AN ANALYSIS OF CROSSING OVER WITHIN A HETEROZYGOUS INVERSION IN DROSOPHILA MELANOGASTER [1]

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THE study of crossing over between chromosomes differing by an inversion provides information on the capability of chromosomes to synapse and undergo exchange despite the mechanical obstacle imposed by the inversion loop configuration. Extensive information is available in *Drosophila melanogaster* on the frequencies of crossing over outside the inverted region of inversion heterozygotes, but measurement of crossing over within the inversion presents difficulties, since single crossovers are ordinarily lost by the formation of dicentrics or duplication-deficiency chromosomes. Frequencies of double crossing over in very long inversion heterozygotes are known; this type of heterozygote, however, constitutes the least interesting and informative case because the chromosomes may pair in reverse direction without the formation of a typical inversion loop. The ideal conditions for this sort of study, then, include the use of a medium-sized inversion centrally located in a chromosome arm with an appreciable pairing segment on both sides of the inversion and some specially devised constitution such that crossovers both within and outside of the inversion yield genetically detectable products. These conditions have been fulfilled by the use of a tandem metacentric compound X chromosome heterozygous for the *dl-49* inversion. An analysis of crossing over in this compound is presented below.

CONSTRUCTION OF THE TANDEM METACENTRIC COMPOUND

The compound X chromosome used in these experiments was made up according to a method described previously (NOVITSKI 1954) and the appropriate scheme is given in figure 1 of that work. Bar males were mated to females heterozygous for a chromosome in normal sequence, except that it carried the *dl-49* inversion, and a second X chromosome which was in inverted order, with the base of *In(1)EN* and the tip of *In(1)sc*^{4}, and which had, in addition, a short arm consisting of the proximal segment of the X chromosome derived from the B^8 translocation. Crossing over between the base of the first chromosome and the short arm of the second should give rise to a metacentric compound X chromosome with its components tandemly arranged, and an egg with such a chromosome, if fertilized by a Y-bearing sperm, would produce a non-Bar female, an exceptional type. From a large

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number of progeny examined from the above mating, five such exceptions were found. Their subsequent genetic behavior indicated them to be of the desired type.

The *dl-49* chromosome carried the mutants, *y*, *v*, *f* and *car*; the other carried *y* and *m*. The relative positions of these mutants, the extent and location of the *dl-49* inversion, and the configuration achieved by this compound after complete synapsis is given in figure 1 under type 1. The proportions of the various regions are drawn approximately to scale from the genetic data on the X

