

A GENETIC ANALYSIS OF REVERSED ACROCENTRIC
COMPOUND X CHROMOSOMES IN *DROSOPHILA*
*MELANOGASTER*¹

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TWO X chromosomes in *Drosophila* can be joined together to produce a single chromosome which is called a compound X chromosome, the best known example of which is the attached-X chromosome. There are five other types of compound X chromosomes possible, however, since the two component chromosomes may either be in tandem or reversed order with respect to one another; the centromere may either be between them or at one end; and the compound may either be closed to form a ring or not. All six of the possible compound X chromosomes have now been synthesized (NOVITSKI 1954). Of these six, however, only two, the attached-X and the tandem metacentric (equals tandem attached) X, have been analyzed to any great extent.

X-ray induced reversed acrocentric compound X chromosomes, known also as double X chromosomes, were first described by MULLER (1943, 1944) and by VALENCIA, MULLER and VALENCIA (1949), who pointed out the consequences of crossing over in this type of compound. The reversed acrocentric compound pairs with itself in the same way as a simple attached-X chromosome, but differs from the attached-X in that the centromere is located at the free end of one of the components. The chromosomes produced by these workers were limited in their usefulness for purposes of analysis, however, since one of the component chromosomes carried an inversion, dl-49. The detection of spontaneous reversed acrocentric compound X chromosomes, and crossover data from these, have been reported by NOVITSKI (1954).

The present work represents an analysis of the origin and meiotic behavior of spontaneous reversed acrocentric compound X chromosomes. It will be shown below that a large proportion of the newly arisen compounds are of such a nature as to exclude their origin by a simple event; other possibilities will be discussed. In addition, it will be shown that crossing over within the compounds is markedly different from that which would be predicted on the basis of studies of other compounds and unattached X chromosomes in that there appears to be a pronounced deficiency of single, as compared with double, exchanges.

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ORIGIN OF REVERSED ACROCENTRICS

Production of reversed acrocentrics

The general method for detecting compound X chromosomes is to mate a female, heterozygous for the chromosomes to be attached, to a male carrying some sex-linked dominant marker, as, for instance, *B*. The regular female class will be heterozygous *B*, but there will appear occasional exceptional females, that is, non-*B*. These females carry both X chromosomes from the parental female. Upon testing, some of these will prove to be simple non-disjunctive females, while others may carry the two X chromosomes attached. The type of compound produced will depend, of course, upon the chromosomes carried by

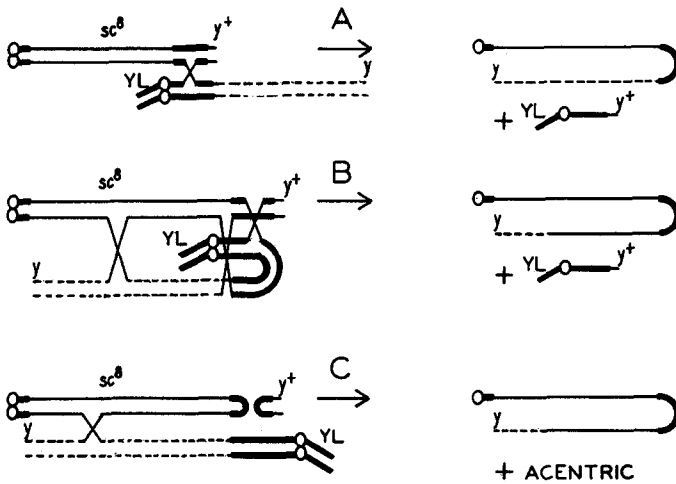


FIGURE 1.—Possible modes of origin of reversed acrocentric compound X chromosomes from a combination of $In(1)sc^8$ and a chromosome in normal sequence carrying the long arm of the Y chromosome (YL) attached to the centromere. Event C is sister-strand union. It is shown occurring in the distal heterochromatic section of the sc^8 chromosome, but it could presumably occur in the proximal heterochromatic region of the chromosome in normal sequence instead.

the parental female. A method for producing reversed acrocentric compound X chromosomes has been described by NOVITSKI (1954). It consists of attaching $In(1)sc^8$ (a long inversion of the X chromosome which places a considerable section of the basal heterochromatin including *bb* and Block A distally) and a chromosome in normal sequence carrying appropriate markers. The simplest way in which such an attachment might occur is shown in line A of figure 1. Since the compounds produced can be shown to be hemizygous for the *y* locus, it appears as though the attachment occurs in such a way as to exclude both the centromere region of the chromosome in normal sequence and the distal uninverted section of the sc^8 chromosome from the resulting compound. The results from four experiments designed to produce reversed acrocentrics are given in table 1. The genetic constitution of twelve of the fifteen compounds recovered was determined immediately after the compounds were obtained. The results of these determinations are given in table 2.

TABLE 1

The results from four crosses made for the purpose of detecting spontaneous reversed acrocentric compound X chromosomes. The parental males in every case carried the attached XY (= YSX.YL), yB chromosome. Experiment II, and all subsequent data from lines ND1 and ND9, are taken from Novitski (1954).

Experiment number	Constitution of parental ♀	Number of regular ♀♀	Exceptional females		
			Non-disjunctional	Sterile	Compound
I	$\frac{sc\ cv\ v\ /car}{sc^a}$	15,575	4	0	0
II	$\frac{sc\ cv\ v\ / (C-2).YL}{sc^a}$	8,897	0	5	4
III	$\frac{sc\ cv\ v\ / .YL}{sc^a}$	9,972	5	3	5
IV	$\frac{y\ cv\ v\ /car.YL}{sc^a}$	35,836	2	3	5*

* One of the females included in this figure was lost before tests could be made. It is included here as a compound because it was phenotypically *y cv v / car*.

Although it is true that theoretically other kinds of compounds (e.g., attached-X's) could be produced from these experiments, it is possible, upon testing, to distinguish reversed acrocentrics from these (see below). All of the compounds which were tested have proved to be reversed acrocentrics.

