CROSSING OVER IN A REVERSED ACROCENTRIC COMPOUND X CHROMOSOME OF *DROSOPHILA MELANOGASTER*¹

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A compound X chromosome is defined as two X chromosomes attached to the same centromere. There are six different compound X chromosomes, all of which have been synthesized and described (NOVITSKI 1954). An extensive genetic analysis has been made of five of these six types (SANDLER 1954, 1957; WELSHONS 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 free X chromosomes. Furthermore, he showed 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.

The reversed acrocentric and reversed ring are synthesized quite differently, yet their common behavior suggests that the abnormal exchange frequencies are due to some feature common to the structure of the two different 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 (1958) has speculated that the physical presence of this block of interstitial heterochromatin is responsible for the reduced frequency of single exchanges, and he has presented a model (SANDLER and KASTENBAUM 1958) to explain this effect. According to this model, a reversed acrocentric deficient for this block of interstitial heterochromatin should have a normal distribution of exchanges, with the single-exchange class being the most frequent.

Thus, it is of interest to study the exchange patterns of a reversed acrocentric that does not have this usual block of interstitial heterochromatin. With this objective in mind, the present study was undertaken.

MATERIALS AND METHODS

The reversed acrocentric (RA) used in this study arose spontaneously in a triploid stock of *Drosophila melanogaster* (MOHLER 1960). A reversed acrocentric is normally hemizygous for the yellow and achaete loci, and contains a large block of interstitial heterochromatin. However,

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this compound chromosome is deficient for the interstitial heterochromatin. This includes all, or nearly all, of the heterochromatin from the proximal *sc^s* break through the suppressor-of-forked locus. BROSEAU has examined this chromosome cytologically (MOHLER, personal communication) and found it to be a short reversed acrocentric, approximately the same length as a normal X chromosome.

The reversed acrocentric is lethal unless the missing heterochromatin is present. Two duplications which cover the deficiency were used in this study. The first was Dp60 (MOHLER 1960), which was formed as the complementary product of the reversed acrocentric, and is marked by γ^{s1d} , an allele of yellow. The second duplication used was Y, *su⁺-f*, a male-fertile Y chromosome, marked by the normal allele of suppressor-of-forked (GETHMANN 1967).

The heterozygous, diploid females used for the crossover tests were recovered from triploid stocks. The appropriate markers were placed on the RA by crossing over between the compound chromosome and the free X chromosome in the triploids. It was not possible to construct triploid stocks homozygous for the mutant markers, since triploid females homozygous for the region marked by crossveinless were sterile; thus, it was not possible to determine the exact linkage configuration of the markers. Crosses which failed to produce homozygosis for all the markers were not included in the final count.

Two series of crosses were made. The first series used females hemizygous for yellow (γ , 0.0), and heterozygous for spotted-white (*sp-w*, 1.5), crossveinless (*cv*, 13.7), vermilion (*v*, 33.0), and forked (*f*, 56.7). In the second series, the females were hemizygous for yellow, and heterozygous for *In(1)dl49*, spotted-white or white apricot (*w^a*, a pseudoallele of spotted-white), vermilion, miniature (*m*, 36.1), forked, and Bar (*B*, 57.0). (All values taken from BRIDGES and BREHME 1944). In both series, the procedure was identical. Females carrying Dp60 were mated to X, γ /Y, *su⁺-f* males for the first test, and apparent noncrossover daughters, now carrying Y,*su⁺-f* to cover the deficiency, were backcrossed to X, γ /Y,*su⁺-f* males for the second test.

All matings were made with single females, less than 36 hours old, and 4 to 6 males, less than 3 days old. The parental flies were transferred at 2-day intervals for the first 6 days, and 3-day intervals for the second 6 days. Progeny were counted from eggs laid over the first 12 days. All matings were made in shell vials, and transfers were made to half-pint bottles. The flies were raised at $25 \pm 1^\circ\text{C}$, and fed on a cornmeal, agar, molasses media, supplemented with live yeast.

RESULTS AND DISCUSSION

The consequences of exchanges within a reversed acrocentric have been worked out by SANDLER (1954). There are two relevant consequences. First, one fourth of the double exchanges about a heterozygous mutant marker will result in homozygosis for the mutant. Second, 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 exchange is dependent upon two numbers: (1) the count of females homozygous for the mutant markers, and (2) the count of the lethal class. The first is obtained directly and the second is obtained from the difference between the number of recovered females and males.