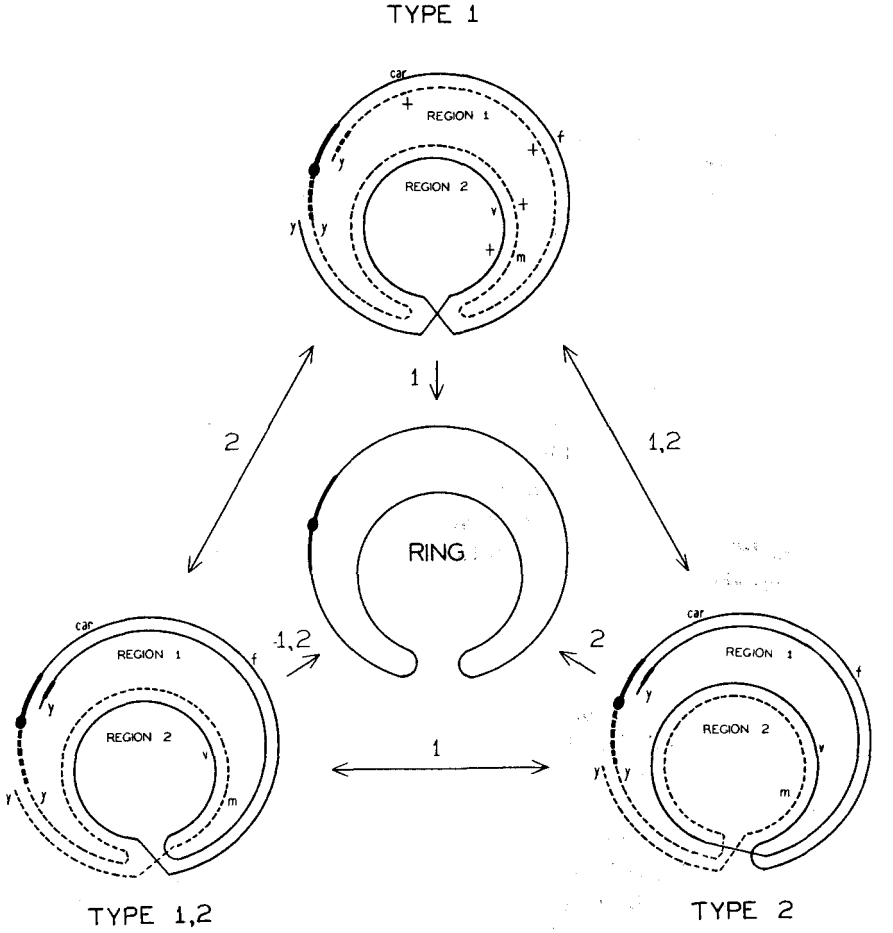

FIGURE 1.—The pairing configurations of the three types of compounds, assuming complete pairing. Type 1 is the original, types 1,2 and 2 are the derived types. Region 1 refers to all the chromosome except the *dl-49* inversion, which is region 2. The numbers by the arrows indicate the position of the exchange that will make the indicated transformations. The positions of the mutants and their normal alleles are given for type 1, as it was synthesized, but only the loci are indicated for the other two types since the exact position of the mutants will depend on the position of the exchange responsible for the transformation. The heterochromatic regions are represented by thickened lines.

chromosome found in BRIDGES and BREHME (1944). For convenience, the regions adjacent to the centromere and outside of the *dl-49* inversion are referred to jointly as region 1 and the inverted region as region 2.

CROSSING OVER IN THE TANDEM METACENTRIC, TYPE 1

Region 1. As illustrated in figure 1 under type 1, a single crossover in region 1 between the two arms of the tandem metacentric may produce a ring chromosome consisting of a single X chromosome. It should be noted that in this compound there are two distinct kinds of single exchanges, one in which the two chromatids involved are attached to the same centromere and another in which the two chromatids are attached to sister centromeres. A schematic diagram of the latter requires the four-strand stage; all crossover products from it are inviable. The two-strand schematic, as in figure 1, is adequate to show the consequences of the first type of exchange which produces viable

TABLE 1

Types of progeny produced from matings of females with the tandem metacentric compound X chromosome heterozygous for dl-49 to B, X-Y males.

1. Noncrossovers:		
a. Matroclinous	*y* ♀	2456
b. Patroclinous	*B* ♂	2804
2. Single crossovers:		
a. Region unidentifiable	*B* ♀	322
b. Exchange between		
y and *dl-49*	*y v f car* ♂	3
dl-49 and *f*	*y m f car* ♂	90
f and *car*	*y m car* ♂	132
car and centromere	*y m* ♂	165
3. Double crossovers:	*y v* ♀	2
	y m ♀	1
	y v f car ♀	2

products. For simplicity, the term exchange will usually be used here only with reference to the kind giving viable products; however, estimates of exchange frequencies must take both kinds into consideration.

Exchanges occurring in the various sections of region 1 may be readily identified. Table 1 gives the frequencies of the various types recovered from a mating of females with the type 1 chromosome to X-Y, *B* males. Single crossovers are recovered as single rings in both sexes, whereas the compound may be recovered only in the female sex; consequently the relative frequency of single rings may be calculated as $(322 + 390)/2(2456 + 5) + (322 + 390) = 712/5634 = 12.6\%$.

Of the 390 single rings recovered as males, only 3 resulted from exchange between *y* and the distal breakpoint of *dl-49*. All the others resulted from exchange between the proximal break and the centromere. The relative proportions of exchange in the regions *y* to distal breakpoint *dl-49*, proximal breakpoint *dl-49* to *f*, *f* to *car*, and *car* to the centromere are, respectively, 0.8%, 23.1%, 33.8% and 42.3%.

Region 2. A crossover within the *dl-49* inversion, region 2, does not produce an immediately identifiable product. Instead, a new chromosome type, that drawn in figure 1 as type 1,2 results. It can be seen upon inspection of the type 1,2 configuration that it will not produce single ring chromosomes by a single exchange; a double exchange, one in region 1 and one in region 2, is required. It is labelled type 1,2 for this reason.