Event giving rise to reversed acrocentrics

Since homozygosis for loci in reversed acrocentrics requires a double exchange or one of higher rank (MULLER 1943), and since, as will be shown below, the frequency of homozygosis in reversed acrocentrics is low in general, it seems most probable that the homozygosis exhibited by the seven of the

TABLE 2

The genetic constitution of females carrying newly formed reversed acrocentric compound X chromosomes. The Roman numerals in parentheses refer to the experiment number as given in table 1.

Line	Parental female heterozygous for	Constitution of compound
ND1	<i>cvv</i> / (II)	<i>cv</i> / +, <i>v</i> / +, + / +
ND9	<i>cvv</i> / (II)	+ / +, + / +, + / +
ND18	<i>cvv</i> / (III)	<i>cv</i> / +, <i>v</i> / +, <i>f</i> / +
ND23	<i>cvv</i> / (III)	<i>cv</i> / +, <i>v</i> / +, <i>f</i> / +
ND25	<i>cvv</i> / (III)	<i>cv</i> / +, <i>v</i> / +, <i>f</i> / <i>f</i>
ND27	<i>cvv</i> / (III)	<i>cv</i> / +, <i>v</i> / <i>v</i> , <i>f</i> / <i>f</i>
ND28	<i>cvv</i> / (III)	<i>cv</i> / +, <i>v</i> / +, <i>f</i> / +
ND33	<i>cvv</i> / <i>car</i> (IV)	<i>cv</i> / +, <i>v</i> / +, <i>f</i> / <i>f</i> , <i>car</i> / <i>car</i>
ND34	<i>cvv</i> / <i>car</i> (IV)	<i>cv</i> / +, <i>v</i> / +, <i>f</i> / +, <i>car</i> / +
ND35	<i>cvv</i> / <i>car</i> (IV)	<i>cv</i> / <i>cv</i> , <i>v</i> / <i>v</i> , <i>f</i> / <i>f</i> , <i>car</i> / <i>car</i>
ND36	<i>cvv</i> / <i>car</i> (IV)	<i>cv</i> / +, <i>v</i> / <i>v</i> , <i>f</i> / <i>f</i> , <i>car</i> / <i>car</i>
ND37	<i>cvv</i> / <i>car</i> (IV)	<i>cv</i> / +, <i>v</i> / +, <i>f</i> / +, <i>car</i> / +

twelve tested compounds (see table 2) occurred coincidentally with their formation. If the compounds were formed in the manner indicated by line A of figure 1, it would require at least a double exchange in addition to the heterochromatic exchange giving rise to the compound to account for this homozygosis. The heterochromatic exchange, furthermore, would have had to take place in a region which was paired in an inversion configuration. Such an event is diagrammed in line B of figure 1. In each instance, moreover, were this the case, one of the exchanges responsible for the homozygosis would have had to take place between the marker nearest the interstitial heterochromatin (*f* in experiments II and III; *car* in experiment IV) and the exchange giving rise to the compound. This is so for the reason that when any locus was homozygous, all of the loci between that one and the interstitial heterochromatin were also homozygous (see table 2). That such a high proportion of the compounds would be produced by triple exchanges having a non-random distribution along the length of the chromosome seems extremely unlikely, and it appears more reasonable, therefore, to assume that reversed acrocentrics arise in two distinctly different ways: (1) by a simple exchange in the heterochromatic regions of the chromosomes (as shown in line A of figure 1) giving rise to those compounds heterozygous for all of the markers which were heterozygous in the parental female; and (2) by a more complicated event which may be thought of simply as the union of sister chromatids in the heterochromatin, plus a single exchange in the euchromatin (as diagrammed in line C of figure 1) giving rise to those compounds homozygous for all of the loci between the euchromatic single exchange and the interstitial heterochromatin. This sister-strand union can apparently occur either in the distal heterochromatic section of the sc^8 chromosome or in the proximal heterochromatin of the chromosome in normal sequence, since some of the compounds recovered were homozygous for alleles carried on the sc^8 chromosome and others were homozygous for alleles on the chromosome in normal sequence.

Another argument can be applied in support of the assumption that compounds showing homozygosis at the time of their origin arise from sister-strand union. Since the base of the sc^8 chromosome, which provides the centromere region of the compounds, lacks the *bb* locus and the nucleolus organizing region (*NO*), these two regions must be present in the interstitial heterochromatin of the recovered compounds for the following reason. The parental males carried the attached XY (= YSX.YL) chromosome (LINDSLEY and NOVITSKI 1950), which is a single chromosome containing the essential elements of the X chromosome plus the fertility factors of the Y chromosome. The compound-bearing females recovered, consequently, did not carry a Y chromosome which would cover heterochromatic deficiencies. Therefore, if a compound arises from sister-strand union, the event would have to take place distal to *bb* and *NO* in the sc^8 chromosome or proximal to these regions in the chromosome in normal sequence in order for the compound-bearing females to be viable. Experiment II, table 1, is of special interest in this connection. The chromosome in normal sequence carries the C-2 deficiency (NOVITSKI

1952), which is a deficiency for some of the basal X chromosome heterochromatin including *bb* and presumably *NO*. Because this is the case, sister-strand union in this chromosome could not give rise to viable products. Since, in experiment II, the C-2 chromosome carried the mutant alleles in the parental female, any compounds recovered from this cross which showed homozygosis would have had to be homozygous for wild-type alleles only, if the assumption of sister-strand union is correct. Two of the compounds recovered from this cross were tested. One of these (ND1) was homozygous for the wild-type allele of *f*; another (ND9) was homozygous for the wild-type alleles of *cv*, *v* and *f*; and there were four others which, although they were not tested sufficiently to determine which markers were heterozygous, nonetheless were not homozygous for mutant alleles.

