Dp60 1st 6 days	2.75	6.73	2.52	0.35
RA/Dp60 2nd 6 days	3.38	6.85	2.08	0.35
ND34 (Sandler)	2.56	1.56	0.56	----
ND34/FR2 (Sandler)	7.11	8.64	2.81	----

since both contain Y heterochromatin as a pairing partner to the RA. The difference that Sandler observed with and without a Y chromosome is absent in this reversed acrocentric, in fact, there is no significant difference between the frequencies of homozygosis for the individual mutants. The frequencies of homozygosis are comparable to Sandler's FR2 series with the exception of the region marked by forked. However, since our RA was deficient for the

interstitial heterochromatin and Sandler's was not, his reversed acrocentric would contain a longer region distal to forked. Since an exchange is required in this region to recover forked homozygous, his RA would be expected to have a higher frequency for distal markers because of the longer region available for an exchange.

Another comparison that can be made is the frequency of non-disjunction between the different duplications, and between the inversion and inversion free crosses. The Grell hypothesis (R. F. Grell, 1962a, 1962b) postulates two distinct types of chromosomal pairing at meiosis. The first of these she calls exchange pairing. Only homologous chromosomes can enter into exchange pairing. The second pairing is called distributive pairing, and non-homologous chromosomes may pair for distribution, provided neither has undergone an exchange. If two homologs have undergone an exchange, they are automatically paired for distributive pairing. In a compound X chromosome, a pairing partner for the compound might be expected to segregate at random when the compound has undergone an exchange. This would be recognized by a high frequency of non-disjunction. E. H. Grell (1963) has studied distributive pairing in compound X chromosomes, and has reported that pairing partners regularly disjoin from the compounds, regardless of whether or not the compound had undergone an exchange. Table 11 lists the frequencies of non-disjunction for the inversion and inversion free

series, and it can be seen that there is no high frequency of non-disjunction associated with the high frequency of exchange found in the inversion free series. Thus, these findings are in agreement with those of Grell.

Table 11. Frequency of non-disjunction of all RA crosses. Data is pooled where disjunction frequencies are the same.

Duplication	Compound X			
	RAd149		RA	
	females	males	females	males
$Dp65X^{c2}$	0.052	0.147	0.054	0.228
Dp60	0.003	0.020	0.011	0.129
Y, su^{+}-f	0.002	0.038	0.007	0.041

DISCUSSION

The high frequency of single exchanges found in this reversed acrocentric confirm Sandler's hypothesis that the block of interstitial heterochromatin was responsible for the reduction of single exchanges. Also, it was demonstrated that the presence of a Y chromosome had no effect upon the frequency of double exchanges. Certain modifications of Sandler's analysis of the exchanges were necessary in this analysis. The location of the mutants relative to one another (i. e., cis versus non-cis linkage) was considered, and it was demonstrated that this did not greatly bias the final calculated frequencies.

There are, of course, other events which could have lead to an erroneous conclusion as to the frequencies of exchange. These include a reduced viability of either the duplications or the reversed acrocentric, non-random disjunction, or non-random exchange between the strands. A reduced viability was demonstrated for the ring duplication, and it was shown how this could have lead to an erroneous conclusion as to the effect of the ring duplication upon the exchange patterns.

Non-random disjunction involves an unequal production of the two gamete types. An excess of the duplication bearing gametes over those bearing the reversed acrocentric would lead to an overestimation of the reduction in females. A reduced viability of the

reversed acrocentric would have a similar effect. Although the negative no exchange class provides indirect evidence for this, neither event could be great enough to reduce the single exchange class to values comparable to those Sandler found. This is shown as follows. If one were to assume d149 eliminated all exchange, and then to assume the 20% reduction in females found in the d149 series measured either non-random disjunction or a viability effect of the reversed acrocentric, the resultant calculated frequency of the single exchange class would still be high, ranging from 50% to 75%.

The third possibility for overestimating the single exchange class would be if the strands involved in the exchanges were not randomly distributed. In the case of double exchanges this would be chromatid interference, in the case of single exchanges it would be strand preference. Both of these would have to operate such that there would be an excess of exchange types which produce anaphase bridges. However, if this were the case, then there should be a high frequency of the no exchange tetrads. Since many of the females used for the crossover tests were selected from progeny of other crossover tests, this possibility can be checked by observing how many of the females were linked in the same linkage configuration as their mothers. A check of this shows that all of the females linked similar to their mothers can be accounted for by the non

exchange chromosomes from single and double exchanges.

Thus, the presence of a large block of interstitial heterochromatin reduces single exchanges, and its absence allows a normal distribution of exchanges. This leads one to speculate as to the nature of this event, and ask whether the frequency of single exchanges is quantitatively related to the amount of heterochromatin, or is this effect due to a single element within the heterochromatin?

BIBLIOGRAPHY

Brosseau, G. E. 1966. Unpublished research on Y killer chromosomes. Iowa City, University of Iowa, Department of Zoology.

Grell, E. H. 1963. Distributive pairing of compound chromosomes in females of Drosophila melanogaster. Genetics 48:1217-1229.

Grell, R. F. 1962a. A new hypothesis on the nature and sequence of meiotic events in the female of Drosophila melanogaster. Proceedings of the National Academy of Sciences 48:165-172.

Grell, R. F. 1962b. The role of distributive pairing. Genetics 47: 1737-1754.