It was anticipated that a female with type 1,2 would produce fewer single-ring-bearing progeny than those with type 1. Accordingly, progeny tests were set up to determine the relative frequencies of types 1 and 1,2 chromosomes from mothers with type 1. Since there was no specific expectation for the frequency of production of single rings from type 1,2, progeny of females showing a lower than average production of rings were tested. It was found that only in cases where a female had produced very few (not more than two) single-ring-bearing progeny was this characteristic of low ring production repeated by her progeny. In every case females suspected of carrying the type 1,2

TABLE 2

The percentages of cultures of females carrying types 1 and 1,2 chromosomes with 0, 1,2 and 3 or more single rings produced by crossing over and the average number of female progeny per culture for those cases where complete counts were necessary.

No. of rings per culture	Chromosome type 1		Chromosome type 1,2	
	%	Average yield	%	Average yield
None	0.6	29.3	73.9	48.4
One	1.5	44.6	21.7	59.3
Two	2.4	48.7	4.3	63.0
Three or more	95.5	....	00.0	....

chromosome were checked by tests of their progeny. In a few cases the tests were not completed; these were classified as doubtful.

Of 1313 progeny of type 1 females tested, 116 were retested because they produced three or less single-ring-bearing F_1 and 46 proved to carry the type 1,2 chromosome. In two cases the tests were inconclusive and a third case involved a still different chromosome type, 2, to be described below, resulting from a double exchange in the type 1 compound. That the criterion for the classification of the type 1,2 chromosome is reliable is shown by table 2. It should be noted that the cultures of type 1 females producing no rings tend to be somewhat lower in productivity than average.

The 46 instances of conversion of type 1 compound into a type 1,2 out of 1313 tests give a frequency of recovery of crossovers within the *dl-49* inversion in this type configuration of 3.5%. The two doubtful cases would increase this figure to 3.7% if they were, in fact, crossovers also.

It has been possible, by further analysis of the crossover types 1,2, to determine where in the *dl-49* inversion the exchange took place. Discussion of these results is deferred until the characteristics of all new crossover types have been presented.

CROSSING OVER IN THE TYPE 1,2 COMPOUND

Region 1. An exchange in region 1 of the type 1,2 produces a new compound, type 2, so named because it will produce single ring chromosomes by single exchange in region 2 (fig. 1). It is difficult to distinguish between types 1,2 and 2 because they behave in much the same way. It is not possible for this reason to present any specific estimate for the conversion from type 1,2 into 2; i.e., the crossover frequency in region 1 of the type 1,2 chromosome. It is known however that this conversion does take place. In tests of 540 progeny of females carrying the 1,2 chromosome, five were shown definitely to carry the type 2 chromosome. The nature of this identification is described below.

Region 2. An exchange in this region results in the conversion of type 1,2 back to type 1. Since these two types can be distinguished without undue difficulty, a more or less precise figure can be arrived at for the frequency of conversion. Of the 540 tests of type 1,2, there were 12 cases of conversion to type 1, and two additional cases where the classification was doubtful, giving a frequency of 2.2 to 2.6%.

Regions 1 and 2. Crossovers in regions 1 and 2 simultaneously will produce a single ring. Although these doubles are rare, an estimate may easily be made of their frequency since the single rings are recovered as individuals and progeny tests are unnecessary. The tests of the 46 females that proved to carry the type 1,2 compound yielded 14 progeny with single rings to 2363 with the compound. The latter figure must be multiplied by two to correct for the loss of half of the compounds by fertilization by an X-bearing sperm; the frequency of production of single rings is therefore $14/(4726+14)$, or 0.3%.

CROSSING OVER IN THE TYPE 2 COMPOUND

Region 1. A crossover in region 1 converts type 2 into type 1,2 (fig. 1). No estimate has been made of the frequency of this change.