Still another argument in favor of the view that compounds showing homozygosis at the time of their origin result from sister-strand union is as follows. If the event giving rise to the compounds were an ordinary exchange between the distal heterochromatin of the *sc*⁸ chromosome and the proximal heterochromatin of the chromosome in normal sequence, there would necessarily be produced, as a complementary product, a chromosome fragment carrying the centromere of the chromosome in normal sequence (which has the long arm of the Y chromosome attached to it), plus the uninverted section of the *sc*⁸ chromosome including the normal allele of *y* (see lines A and B of figure 1). Irrespective of whether this event is meiotic or mitotic, the fragment should be recoverable either in the compound-bearing females or in patroclinous males. The latter type would be detectable as non-*y* (and hence fragment-bearing), *B* males; the former type of course would have routinely been tested, since they would be exceptional females. Two such fragments have been reported from experiments similar to those described here (NOVITSKI 1954), but no such fragments have been recovered from experiments III and IV, although from these same experiments a total of ten compound-bearing females were recovered.

This discrepancy between the number of fragments recovered as compared with the number of compounds recovered is consistent with the assumption that sister-strand union is responsible for a large proportion of the compounds, since under this assumption the fragments would be eliminated as acentrics (see line C of figure 1), and is inconsistent with the alternative that simple exchange in the heterochromatic regions of the chromosomes to be combined accounts for most of the compounds.

Further information relative to sister-strand union

In all of the experiments described above, the only place sister-strand union could have occurred and given rise to recoverable products was in the heterochromatic regions such that *bb* and *NO* were included in the resulting compounds. In order to test whether the event is limited to these regions or not, it becomes necessary to be able to recover compounds carrying homozygous deficiencies for regions in and around the interstitial heterochromatin. For this

purpose the following cross was made. Females heterozygous for $In(1)sc^8$, $cv\ v\ f$ and a chromosome in normal sequence carrying the markers $y\ w$, the $C-2$ deficiency (for description see above), and the long arm of the Y chromosome attached to the centromere ($= sc^8, cv\ v\ f/y\ w\ (C-2).YL$) were mated to males carrying $T(1,4)B^S$. The right end of the B^S translocation is a chromosome fragment consisting of the centromere of the X chromosome, all of the basal heterochromatin, and the euchromatin up to and including the B locus. The fragment is marked by the dominant allele B . In this cross, compound-bearing females could be recognized since they would be phenotypically y . Compounds which were deficient for any of the heterochromatic regions, or euchromatic regions adjacent to the interstitial region, would be recoverable when fertilization was effected by a sperm carrying the B^S duplication. Compounds which did not receive the B^S duplication would receive a Y chromosome and might therefore still be recoverable as homozygous deficiencies for heterochromatic regions.

TABLE 3

The results of tests of y (and, hence, compound-bearing) female progeny from a mating of $yw(C-2).YL/sc^8, cv\ v\ f$ ♀♀ × $T(1,4)B^S$ ♂♂. The total of ten lines includes one cluster of four (ND39) and one cluster of two (ND41).

Line	Phenotype when obtained	Constitution	Viable as XX/Y	Viable as XX/O
ND39a	$y\ B^S$	Sterile	?	?
ND39b	$y\ B^S$	$cv/+,\ v/+,\ +/+$	+	-
ND39c	y	Sterile	+	?
ND39d	y	Sterile	+	?
ND40	y	$cv/+,\ +/+,\ +/+$	+	-
ND41a	$y\ B^S$	$cv/+,\ v/+,\ +/+$	+	-
ND41b	y	$cv/+,\ v/+,\ +/+$	+	-
ND42	$y\ f$	$cv/+,\ v/+,\ f/f$	+	+
ND43	$y\ f$	Sterile	+	?
ND44	$y\ v\ f$	$cv/+,\ v/v,\ f/f$	+	+

The progeny from approximately 450 matings were examined (no counts were made). This represents an experiment approximately twice the size of experiment IV, table 1. Six separate compounds were recovered from this experiment, two of which, however, occurred as clusters of identical individuals. Tests were carried out on all of these compounds. The results of these tests are summarized in table 3. Those compounds which were inviable in the absence of a Y chromosome (as XX/O) were tested for viability with a chromosome fragment equivalent to the long arm of the Y ($= Y^{el}$). None of them were viable with this chromosome fragment. These compounds, therefore, must lack some factor necessary for survival which can be supplied by the short arm of the Y chromosome. Since all of the compounds were homozygous for loci adjacent to the interstitial heterochromatin, it appears as though all of these resulted from sister-strand union.

From these data, there are three points of interest. First, since all of the fertile compounds were viable with a Y chromosome and therefore carried no

euchromatic deficiencies, it would seem as though sister-strand union in euchromatic regions is at least as rare as sister-strand union in heterochromatic regions. Secondly, in this cross the C-2 chromosome carried the wild-type alleles of the markers used, and hence, compounds which were homozygous for wild-type alleles would be expected to be lethal without a Y chromosome according to the assumption of sister-strand union since they should lack the *bb* locus and *NO*. Those compounds which were homozygous for mutant alleles (and therefore had interstitial heterochromatin derived from the *sc*⁸ chromosome) might be expected to be viable under the same condition. Table 3 shows that this is, in fact, the case, and hence this experiment provides the most convincing evidence for sister-strand union thus far.

Finally, it should be noted that ND39 and ND41 each occurred as a cluster. Since the frequency of reversed acrocentric formation is very low, the occurrence of a cluster of compound-bearing individuals provides very strong evidence that these compounds arose during the gonial divisions; that is, a single compound was formed in some pre-meiotic mitosis, resulting, as a consequence of subsequent mitotic divisions, in a group of cells each carrying this compound.

Effect of YL on reversed acrocentric formation

Experiment I, table 1, differed from the other experiments designed to produce reversed acrocentric compounds in that the chromosome in normal sequence lacked the long arm of the Y chromosome (YL) attached to the centromere. From this experiment no compounds were recovered although the total number of flies examined was greater than for experiments II and III from which compounds were obtained. These data suggest the possibility that a Y chromosome arm at the base of one of the chromosomes to be attached is necessary for the production of reversed acrocentrics. In order to test this point, five different chromosome combinations which might be expected to yield reversed acrocentric compounds, but in which a Y chromosome arm was not involved, were tested in much the same manner as were the *sc*⁸/normal combinations described above. No compounds were recovered from a total of 43,379 female progeny examined. It appears most likely, therefore, that Y chromosome heterochromatin at the base of one of the chromosomes to be attached is necessary for the production of reversed acrocentric compounds. It should be noted that there is no simple scheme whereby YL could be directly involved in the exchange giving rise to the compound, as such an exchange would ordinarily lead to the production of a dicentric.