Since one fourth of the double exchanges result in homozygosis for included mutant markers, 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. The remainder will be the frequency of lethals due to single-exchange bridges. Since one half of the single exchanges result in lethal bridges, the frequency of the single-exchange class 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 females homozygous for at least one marker depends upon the linkage configuration of the reversed acrocentric. If the markers are linked *cis* (all linked on the same arm), the number of homozygotes will be less than if the markers are linked in *trans*. The effect is as follows. If two markers are in a *cis* linkage, from any double exchange that includes both marked loci, one compound chromatid would be homozygous for both mutants, while the other would be homozygous for the wild markers. Only one product would be counted. If, however, the markers are on different arms, from the same double exchange, one compound chromatid would be homozygous for one mutant, and the other homozygous for the other mutant. In this last situation, both products would be counted, instead of only one of them.

Progeny from females linked in *cis* 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 has a high no-exchange class, and therefore, he would have many noncrossover progeny from which to select his females for the crossover tests. However, in this case, it was impractical to collect data only from *cis* linked females, since, as will be shown, the no-exchange class was very small. Therefore, by counting progeny from *trans* linked females, the calculated frequency of the E_2 class becomes an overestimation of the value that would be calculated from *cis* linked females, while the single- and no-exchange classes would be underestimated.

Alternatively, if the frequency of homozygosis is calculated on the frequency of a single mutant, then the double-exchange class would be underestimated, and the other two classes would be overestimated. The phenotypic classes from the crossover tests are listed in Table 1, and the information necessary for calculation of the exchange frequencies is listed in Table 2.

Using the data from the RA/Dp60 females (Table 2), the exchange frequencies can be calculated as follows: Since one fourth of the double exchanges about a mutant marker result in homozygosis for that mutant, the frequency of double exchanges would be four times 0.050, or 20.0%. The reduction in females is one minus the sex ratio, or $(1 - 0.483)$, which is 51.7%. Since one fourth of all double exchanges result in lethal bridges, the frequency of single-exchange bridges must be $51.7 - 5.0$, or 46.7%. The frequency of single exchanges would be twice this value, or 93.4%. This leaves a minus 13.4% for the no-exchange class. A similar analysis of the data, but with the frequency of vermilion, the mutant most often homozygous, substituted as an estimate of the double-exchange class, gives the following exchange frequencies: $E_0 = -10.0$, $E_1 = 96.8$, and $E_2 = 13.2$.

A negative value for E_0 has no biological meaning and points to the fact that the estimates for the single-exchange class must be too high. Since the single-exchange class is estimated from the sex ratio, events other than exchange bridges which alter the sex ratio could lead to an overestimation of the single-exchange class. Such events might include nonrandom disjunction or a reduced viability of the reversed acrocentric. The very high single-exchange class indicates that

TABLE 1

Number and phenotypes of progeny from the crossover tests. Genotypic details are given in Materials and Methods

Type of progeny	Female parent			
	RA/Dp60	RA, <i>dl49</i> /Dp60	RA/Y, <i>su</i> ⁺ -f	RA, <i>dl49</i> /Y, <i>su</i> ⁺ -f
Regular females				
<i>γ</i>	2538	969	2545	1680
<i>γ f</i>	69	0	58	0
<i>γ f v</i>	10	0	39	0
<i>γ f v cv</i>	2	0	9	0
<i>γ f v cv sp-w</i>	0	0	2	0
<i>γ v</i>	148	0	136	0
<i>γ v cv</i>	31	0	44	0
<i>γ v cv sp-w</i>	2	0	0	0
<i>γ cv</i>	21	0	27	0
<i>γ cv sp-w</i>	5	0	5	0
<i>γ sp-w</i>	1	0	1	0
<i>γ f cv</i>	4	0	3	0
<i>γ f cv sp-w</i>	0	0	1	0
<i>γ v m</i>	0	1	0	0
Exceptional females				
<i>γ^{31d}</i>	27	3	..	..
<i>γ^{31d} f</i>	1	0	..	..
<i>γ^{31d} v</i>	1	0	..	..
<i>γ^{31d} cv sp-w</i>	2	0	..	..
Males				
Regular	5157	1221	5274	2061
Exceptional	764	25	..	..

such events might be taking place; however, it can be shown that correction for these events cannot reduce the single-exchange class to values as low as those SANDLER found. This can be illustrated as follows: STURTEVANT and BEADLE (1936) and NOVITSKI and BRAVER (1954) have shown that *In(1)dl49* greatly reduces exchanges when heterozygous. Therefore, if one were to assume that *dl49*

TABLE 2

Number and types of progeny from crossover tests, female to male ratio, and frequency of all recessive mutants and vermilion

Parental females	Heterozygous females	Homozygous females	Males	Female to male ratio	Homozygous female/male ratio	
					All mutants	Vermilion
RA/Dp60	2565	297	5921	0.483	0.050	0.033
RA/Y, <i>su</i> ⁺ -f	2545	325	5274	0.544	0.062	0.044
RA, <i>dl49</i> /Dp60	973	1	1246	0.782		
RA, <i>dl49</i> /Y, <i>su</i> ⁺ -f	1680	0	2061	0.815		

Frequencies were calculated on the basis of expected females (total males). Genotypic details of the crosses are given in the MATERIALS AND METHODS, and phenotypic classes are given in Table 1.