Region 2. A single in region 2 gives rise to a single ring. Crossing over in this region (*dl-49*) is not common; the identification of a chromosome as type 2 rests upon the distribution of the mutants *f* and *car* in the recovered rings. Reference to figure 1 will show that the specific allele of *f* and *car* present on the single ring is determined by the location of the exchange in region 1 responsible for the initial occurrence of the type 1,2 compound. Consequently, all single rings from a given female will be identical with respect to these mutants. Thus, a given type 2 compound will produce rings all of which carry *car*, or all with *f car*, or all with neither, unlike types 1 and 1,2 which produce rings with all three possible combinations in a single test. For example, a set of thirty daughters from one female with a type 2 compound produced the following kinds of single rings: 29 *m car*, 4 *v m car* and 3 *v car*. The alleles within *dl-49* will, of course, vary within a culture since they will depend upon the position of the exchange giving rise to the ring. This will be discussed in more detail in the section on the distribution of exchanges within the *dl-49* inversion. Nine females, subsequent tests of whose progeny showed that they carried the type 2 compound, produced 667 compound X progeny to 21 rings,

or 1.5%. Tests in later generations gave a lower value, presumably because of the presence of a number of type 1,2 chromosomes newly formed by crossing over in region 1 of the type 2.

Regions 1 and 2. No reversions to type 1 by a double in type 2 were observed in the small number of tests of type 2 compounds.

THE DISTRIBUTION OF EXCHANGES IN REGION 2

The position of the crossover in the *dl-49* inversion responsible for the conversion of type 1 into type 1,2 cannot be determined immediately. The genetic

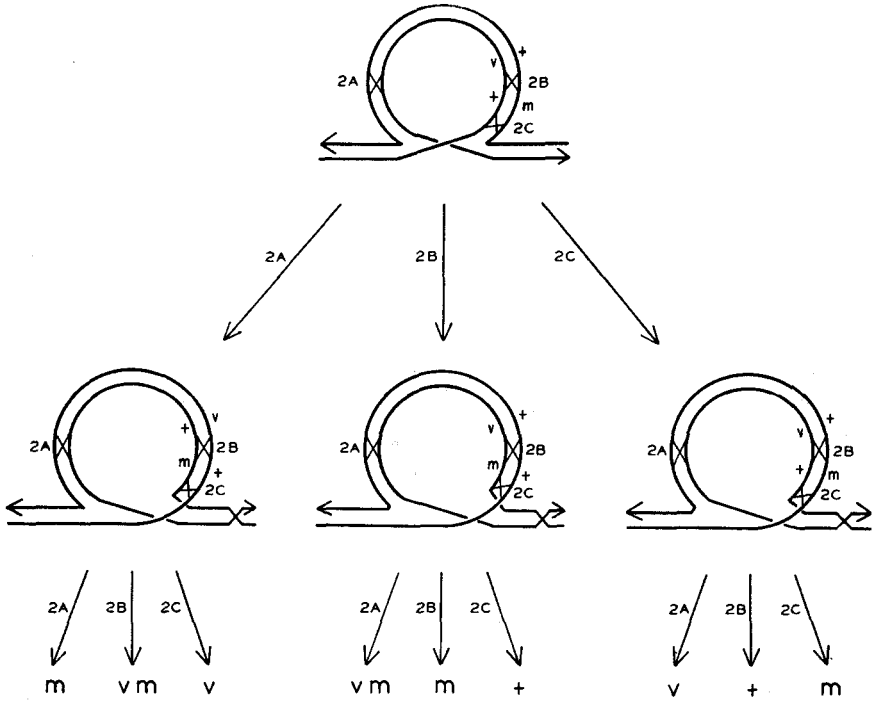

FIGURE 2.—The method for locating the position of exchanges within the *dl-49* inversion, showing how different initial exchanges alter the distribution of mutants carried by the single rings produced in later generations.

composition of the kinds of rings produced by type 1,2, however, do indicate the location of the original region 2 exchange. This is diagrammed in figure 2. Region 2 is divided into three subregions: from the breakpoint of *dl-49* to *v*, from *v* to *m*, and from *m* to the other breakpoint. These are labelled 2A, 2B, and 2C, respectively. The chromatids ending in arrows are those joined to the centromere. From the figure it is seen that an exchange in each of the three regions gives rise to three possibilities with respect to the distribution of the mutants and their normal alleles on the two chromatids, although all three are structurally type 1,2. In each of these types, once again exchange may occur in each of the three subregions. In combination with an exchange out-

side of the inversion, single rings may be produced. The mutants carried by the rings will be different for the different combinations of exchanges, but more diagnostic, perhaps, is the absence of one of the possible combinations in each of the three sets. Thus, if the original exchange was in region 2A, none of the single rings appearing from later crossing over should be non-*m* non-*v*. On the other hand, if it was in 2B, rings with *v* only should be absent and if in 2C, the *v m* class should be absent.