MEIOTIC BEHAVIOR OF REVERSED ACROCENTRICS

Meiotic loss of unpaired reversed acrocentrics

Since it has been shown previously (SANDLER and BRAVER 1954) that chromosomes in *Drosophila* which fail to pair with a homolog may be lost during the meiotic divisions, it becomes of interest (both in its own right and for purposes of the analysis to follow) to determine whether or not reversed

TABLE 4

The results from a series of parallel matings of females, each of which carries a reversed acrocentric compound X chromosome hemizygous for the mutant allele of y and heterozygous for $In(1)AB$ and v , both with and without a pairing partner (=FR2), to $YSX.YL, yB$ males both with and without FR2. The compound-bearing females are symbolized as y ; the $YSX.YL, yB$ males as $X-Y$.

Cross	Regular		Non-disjunctional		$(y \text{ or } y^+)v \text{ } \text{♀♀}$
	♀♀	♂♂	♀♀	♂♂	
$y \times X-Y$	1,944	1,851	13
$y/FR2 \times X-Y$	3,644	3,057	6	47	8
$y \times X-Y/FR2$	1,824	2,175	0	1	5
$y/FR2 \times X-Y/FR2$	3,790	3,593	97	17

acrocentrics undergo meiotic loss. For this purpose, a series of crosses was made using females carrying reversed acrocentric compounds hemizygous for the mutant allele of y , heterozygous for $In(1)AB$ (a small inversion extending from near v to between g and sd) and v , both with and without FR2 (NOVITSKI 1952), by yB males carrying the $YSX.YL$ chromosome both with and without FR2. FR2 is a heterochromatic fragment consisting of the long arm of the Y chromosome, and is marked by the normal allele of y from $In(1)sc^8$. The results from this series of experiments are given in table 4. Since $In(1)AB$ is inseparable from the normal allele of v , females which lose the inversion by a double crossover are recognizable as homozygotes for the mutant allele of v . Females of this type were collected and used in a series of experiments identical with that described above. The results from this series of experiments are given in table 5. The relevance of these two series, in connection with the calculation of exchange values, is shown below. From the frequency of the exceptional classes it can be seen that FR2 provides a homolog for both the reversed acrocentric compound and the $YSX.YL$ chromosome. As a consequence, the disjunctional behavior of both of these chromosomes can be studied with and without a pairing partner.

There are, in each set of experiments, two comparisons that can be made to obtain the frequency of meiotic loss for the compound chromosome, and two comparisons for the loss of the $YSX.YL$ chromosome. For loss of reversed

TABLE 5

The results from a series of parallel matings of females, each of which carries a reversed acrocentric compound X chromosome hemizygous for the mutant allele of y and homozygous for v , both with and without a pairing partner (=FR2) to $YSX.YL, yB$ males both with and without FR2. The compound-bearing females are symbolized as yv ; the $YSX.YL, yB$ males as $X-Y$.

Cross	Regular		Non-disjunctional	
	♀♀	♂♂	♀♀	♂♂
$yv \times X-Y$	945	1,352
$yv/FR2 \times X-Y$	2,441	2,806	16	51
$yv \times X-Y/FR2$	513	945	0	0
$yv/FR2 \times X-Y/FR2$	2,001	3,161	81

acrocentrics the first two lines in tables 4 and 5 give a comparison in which the male is a constant, while the compound in one case has a homolog (FR2) and in the other case has not, and a similar comparison is afforded by the last two lines in each table. For loss of the YSX.YL chromosome, the first and third lines of each table represent cases in which the female is a constant, while the YSX.YL chromosome in one case has a homolog and in the other case has not, and the other comparison of this type is between the second and fourth lines in each table. Loss is calculated as the percentage discrepancy between the two experiments compared; that is, $100\% - (\text{observed ratio without homolog} / \text{observed ratio with homolog})$.

For the experiments in which the reversed acrocentrics were heterozygous for In(1)AB, loss of the compound chromosome was 12 percent in one case, $100\% - (1,944/1,851) / (3,644/3,057)$, and 20 percent in the other case, $100\% - (1,824/2,175) / (3,790/3,593)$. Loss of the YSX.YL chromosome was 20 percent in the first comparison, $100\% - (1,851/1,944) / (2,175/1,824)$, and 12 percent in the other, $100\% - (3,057/3,644) / (3,593/3,790)$. Similar calculations for the set in which the compounds were not heterozygous for an inversion gives the following results. Loss of the compound chromosome was 20 percent in the first case, $100\% - (945/1,352) / (2,441/2,806)$, and 14 percent in the other case, $100\% - (513/945) / (2,001/3,161)$. Loss of the YSX.YL chromosome was 22 percent in the first comparison, $100\% - (1,352/945) / (945/513)$, and 27 percent in the other, $100\% - (2,806/2,441) / (3,161/2,001)$.

Although the magnitude of the discrepancy varies somewhat from experiment to experiment, the data indicate that the reversed acrocentric is lost in 12 to 20 percent of all cases, when it has no homolog.

Exchange in reversed acrocentrics

The genetically distinct types of exchanges in the reversed acrocentric compound X chromosome are shown in figure 2. The genetic consequences of all combinations of these exchanges are given in table 6. Extensive tests were

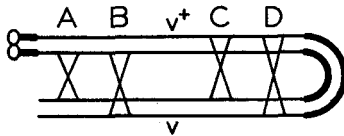


FIGURE 2.—The genetically distinct types of exchanges in the reversed acrocentric compound X chromosome. The consequences of all possible combinations of these exchanges are given in table 6.

made of lines ND1, ND18, ND23, ND33, ND34 and ND37. In certain of these tests the females carried FR2, while in others the females did not. The males in every case carried the YSX.YL, yB chromosome. The results from these crosses are given in tables, 7, 8, 9 and 10. Table 11 gives a summary of the frequencies of homozygosis for all of the tests made.