Lindsley, D. L. and L. Sandler, 1965. Meiotic behavior of tandem metacentric compound X chromosomes in Drosophila melanogaster. Genetics 51:223-245.

Mohler, J. D. 1960. New mutants. Report of J. D. Mohler. Drosophila Information Service no. 34:52.

Mohler, J. D. 1966. Unpublished research on crossing over in triploids. Corvallis, Oregon State University, Department of Zoology.

Morgan, L. V. 1922. Non-crisscross inheritance in Drosophila melanogaster. Biological Bulletin 42:267-274.

Neeley, J. C. 1963. Crossing over in a reversed acrocentric X chromosome in triploid Drosophila melanogaster (Meigen). Master's thesis. Corvallis, Oregon State University. 58 numb. leaves.

Novitiski, E. 1954. The compound X chromosomes in Drosophila. Genetics 39:127-140.

Sandler, L. 1954. A genetic analysis of reversed acrocentric compound X chromosomes in Drosophila melanogaster. Genetics 39:923-942.

Sandler, L. 1957. The meiotic behavior of reversed compound ring X chromosomes in Drosophila melanogaster. Genetics 42: 764-782.

Sandler, L. 1958. Genetic studies on exchange in the compound X chromosomes of Drosophila melanogaster. Cold Spring Harbor Symposia on Quantitative Biology 23:211-223.

Sandler, L. and M. Kastenbaum. 1958. A note on the frequency distribution of tetrads by rank in Drosophila melanogaster. Genetics 43:215-222.

Sandler, L. and D. L. Lindsley. 1963. The meiotic behavior of tandem acrocentric compound X chromosomes in Drosophila melanogaster. Genetics 48:1533-1543.

Welshons, W. J. 1955. A comparative study of crossing over in attached-X chromosomes of Drosophila melanogaster. Genetics 40:918-936.

APPENDIX

APPENDIX

Table 1. Progeny from $\overline{XY}$, yv/Dp60 males and Y, su^+-f males, mated to XX, y w females.

Age of males in days	Progeny of Dp60 males		Progeny of Y, su^+-f males	
	females	males	females	males
1-2	653	684	163	190
3-4	1492	1511	498	523
5-6	835	877	480	451
7-8	262	477	359	339
9-10	236	491	395	411
11-12	370	600	446	461
13-14	----	----	373	353

Table 2. Progeny from RA females over Dp60, mated to X, y/Y, su^+-f males. All females were heterozygous for cv, v, and f.

Type of progeny	1st 6 days	2nd 6 days
Regular heterozygous females	1525	1013
Exceptional heterozygous females	12	15
Regular homozygous females	170	123
Exceptional homozygous females	2	2
Total females	1709	1153
Regular males	3118	2039
Exceptional males	437	327
Total males	3555	2366

Table 3. Progeny from RA females over Y, su^+-f, mated to X, y/Y, su^+-f males. All females were heterozygous for cv, v, and f.

Type of progeny	1st 6 days	2nd 6 days
Heterozygous females	1508	1037
Homozygous females	176	149
Total females	1684	1186
Total males	3150	2124

Table 4. Progeny from RA females over $Dp65X^{c2}$, mated to either $\overline{XY}$, y v/Dp60 males, or X, y/Y, su^+-f males. All females were heterozygous for cv, v, and f.

Type of progeny	1st test	Repeat
Regular heterozygous females	390	466
Exceptional heterozygous females	26	23
Regular homozygous females	54	55
Exceptional homozygous females	1	5
Total females	471	549
Regular males	469	613
Exceptional males	169	152
Total males	638	765

Table 5. Progeny from RA females, heterozygous for In(1)d149, over Dp60, mated to X, y/Y, su^+-f males.

Type of progeny	1st 6 days	2nd 6 days
Regular heterozygous females	531	442
Exceptional heterozygous females	0	3
Regular homozygous females	1	0
Total females	532	445
Regular males	644	577
Exceptional males	8	17
Total males	652	594

Table 6. Progeny from RA females, heterozygous for In(1)d149, over Y, su^+-f, mated to X, y/Y, su^+-f males.

Type of progeny	1st 6 days	2nd 6 days
Heterozygous females	1077	603
Homozygous females	0	0
Total females	1077	603
Total males	1303	758

Table 7. Progeny from RA females, heterozygous for In(1)d149, over $Dp65X^{c2}$, mated to either $\overline{XY}$, y v/Dp60 males or X, y/Y, su^+-f males.

Type of progeny	X Y, su^+-f male 1st 6 days	X Y, su^+-f male 2nd 6 days	X Dp60 males 1st 6 days
Regular heterozygous females	1072	546	786
Exceptional heterozygous females	49	37	45
Regular homozygous females	0	0	0
Total females	1121	583	831
Regular males	979	480	674
Exceptional males	146	107	116
Total males	1146	587	790

Table 8. Progeny from RA females, either with or without In(1)d149, over Y, su^+-f, mated to y cv v f car su-f males.

Cross	Type of progeny: Exceptional females	Regular males	Exceptional males
RA, d149/Y, su^+-f 1st 6 days	2	643	27
RA, d149/Y, su^+-f 2nd 6 days	0	459	16
RA/Y, su^+-f 1st 6 days	4	801	32
RA/Y, su^+-f 2nd 6 days	2	665	31