The exchange in region 1 necessary for ring production in addition to the one in region 2 may occur simultaneously with the latter as a double, or it may occur in an intervening generation, converting the type 1,2 into type 2. In either case the analysis is the same, although the latter is more efficient since rings are produced more readily by type 2 than by type 1,2.

In practice, the above expectations have been fulfilled and it has been possible to specify the location of the original exchange in 15 crossovers in the *dl-49* inversion. Ten gave a distribution of single rings indicating that the

TABLE 3

The distribution of exchanges in the type 1,2 configuration when one occurs in region 1 and the second in region 2. The numbers in parentheses give expected distribution if the exchanges were occurring singly.

Region 1	Region 2			Total	Expected
	2A	2B	2C		
breakpoint-*f*	6	2	1	9	(6.7)
f-car	6	2	1	9	(9.9)
car-centromere	11	0	0	11	(12.4)
Total	23	4	2	29	(29.0)
Expected	(22.5)	(3.2)	(3.3)	(29)	

original exchange must have been in 2A, one proved to be a 2B exchange and four were of the 2C type. An additional one gave only two crossovers, both of which were *m*, suggesting that here too the original exchange was located in 2A, since this is the most frequent class from the 2A group and least frequent from the other two.

When the type 1,2 is converted into type 2, single rings are produced with a higher frequency, as noted above. From these type 2 compounds it is possible to designate the specific region of exchange directly involved in the production of the rings, provided only that it is possible to determine the exact constitution of the type 2 chromosome. Of 510 rings from type 2 females, 395 came from crossing over in 2A, 57 from crossing over in 2B and 58 from crossing over in 2C.

A small amount of data has been collected on the location of the exchanges occurring simultaneously in the two regions 1 and 2 when rings are produced from the type 1,2 compound. These cases are given in table 3, along with the expected distribution calculated from the relative frequencies of recovered crossovers in region 1 in type 1 and in region 2 in type 2.

DISCUSSION

The use of this specially constituted chromosome which is not only a compound X chromosome but has effectively two inversions, one very long involving an entire chromosome arm and the other *dl-49,* may be open to question because its synaptic properties could be basically different from those of two unattached homologues differing only by the *dl-49* inversion. To some extent there must be differences; it is nevertheless surprising that the similarities in exchange values between this type and others from which information can be obtained are so striking.

Crossing over outside of the *dl-49* inversion in normal chromosomes has been studied extensively (STURTEVANT and BEADLE 1936). Crossovers between *y* and the left break of *dl-49* are rare (0.5%), as they are in the compound. The other regions agree in their crossover rates also, but the comparisons depend upon the conversion of observed rates into exchange frequencies. As pointed out earlier, half of all exchanges give rise to inviable zygotes. In tetrads of the type 1 compound, when a ring is formed by exchange in region 1 there is an unaffected type 1 compound sister chromatid not shown in figure 1 which has been drawn for simplicity as the two-strand stage. The estimation of the exchange frequency must take all these into account; calculated most simply the exchange frequency would be four times the number of single rings recovered, since only one quarter of all exchanges in region 1 produce single rings, divided by a total which has been corrected for the loss of zygotes due to inviable exchange products and to fertilization of compound X eggs by X-bearing sperm. Arithmetically this becomes $4 \times \text{rings}/(2 \times \text{compound X's} + 3 \times \text{rings})$. If we accept the best value (table 1) for the frequency of production of rings as $2 \times 390 = 780$ (since it has been shown by NOVITSKI (1954) that the recovery of ring-bearing females from crosses of this sort tends to be depressed and that the best estimate of ring production is made by doubling the ring-bearing male class) and the frequency of compounds as 2461, then the exchange frequency in region 1 becomes 0.43 which would correspond to a crossover value of 0.215 in an ordinary *dl-49* inversion heterozygote. More complicated calculations which take into account other products, such as the double exchanges, give the same value for the exchange frequency. This is much higher than the 0.13–0.15 value found in such heterozygotes (STURTEVANT and BEADLE 1936). While it is conceivable that exchange is more frequent in the compound than in unattached or ordinary attached-X chromosomes heterozygous for *dl-49,* the high exchange value is probably best interpreted as a manifestation of nonrandom disjunction, and it should be noted that this particular phenomenon, an excessive recovery of rings from the tandem metacentric compound, has been well established (NOVITSKI 1951). If the excess of rings is about the same as in previous experiments, then the calculated exchange value of 0.43 may be about 50% higher than the true value, which would be 0.29, a figure in close agreement with the exchange values from the more orthodox chromosome types.