From the data presented, a number of points of interest arise. First, there

TABLE 6

The consequences of exchange of ranks 0, 1, and 2 in the reversed acrocentric compound X chromosome. 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 products from the various types of exchanges. E_0 = no-exchange; E_1 = single exchange; and E_2 = double exchange. The "cross-overs involved" are shown in figure 2.

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

is an unexpectedly high frequency of homozygosis for loci adjacent to the interstitial heterochromatin (*f* and *car*) as compared with the frequencies obtained from simple attached-X experiments. Secondly, in the presence of FR2 the frequency of homozygosis in the compound chromosome is markedly increased. Finally, in the case of ND33, ND34 and ND37 there appears to be no appreciable reduction in the number of females, although from table 6 it

TABLE 7

The results from crosses of females, each of which carries a reversed acrocentric compound X chromosome, to yB males carrying the YSX.YL chromosome. ND18 and ND23 are heterozygous for *cv*, *v*, and *f*; the parental females carry a heterochromatic fragment marked by the normal allele of *y* (=FR2). ND1 is heterozygous for *cv* and *v* only, and the parental females did not carry FR2.

Phenotype of progeny	ND1	ND18	ND23
+ ♀♀	1,479	2,320	2,327
regular ♂♂	1,974	3,412	3,568
<i>cv</i> ♀♀	4	6	52
<i>v</i> ♀♀	27	77	106
<i>f</i> ♀♀	65	84
<i>cvv</i> ♀♀	5	23	0
<i>vf</i> ♀♀	...	67	73
<i>cvv/f</i> ♀♀	...	15	0
exceptional ♂♂	55	101

TABLE 8

The results from a mating of females, each of which carries a reversed acrocentric compound X chromosome hemizygous for the mutant allele of *y*, homozygous for the mutant alleles of *f* and *car*, and heterozygous for *cv* and *v*, to YSX.YL, *y*B males. The results given in the second column are from females which carried a heterochromatic fragment marked by the normal allele of *y* (=FR2) in addition to the compound.

Phenotype of progeny.	ND33	ND33/FR2
<i>y/car</i> ♀♀	959	2,235
Regular ♂♂	1,105	2,281
<i>ycv/car</i> ♀♀	11	64
<i>yv/car</i> ♀♀	31	194
<i>ycvv/car</i> ♀♀	1	4
Exceptional ♀♀	11
Exceptional ♂♂	52

can be seen that one half of the single exchanges plus one quarter of the double exchanges should produce anaphase II bridges which would cause a reduction in the female class (STURTEVANT and BEADLE 1936). Each of these points will be considered separately.

Crossing over in the interstitial region

Reversed acrocentrics differ structurally from simple attached-X chromosomes primarily with respect to the position of the centromere; it being sub-terminal in the case of reversed acrocentrics and median in the case of the attached-X. The basal heterochromatin of attached-X chromosomes, therefore, is analogous to the interstitial heterochromatin of reversed acrocentrics. With respect to this comparison, it seems most likely that attached-X chromosomes must have, at the very least, as much or more basal heterochromatin than reversed acrocentrics have interstitial heterochromatin, for the reason that an attached-X presumably possesses two complete basal regions, while the inter-

TABLE 9

The results from a mating of females, each of which carries a reversed acrocentric compound X chromosome hemizygous for the mutant allele of *y* and heterozygous for *cv*, *v*, *f*, and *car*, to YSX.YL, *y*B males.

Phenotype of progeny	ND34	ND37
<i>y</i> ♀♀	863	694
<i>y</i> B ♂♂	966	849
<i>ycv</i> ♀♀	3	3
<i>yv</i> ♀♀	8	8
<i>yf</i> ♀♀	3	3
<i>ycar</i> ♀♀	1	0
<i>ycvv</i> ♀♀	1	8
<i>yvf</i> ♀♀	3	2
<i>yf/car</i> ♀♀	16	12
<i>ycvvf</i> ♀♀	1	0
<i>yvf/car</i> ♀♀	1	3
<i>ycvvf/car</i> ♀♀	0	0

TABLE 10

The results from a mating of females, each of which carries a reversed acrocentric compound X chromosome hemizygous for the mutant allele of *y* and heterozygous for *cv*, *v*, *f*, and *car*, plus a heterochromatic fragment marked by the normal allele of *y* (=FR2), to YSX, YL, *y*B males.

Phenotype of progeny	ND34	ND37
<i>y</i> ♀♀	2,162	1,886
<i>B</i> ♂♂	2,457	2,279
<i>ycv</i> ♀♀	7	15
<i>yv</i> ♀♀	92	76
<i>yf</i> ♀♀	31	28
<i>ycar</i> ♀♀	2	17
<i>ycvv</i> ♀♀	48	36
<i>yvf</i> ♀♀	32	38
<i>yfcar</i> ♀♀	71	42
<i>ycvvf</i> ♀♀	6	8
<i>yvfcar</i> ♀♀	28	27
<i>ycvvfcar</i> ♀♀	9	13
<i>yB</i> ♂♂	43	40
Exceptional ♀♀	3	14

stitial heterochromatin of reversed acrocentrics is composed of some fraction of two complete basal regions (see fig. 1). Homozygosis for loci in both of these compounds requires a crossover between the locus and the heterochromatic region, although an additional exchange to the other side of the locus is necessary in the case of reversed acrocentrics. Homozygosis for *car* in attached-X experiments normally runs at less than one percent, whereas in the case of reversed acrocentrics, as can be seen from table 11, it ranges from 4.26 percent to 4.53 percent when the parental females carried FR2, and 2.00 percent to 2.05 percent when the parental females did not carry FR2. Since this difference apparently cannot be a function of the length of the heterochromatic regions involved, it would appear to be some function of the position of the locus with respect to the position of the centromere. Suppression of crossing over as a consequence of the proximity of the centromere has been reported

TABLE 11

A summary of the frequencies of homozygosis for mutants in reversed acrocentric compound X chromosomes. The experiment number refers to table 1. The figures presented are calculated from the data given in tables 7, 8, 9, and 10.