TABLE 3

Exchange frequencies in percent from the inversion-free crossover tests, based on total homozygous females, and vermilion females

Parental females	Exchange frequencies calculated on total homozygous females			Exchange frequencies calculated on vermilion females		
	E_0	E_1	E_2	E_0	E_1	E_2
RA/Dp60	-13.4 (8.2)	93.4(66.6)	20.0(25.2)	-10.0(12.6)	96.8(71.0)	13.2(16.4)
RA/Y, <i>su</i> ⁺ - <i>f</i>	- 3.6(16.8)	78.8(52.4)	24.8(30.8)	0.0(21.2)	82.4(56.8)	17.6(22.0)
ND34 (from SANDLER)	78.2	5.4	16.4	...	...	...
ND34/FR2 (from SANDLER)	44.0	3.6	52.4	...	...	...

Included are data from SANDLER (1954). The values given in parentheses represent exchange frequencies corrected for loss of females. See text for discussion.

eliminated all exchange, and that the 22% reduction in females found in the *dl49* series (Table 2) measured either nonrandom disjunction or a viability effect of the reversed acrocentric, or a combination of both, the resultant calculated frequency of the single-exchange class would still be greater than 50%.

The calculated exchange frequencies for all crossover tests are listed in Table 3, along with values from SANDLER (1954). The values given in parentheses are the corrected values, assuming a 20% loss of the female class, as estimated from the *dl49* data. It is immediately evident that the low frequency of single exchanges SANDLER found in his reversed acrocentric is absent in this one. Furthermore, it can be seen that the frequency of single exchanges is still quite high, even when there has been a correction for loss in the female class.

A third possibility for an overestimation of the single-exchange class would occur 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 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 would be a high no-exchange class. 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 configuration as their mothers. A check of this shows that 14 out of 27 females used in the Y, *su*⁺-*f* crossover tests were linked similarly as their mothers. This is as expected if the single-exchange class is very high, since one half of the surviving progeny from single crossovers would be linked the same as their mothers. If the single-exchange class was low, and the no-exchange class high, the number would be much greater than one half.

SANDLER also demonstrated that a Y chromosome has the effect of doubling the frequency of homozygosis in his reversed acrocentric. Since, in data from *trans* linked females, there is some ambiguity in calculating the frequency of

TABLE 4

The frequency of homozygosis of mutants used in the crossover tests

Parental females	<i>f</i>	<i>v</i>	<i>cv</i>	<i>sp-w</i>
RA/Dp60	3.00	6.78	2.84	0.35
RA/Y, <i>su</i> ⁺ - <i>f</i>	3.90	8.01	3.17	0.31
ND34 (from SANDLER)	2.56	1.56	0.56	...
ND34/FR2 (from SANDLER)	7.11	8.64	2.81	...

Calculations are based on the number of recovered females. Included are data from SANDLER (1954).

exchange classes, the effect of the Y chromosome can be judged better by examining the frequencies of homozygosis of the individual mutants (Table 4). RA/Dp60 is equated to ND34, since neither contain any Y heterochromatin; RA/Y, *su*⁺-*f* is equated to ND34/FR2, since both contain Y heterochromatin as a pairing partner to the reversed acrocentric. The difference SANDLER observed with and without a Y chromosome is not observed in this reversed acrocentric.

Finally, it can be seen from Table 1 that Dp60 regularly segregates from the reversed acrocentric, independent of any exchange the compound chromosome may have undergone. This is in agreement with the findings of E. H. GRELL (1963), who reported that crossing over in a compound X chromosome does not affect disjunction between the compound chromosome and its pairing partner.

In conclusion, it would appear that the reduction of single exchanges and the effect of a Y chromosome upon double exchanges is indeed due to the presence of the interstitial heterochromatin. SANDLER's reversed acrocentrics all possessed this heterochromatic block, and his reversed acrocentrics all showed a low frequency of single exchanges, with one exception. The exception was a case in which the long arm of the Y chromosome was attached to the centromere, and here, he recovered a high frequency of single exchanges (1958).

However, removal of this block of heterochromatin results in a normal distribution of exchanges, with little or no effect of a Y chromosome upon double exchanges. SANDLER (1958) has argued that the effect of the heterochromatin is a structural effect, imposed by the presence of the interstitial block, and the results presented here are consistent with this interpretation.

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SUMMARY

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 is due to the interstitial heterochromatin. The critical test of this explana-

tion 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. Two 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 close to that of free X chromosomes. The presence of Y heterochromatin as a pairing partner had little effect upon the frequency of homozygosis of the mutant markers.

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