It is of particular interest that the high rate of exchange between the in-

version and the centromere and the low rate distal to the inversion, found in simple heterozygotes and in attached-X heterozygotes, is found also for this compound where the terms proximal and distal pairing lose their significance since the proximal region of each arm pairs with the distal region of the other. It can be concluded that the relative frequency of crossing over in each of these regions is primarily a function of the nature of the region and that the modification of crossover values by the position of the region with respect to the centromere (the "centromere effect") is of secondary importance.

The frequency of exchange within *dl-49,* region 2, in the experiments reported here is complicated by the fact that the identification of a compound as a crossover, type 1,2 from type 1, depends on progeny tests. Some fraction of the eggs laid in the same generation as those carrying the crossover compounds must have carried rings, or inviable products, and so would not have been tested; these, nevertheless, must be added to the total of 1313 tested to give a more valid estimate of the total sample. The determination of this increment involves drawing the type 1 compound in the four-strand stage, and calculating, from the observed numbers of the different types produced, and from the frequency of ring production from the type 1 compound (15.8%), the number of rings and inviable zygotes. The adjusted total is about 2050, which gives a frequency of exchange of from 9.2 to 9.6%, depending on whether the two doubtful cases are included. If, in these calculations, nonrandomness is assumed to be operating, the values are increased slightly, to 9.6 and 10.0%. STURTEVANT and BEADLE (1936) made an estimate of the exchange frequency in *dl-49* using an attached-X heterozygous for the inversion. In this configuration, crossovers within *dl-49* produce inviable zygotes and the additional depression of the attached-X progeny below the patroclinous male class compared to a control series without *dl-49* was taken as a measure of the exchange frequency. Their experiments gave a value of 12%.

Before considering the rates found in the other two types, it should be pointed out that the three types are not synaptically equivalent. A close examination of types 1,2 and 2 in figures 1 and 2 will reveal that, assuming complete synapsis, the basal region of each arm pairs with the distal region of the same arm, forcing the *dl-49* regions in each arm into a loop. If it is hypothesized that the depression of crossing over within an inversion results from an inability of the chromosomes to synapse simultaneously outside of the inversion and also within the inversion because the relatively inverted segments must become reoriented with respect to each other for pairing, this difficulty in the formation of an inversion loop would disappear in types 1,2 and 2 since here complete pairing outside of the inversion would facilitate loop formation in each arm and synapsis within the *dl-49* region could be completed simply by the juxtaposition of the loops in each arm. It will be shown below that the data do not support this idea; it is presented simply to emphasize the caution that must be observed in equating the three types to each other.

The best estimate for the frequency of exchange in the inversion, region 2, in the type 1 compound is 9.6 to 10.0%. In a way identical to that described

above for the type 1 compound, exchange frequencies in region 2 may be obtained for the other two types. The calculated exchange value in the type 1,2 compound ranges from 7% to 9%, depending on whether two doubtful cases are included, and the exchange value for this region in the type 2 compound is 5%. In both of the latter cases exchange would have been facilitated, giving an expectation above 10% if the mechanism for the supression of crossing over were that described in the preceding paragraph. The differences in the exchange values between type 1 and the other two types are not unequivocal, being based on low numbers of crossovers derived from crosses made at different times. Nevertheless the 5% figure for the type 2 compound strongly suggests that there is in fact a depression since this is based on the recovery of single rings which, as has been shown above, are very likely being recovered preferentially because of the nonrandom effect. It is possible that pairing in region 2 is less often achieved in the types 1,2 and 2 than in type 1, despite the presence of the true inversion configuration in the latter and not in the former.