Line	Experiment number	% homozygosis			
		<i>cv</i>	<i>v</i>	<i>f</i>	<i>car</i>
ND1	II	0.59	2.11
ND18/FR2	III	1.71	7.07	5.71
ND23/FR2	III	1.97	6.78	5.94
ND33	IV	1.20	3.19
ND33/FR2	IV	2.72	7.93
ND34	IV	0.56	1.56	2.56	2.00
ND34/FR2	IV	2.81	8.64	7.11	4.26
ND37	IV	1.50	2.86	2.73	2.05
ND37/FR2	IV	3.29	9.06	7.14	4.53

previously for other situations in *Drosophila* (BEADLE 1933), and is known generally as the centromere effect.

Effect of FR2 on crossing over

From the crossover data presented it can be seen that the experiments fall into two distinct classes; those in which the females carried FR2, giving crossover values ranging from 13.7 percent (ND37/FR2) to 9.8 percent (ND18/FR2), and those in which the females carried no pairing partner for the reversed acrocentric, giving crossover values ranging from 5.3 percent (ND37) to 2.4 percent (ND1). The best comparisons come, of course, from lines ND33, ND34 and ND37 for which there are parallel experiments both with and without FR2. It is clear that the fragment has the effect of approximately doubling the rate of homozygosis. It should be noted, however, that the relative proportions of the F_1 classes showing homozygosis for markers near the interstitial region appears to be higher in those cases in which the parental females did not carry FR2.

Certain other information relevant to this effect has been collected. FR2 consists mainly of the heterochromatin from the long arm of the Y chromosome, and it might be wondered, therefore, whether the increase in homozygosis is simply a consequence of the presence of a heterochromatic fragment, or whether the effect is due particularly to Y chromosome heterochromatin. In order to test this point, females carrying reversed acrocentrics hemizygous for the mutant allele of y , heterozygous for v , and carrying a free fragment consisting of X chromosome heterochromatin (the right end of $T(1,4)B^8$; for description see above) were crossed to $YSX.YL, yB$ males. The progeny included 1,325 y ♀♀; 455 yB/B^8 (hyperploid) ♂♂; 30 yv ♀♀; 58 exceptional females (one of which was v); and 130 exceptional males. This represents a frequency of homozygosis for v of 2.21 percent (30/1,355). From these data it appears, then, that the effect of increasing crossing over does not extend to X chromosome heterochromatin.

It becomes of interest, consequently, to determine whether this effect is confined to the compound X chromosome, or whether it is a general effect extending to all of the chromosomes in the complement. To test this, females carrying reversed acrocentrics hemizygous for the mutant allele of y , heterozygous for v and the third chromosome dominants D^8 and H , and carrying the $sc^8.Y$ chromosome (MULLER 1948) were crossed to $YSX.YL, yB$ males. The $sc^8.Y$ is a full Y chromosome marked by the normal allele of y . The frequency of homozygosis for v in this experiment was 9.60 percent (117/1,218), and there was 28 percent crossing over between D^8 and H . From among the progeny of this cross y , non- v, D^8H females were collected and crossed to $YSX.YL, yB$ males. These females are identical with the ones used in the experiment described above except that they do not carry a Y chromosome. Homozygosis for v in this case was 4.48 percent (16/357), and there was 29 percent crossing over between D^8 and H .

From these data it would appear that the effect of a Y chromosome on

crossing over is confined to the compound X chromosome. It might in addition be noted that these results also show that the effect is produced by a full Y chromosome as well as by FR2.

Analysis of exchange with respect to rank

The frequencies of no, single and double exchanges for any test can be arrived at from the frequency of homozygosis and the discrepancy between males and females. For example, in the case of ND34 carrying FR2 (table 10) there were a total of 2,488 regular females and 2,457 regular males. Since meiotic loss of the YSX.YL chromosome results in a 16 percent deficiency (the average loss of this chromosome as measured in a number of different experiments) of the male class, the males must be increased by that amount. This means that there should actually have been 2,925 ($2,457/0.84$) males. The observed frequency of homozygosis equals 13.1 percent ($326/2,488$). This represents a total frequency of homozygosis of 26.2 percent since only one half of the homozygotes are homozygous for mutant alleles. The total frequency of double exchange is equal to twice the frequency of homozygosis (see table 6) or 52.4 percent. One quarter of these doubles produce anaphase II bridges which are lethal and hence cause a corresponding depression in the female class. This amounts to a 13.1 percent depression of females attributable to lethal bridges coming from double exchanges. The total observed depression in the female class amounts to 14.9 percent ($2,925 - 2,488/2,925$), and hence the anaphase II bridges that come from single exchanges equals 1.8 percent ($14.9\% - 13.1\%$). One half of all singles should produce lethal bridges, and therefore the total frequency of singles becomes 3.6 percent. Since doubles equal 52.4 percent, and singles equal 3.6 percent, no-exchange tetrads must have a frequency of 44.0 percent.

A similar analysis for ND37/FR2 (table 10) gives the following results: no-exchange = 33.8% ; singles = 11.4% ; and doubles = 54.8%. For ND34 not carrying FR2 (table 9), if it is assumed that meiotic loss is the same for the compound X chromosome as for the YSX.YL chromosome (and may therefore be ignored), no-exchange = 78.2% ; singles = 5.4% ; and doubles = 16.4%. For ND37 not carrying FR2 (making the same assumption about meiotic loss) no-exchange = 62.2% ; singles = 16.6% ; and doubles = 21.2%.