Examination of the distribution of exchanges within the *dl-49* inversion does not lead to the conclusion that there are extensive synaptic difficulties because of inversion loop formation. Of sixteen crossovers analyzed, four were found to have occurred between the miniature locus and the adjacent breakpoint. Furthermore, of the 510 crossovers in type 2 giving rise to rings, eleven percent occurred in this region. This high frequency in the region adjacent to the breakpoint may be related to the low frequency from *y* to the inversion; i.e., a low degree of synapsis in the latter region may allow for greater synapsis near the breakpoint within the inversion. The relative frequencies of exchange in the three sections of region 2 in the type 2 compound may be compared with expectation in the following way. The approximate genetic lengths of regions 2A, 2B and 2C on a normal chromosome are 22, 4 and 5 units respectively. The relative lengths are 0.71, 0.13 and 0.16. The frequencies of exchange in those regions are 395, 57 and 58, or, as decimals, 0.77, 0.11 and 0.11. An agreement between the two sets of figures is reached by assuming simply a slight deficiency of exchange in region 2C and a slight excess in region 2A.

It might be argued that this random distribution holds because pairing within the *dl-49* region is complete when it occurs (except, of course, for the sections immediately adjacent to the breakpoint) and that the reduction in crossing over comes from competition between the inverted and uninverted segments for pairing partners. This would give rise to interference between simultaneous pairing in the two regions and could be detected as a reduced number of doubles below expectation. For the type 1 compound the data are meager. If the frequency of exchange in region 1 is taken as 0.43 and that in region 2 as 0.10, the frequency of doubles should be 0.043. Examination of all possibilities of double exchange in the type 1 configuration shows that only one out of every sixteen gives the type 2 compound. Of the 1313 tests of progeny of type 1 compound bearing mothers, only one type 2 compound was

identified when five might have been expected. There are two reasons why fewer than the expected number would have been observed. In the first place, the difficulty in making a simple distinction between types 2 and 1,2 may have led to the inclusion of a few of the former with the latter. Secondly, it has been mentioned earlier that the exchange value in region 1 of type 1 is probably in error by as much as 50% of the true value, since it is based on rings derived from the type 1, a class subject to nonrandom disjunction. If this value is corrected, the expectation becomes three rather than five.

Additional information comes from the type 1,2 compound. The frequency of exchange in region 2 is about 0.08 and that for region 1 can again be taken as 0.43. The expected frequency of double exchanges is simply the product of the two, or 0.034. Taking into consideration all possible combinations of double exchanges occurring in the four-strand stage, one-eighth of the total doubles, or 0.004 of all tetrads, should give rise to rings. The observed frequency was 0.003, which may be corrected to about 0.002 by including in the total a figure for the frequency of inviable zygote production by single exchanges based on the frequencies given above. Note that in this case no correction need be made for the nonrandom effect because both calculated and observed values would have to be altered in the same proportion. This comparison indicates that if there is any interference between exchanges in regions 1 and 2 in the type 1,2 compound, it is probably no more than that which would be expected if there were no inversion present.

Further evidence for the absence of extensive competition between the *dl-49* region and the rest of the chromosome in the type 1,2 compound is given by the breakdown of the doubles into the specific subregions involved (table 3). Although only 29 cases were analyzed, there appears to be a distribution of exchanges in region 2 similar to the distribution when no exchange occurs in region 1, and vice versa. Also, there is no apparent tendency for an exchange in a specific part of region 2 to occur preferentially with an exchange at a specific point in region 1.

The essential points that must be considered in an attempt to develop a self-consistent scheme to explain the behavior of this inversion heterozygote are: (1) a reduction of exchange value in the *dl-49* inversion to about one quarter that found in the same region when no inversion is present, similar in magnitude in both the attached-X and tandem metacentric compounds which pair in quite different ways, (2) a high level of exchange between the proximal break of *dl-49* and the centromere, irrespective of whether the inversion is heterozygous in normal chromosomes, attached-X or tandem metacentric compounds and, contrarily, a low value between *y* and the distal breakpoint in all three cases, (3) a distribution of the exchanges that occur within the *dl-49* region not much different from that expected from a comparison with the standard map, except for a small depression near the breakpoints of the inversion and (4) the apparent absence of any strong interference between exchanges occurring within and outside of the *dl-49* region. In addition, there is an indication of a lower value of exchange in the *dl-49* region found in the

two types with intra-arm pairing compared to the value from the type 1 compound which is a true inversion heterozygote.

All of these relationships can be rationalized if it is simply assumed that the synaptic tendency along the euchromatic length of the chromosome is very weak and ordinarily becomes manifest after homologous regions have been brought into proximity by stronger (but not necessarily specific) pairing centers located in the heterochromatic regions adjacent to the centromeres. These pairing regions would be, presumably, similar or identical to those postulated by COOPER (1951) on cytological grounds. According to this idea, the distal heterochromatin of the sc^4 inversion at the tip of one arm of the tandem metacentric, by pairing with the heterochromatin region near the centromere, is responsible for bringing the normally basal regions together, whereupon the euchromatic pairing tendency takes over and causes a nearly normal degree of synapsis, and crossing over, in that region. The homologous *dl-49* regions will be brought closer together by this and in some cases, presumably in about 25% of the total, are sufficiently near to allow those euchromatic regions to synapse. The effect of the inversion, then, in reducing crossing over is not so much a consequence of the formation of the inversion loop as it is of the interruption of the continuity of euchromatic pairing. The shorter euchromatic segment from *y* to the distal break pairs relatively infrequently. Any reduction of crossing over in region 2 of the types 1,2 and 2 compounds would be explainable on the basis of the intra-arm pairing; here the arm with the heterochromatic tip derived from sc^4 pairs with its own base and the *dl-49* regions of the two arms synapse only when they happen by chance to be suitably disposed to do so.

While this scheme is adequate to account for the results in these experiments, it is clear that it cannot be extended to include cases of inversion heterozygotes studied in maize (RHOADES and DEMPSEY 1953), where it has been quite clearly demonstrated that the Drosophila type of crossover suppression, particularly in the regions adjacent to the inversion, is absent. It could be surmised that there may be basic differences in the constitution of the chromosomes in the two forms, possibly with respect to the presence of knobs in maize. In any case, the postulate of primary heterochromatic pairing centers functional in normal oogenesis of Drosophila is one which is subject to direct experimental test.

SUMMARY

Crossing over has been studied in a specially constituted chromosome, a tandem metacentric compound X chromosome, in *Drosophila melanogaster*, in which it is possible to measure rates simultaneously within a heterozygous inversion, *dl-49*, and outside the limits of the inversion. The frequency of exchange within the inversion is reduced to about twenty-five percent of the amount expected for the same region without an inversion. Crossovers within the inversion appear to be randomly distributed, except for a slight depression near the breakpoints of the inversion, and there is no striking interference

between crossing over outside of the inversion and within it, with respect to either the overall frequencies or the position of the crossovers. This compound is unstable and by crossing over gives rise to new types with different synaptic properties, whose crossover rates have also been studied. It is suggested that the similarities and dissimilarities in crossover values between the tandem metacentric, its derivatives, ordinary attached X chromosomes, and simple chromosomes, all heterozygous for the *dl-49* inversion, are understandable if it is assumed that the pairing of chromosomes is initiated by the heterochromatic regions, with a weaker euchromatic pairing tendency coming into effect after homologous euchromatic regions find themselves in proximity.

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LITERATURE CITED

BRIDGES, C. B., and K. S. BREHME, 1944 The mutants of *Drosophila melanogaster*. Carnegie Inst. of Wash., Publ. 552. 256 pp.

COOPER, K. W., 1951 Studies on spermatogenesis in *Drosophila*. Yearb. Amer. Phil. Soc. for 1951: 146–147.

NOVITSKI, E., 1951 Non-random disjunction in *Drosophila*. Genetics **36:** 267–280.

1954 The compound X chromosomes in Drosophila. Genetics **39:** 127–140.

RHOADES, M. M., and E. DEMPSEY, 1953 Cytogenetic studies of deficient-duplicate chromosomes derived from inversion heterozygotes in maize. Amer. J. Bot. **40:** 405–423.

STURTEVANT, A. H., and G. W. BEADLE, 1936 The relations of inversions in the X chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21:** 554–604.