There are two points which should be noted with respect to this analysis. First, it is assumed that the anaphase II bridges produced by crossing over are lethal. Evidence for this is found in tables 4 and 5, from which it can be seen that the frequency of the exceptional (that is, yB) male class is approximately the same, irrespective of whether the compound was heterozygous for an inversion or not. The comparisons come from those cases in which the compound-bearing females carried FR2. The frequency of exceptional males from females heterozygous for the inversion is 2.1 percent ($144/6,894$), and the frequency from females not heterozygous for the inversion is 2.2 percent ($132/6,099$). The reduced frequency of homozygosis for v in those compounds heterozygous for the inversion indicates that the rate of crossing over is very much reduced.

If the anaphase II bridges were not lethal, but instead were lost to the daughter nuclei (as are anaphase I bridges), then there would be produced nullo-X eggs which, after fertilization by YSX.YL, *yB* sperm, would produce exceptional males. Since the frequency of these males does not vary with crossing over, it seems fairly certain that the anaphase II bridges, when produced, are lethal. The second point to note is that any reasonable assumption about meiotic loss cannot account for the reduced frequency of single exchanges, since not assuming meiotic loss would result in a lower calculated frequency of singles, and the frequency of loss of the YSX.YL chromosome necessary to bring the singles up to where they would compare reasonably with the doubles (the calculated frequency of which is independent of either anaphase II bridges or meiotic loss) is much higher than anything ever observed for this chromosome.

From this analysis it appears as though single exchanges are very infrequent as compared with double exchanges. This estimate, however, comes from that half of the singles which produce lethal anaphase II bridges. The other half of the singles, which correspond to exchange A or D in figure 2, have the effect only of changing the position of the mutants with respect to the strand they are on. For example, a compound symbolized *cv v f car/++++*, with a single exchange of the type not producing an anaphase II bridge between *v* and *f*, would produce, as one of the products, a compound which could be symbolized *cv v +++ f car*. This is not an immediately detectable product, but an estimate of the frequency of this can be arrived at in the following manner. If two mutants are separated by a single exchange and displaced to different strands with respect to each other (as indicated in the above scheme), then it requires at least a quadruple exchange to produce a single individual which is homozygous for both of these mutants. It is therefore possible to arrive at a maximum estimate for the frequency with which such an exchange has occurred simply by examining the progeny of single females and checking for those cases in which adjacent mutants have not appeared simultaneously. This would be a maximum estimate for the reasons that (a) two-strand doubles with one exchange between *car* and the interstitial region, and one half of all four-strand doubles would produce products identical with those produced by this type of single, and (b) the overall frequency of homozygosis being low, the critical types might not appear among the progeny simply by chance. It should be noted, moreover, that there is no selection against compounds resulting from a single exchange of this type, and, as a consequence, they should accumulate in stock. The tests recorded in table 10 were made three generations after the compounds were obtained, and single females were used as parents. The progeny of each individual female was examined, and those cases selected in which all of the mutants appeared at least once. Of the 40 females of this type whose progeny was examined, 35 must have been *cv v f car/++++*, while five could have had mutants displaced with respect to strand. This number must be doubled, since only one of the products from a tetrad with the type of single not producing an anaphase bridge will have mutants displaced. This represents a maximum frequency of this event of 25 percent ($2 \times 5/40$) over three genera-

tions. The overall frequency per generation, then, is 8.33 percent ($0.25/3$); a value which is in agreement with that found in the analysis given above in that they both show surprisingly low values for single exchanges.

The expectations from exchanges of rank 3 are: compounds not showing homozygosis = $1/8$; compounds showing homozygosis (total) = $4/8$; anaphase II bridges = $3/8$. Since the frequency of homozygosis remains the same as for double exchanges, and the frequency of bridges goes up, the analysis given above would admit of some frequency of triple exchanges, but since the frequency of singles is taken from the lethality of anaphase bridges, any frequency of triples would detract from the calculated number of singles.

The analysis thus far has been confined to lines ND33, ND34, and ND37 for the reasons that (a) in these lines there were parallel sets both with and without FR2; (b) these lines were marked by the mutant allele of *y*, and, in the case of ND34 and ND37, were heterozygous for *car*; and finally (c) the other lines, that is ND1, ND18, and ND23, were tested before it was known that the YSX.YL chromosome would be lost in the absence of a homolog, and therefore no special effort had been made to insure the absence of a free Y chromosome in the male stocks. However, evidence that all the lines behave in the same way comes from table 11, from which it can be seen that the frequencies of homozygosis are roughly the same for all of the comparable tests. In addition, ND23 is of interest because it can be seen (from table 7) that all of the females tested (there were 40) must have been of the constitution *cv v +/+ + f*. This test was made three generations after the original compound-bearing female was obtained, and hence it appears that a single exchange (or two-strand or four-strand double) between *v* and *f* occurred once, some time previous to the test, and that all compounds tested subsequently from this line were of this constitution.

It appears from the data then, that single exchanges in reversed acrocentric compounds are extremely rare with respect to doubles; that is, most tetrads have either no exchange or a multiple exchange.

DISCUSSION

The information that a Y chromosome or a Y chromosome fragment can approximately double the rate of homozygosis in reversed acrocentrics is of some interest with respect to the pairing properties of this particular type of compound. First, since this effect is not a general one for the entire chromosome complement but is confined to the compound chromosome, and, since the homology between the compound chromosome and the Y chromosome is indicated by the regular disjunction of each from the other, it seems likely that the effect of the Y chromosome on the rate of homozygosis is related to the pairing of these two chromosomes. Moreover, since there is no such effect evident from attached-X studies, it seems reasonable to suppose that the effect is in some way related to the position of the centromere in the reversed acrocentric compound. The effect might come about in the following manner. If the spatial relationships between the chromosomes are maintained, to some extent, from

any telophase to the following prophase, then at the first meiotic prophase the reversed acrocentric compound would lie straightened out as a consequence of the anaphase movement of the preceding mitosis. If such were the case, then, in order for the components of the compound chromosome to synapse with each other, the compound would have to fold back on itself; a movement which would not be required in the case of the attached-X. It is conceivable that such folding back would be facilitated by a heterochromatic fragment, perhaps by pairing with the interstitial region and thereby mechanically causing the folding back. The fact that homozygosis for markers near the interstitial region was relatively higher when the parental females did not carry FR2 may well be evidence in support of the view that the fragment does pair interstitially with an appreciable frequency. This sort of notion requires, however, that Y chromosome heterochromatin have a different homology with X chromosome heterochromatin than X chromosome heterochromatin has with itself, since the effect is caused specifically by Y chromosome heterochromatin.

It has been shown that a simple analysis of crossover data from reversed acrocentrics indicates that there is a decided deficiency of single, as compared with double, exchanges. The example worked out in text gives the following values for the frequencies of tetrads of different ranks: no-exchange = 44.0%; single exchange = 3.6%; and double exchange = 52.4%. Data indicating that such a distribution of exchanges is atypical for X chromosomes in *Drosophila* is available from other sources. For instance, exchange values for unattached X chromosomes heterozygous for *sc ec cv ct v s f car bb* have been calculated by WEINSTEIN (1936). The frequencies of tetrads of the different ranks are given as: no-exchange = 5.6%; single exchange = 48.5%; double exchange = 42.9%; and triple exchange = 3.0%. It should be noted that the value given above for single exchanges in reversed acrocentrics is a maximum estimate, and it may very well be that the true value for single exchanges is zero. This is so for the following reasons. The frequency of single exchanges is calculated from the discrepancy between the recovery of males as compared with females, and also (independently) from the cases in which adjacent mutants have been displaced to different strands. It can be seen from the treatment given in text that the first method depends to a large extent upon the estimate of the amount of meiotic loss of the YSX.YL chromosome in the parental male, which, if it were overestimated by just a few percent, would erroneously give the appearance of some frequency of single exchanges. The other method depends upon the identification of chromosomes which are produced by single exchanges, but which are also produced by certain of the two-strand doubles and one half of all four-strand doubles.

Although it is not possible to state categorically why the reversed acrocentric compound should give a value for single exchanges of close to zero, certain possibilities may be eliminated from the data now available. The most obvious of these possibilities is that the participation of the strands in exchange is not at random. This would be chromatid interference in the case of double or higher rank exchanges and "strand preference" in the case of single ex-

changes. Both of these possibilities may be eliminated as being responsible for the observed lack of single exchanges. Since that half of the single exchanges which does not produce anaphase bridges are found to occur with a very low frequency, the only way strand preference could operate would be to give an excess of the other type of single. This type of single produces an anaphase bridge which causes a discrepancy between the recovery of the two sexes, and this discrepancy has not been observed. Chromatid interference, on the other hand, only affects the distribution of multiple exchanges, and could, therefore, only affect the calculated recovery of singles if it reduced the contribution of double exchanges to the anaphase bridge class. Since the total number of anaphase bridges is so small, this could not increase the number of singles to any appreciable extent. Moreover, if this were the case, it would mean invoking not only chromatid interference, but also strand preference, since it would mean that more singles of the type which give anaphase bridges were being produced than the type which do not.

Another possibility is that single exchanges occur with the expected frequency, but for some reason are not recovered. This might happen, for instance, if the single exchanges occurred predominately in the gonial divisions just preceding meiosis. This would result in one of two possibilities: (a) the cells containing the bridges would be eliminated before meiosis; or (b) the bridges would be eliminated, but the cells would go through meiosis to produce nullo-X eggs. The latter case would result in a depression of the female class, and this has not been observed. The former possibility, would, it is true, result in the elimination of the bridges without causing a discrepancy in the sex ratio, but it would not explain the lack of that type of single exchange which does not produce an anaphase bridge, since this type of single would not be selected against.

Single exchange chromatids might also not be recovered if there were a preferential recovery of strands not involved in exchange. This should not, however, affect the detection of anaphase bridges, and may, on these grounds, be eliminated as a possible cause for the lack of detected single exchanges.

In this general connection one final point may profitably be made. The reversed acrocentric compound bears a striking resemblance to a heterozygous long inversion in that the pairing properties of both are of such a nature as to give recognizable crossover products only following a double or higher rank exchange. Although it has been generally assumed that single exchanges occur freely within long inversions, the data from reversed acrocentrics might make one wonder whether this is, in fact, the case. To determine this, using unattached X chromosomes, would require some special genetic test.

SUMMARY

Reversed acrocentric compound X chromosomes are essentially like simple attached-X chromosomes except that the centromere is sub-terminal instead of median. They are produced by attaching $In(1)sc^8$ and a chromosome in normal sequence carrying appropriate markers. The simplest way in which such

an attachment might occur is by an exchange between the distal heterochromatin of the *sc*⁸ chromosome and the proximal heterochromatin of the chromosome in normal sequence. However, a large proportion (13 out of 18) of the tested compounds were recovered homozygous for loci adjacent to the point of attachment. Since this appears to negate the above assumption, it is suggested that those compounds which are recovered with homozygous markers arise as the result of a more complicated event which may be thought of simply as the union of sister chromatids in the heterochromatin (sister-strand union) plus a single exchange in the euchromatin. Four lines of evidence in support of this view are presented.

An analysis of exchange in reversed acrocentrics shows that: (1) crossing over in the interstitial region is much higher than that which would be expected on the basis of attached-X studies. This appears to be what is generally termed a centromere effect, and has been reported for other situations in *Drosophila*. (2) The rate of homozygosis is approximately doubled by the presence of a Y chromosome or a Y chromosome fragment. There appears to be no such effect from a fragment containing X chromosome heterochromatin only, and the effect does not extend to the other chromosomes of the complement, but is confined to the compound X chromosome. A possible significance of this effect with respect to the structure of the reversed acrocentric compound is considered. (3) An analysis of exchanges with respect to rank indicates that most tetrads contain either no exchange or a multiple exchange, with single exchanges being very rare or absent. Various explanations for this are considered.

The results from the reversed acrocentric compound may be considered of particular interest in view of the fact that its synaptic configuration is probably very much like that of a heterozygous long inversion.

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