

# CROSSING OVER NEAR THE CENTROMERE OF CHROMOSOME 3 IN DROSOPHILA MELANOGASTER FEMALES<sup>1</sup>

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THE basic approach used in studying the mechanism of crossing over has been to alter crossover frequencies by manipulating extrinsic or intrinsic factors in an attempt to correlate the primary effect of the manipulation with the observed genetic results. These experiments have provided much data on the phenomenon of crossing over but little information on its basic mechanism. This is because very little is known of the primary effects of intrinsic alterations such as chromosome rearrangements or of extrinsic conditions, both of which may result in a spectrum of physiological changes. One result common to all treatments that induce changes in crossover frequencies is that crossing over is invariably increased in regions adjacent to or spanning a centromere. Thus, radiation (MULLER 1925), temperature (PLOUGH 1917), chemicals (BROWNING 1949; SUZUKI 1963a), and chromosome aberrations (see SCHULTZ and REDFIELD 1951 for a review) all increase crossing over in centromeric regions.

The experiments reported here were carried out to determine: (1) whether the different conditions which result in increased crossing over are acting on the exchange process in different ways that lead to a similar end result or whether they all affect a common mechanism; and (2) whether all regions near the centromere are equally sensitive to the induction of increases in crossing over as opposed to a regional variation in response to the treatments.

## MATERIALS AND METHODS

Crossing over was measured in chromosome 3 using the following markers: scarlet (*st*), intumed (*in*), radius-incompletus (*ri*), and pink-peach (*p<sup>p</sup>*) (descriptions listed in BRIDGES and BREHME 1944). The region from *st* to *in* has been designated as 1, from *in* to *ri* as 2, and *ri* to *p<sup>p</sup>* as 3. According to the recent work of THOMPSON (1964), the centromere lies between *in* and *ri*. The genetic length of the *st*-*p<sup>p</sup>* region has been found to increase under intrinsic (SUZUKI 1962a, 1963b) and extrinsic (SUZUKI 1963a) changes, and it is instructive to note that although the *st* to *p<sup>p</sup>* region occupies more than 1/5 of the salivary length of chromosome 3, the same region constitutes less than 1/20 of the genetic length of the third chromosome.

Crossing over was measured in females bearing a reversed acrocentric compound-X chromosome (RA) without a free Y, a condition known to increase crossing over in chromosome 3 (HART and SANDLER 1961; SUZUKI 1962a). RA/0 females heterozygous for the markers were obtained by crossing stock RA females with males which, in addition to the recessive markers, carried an attached-XY chromosome with no free Y. In order to minimize background genetic variability, controls were obtained by crossing reversed metacentric compound-X (RM) females

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to the attached-XY/0 males from the marker stock. Crossover values in chromosome 3 of RM/0 females have been shown to be similar to values obtained in normal rod-X-bearing females (SUZUKI 1962a).

RM/0 females were exposed to 4000 rads of gamma rays from a Cobalt<sup>60</sup> source in order to study the effects of radiation on crossing over. The same controls were used for the RA/0 and the radiation series. In order to study the effect of chemicals on crossing over, actinomycin D dissolved in a 0.7N NaCl solution at a concentration of 50  $\mu\text{g}/\text{ml}$  was injected into RM/0 females. Actinomycin D is an antibiotic which specifically inhibits DNA-mediated RNA (dm-RNA) synthesis (see REICH 1964 for a review) and has been shown to increase crossing over in the *st-p<sup>p</sup>* region (SUZUKI 1963a). RM/0 females injected with a physiological saline solution served as controls.

All of the crosses were made concurrently. The flies were collected within 24 hours of eclosion, treated (radiation, injection) or stored for the next 24 hours and then crossed using five to seven females in each quarter-pint bottle. The females were allowed to lay eggs for five days (Brood I); then they were transferred to fresh bottles for another five-day period (Brood II). Only the male offspring of the RM/0 females were scored as eye markers on the RM chromosome interfered with the scoring of the autosomal genes. All crosses were carried out at  $25^\circ \pm 0.5^\circ\text{C}$ .

### RESULTS

The total number of offspring counted and the crossover values in each region in the two broods are listed in Table 1. The reversed metacentric/0 (0 rad) females serve as controls for both the reversed acrocentric/0 and RM/0 (4000 rads) females. It can be seen in the controls (column 1, Table 1) that region 1 is approximately twice as long as region 3 and that region 2 is extremely short. The decrease in crossing over in Brood II of the controls can be attributed to the maternal-age effect first noted by BRIDGES (1927). The reversed acrocentric increases crossing over in all three regions in both broods (compare columns 1 and 3, Table 1). Since the effects of radiation and actinomycin D are primarily on those cells which are subsequently recovered in the second brood, all further comparisons will be restricted to Brood II.

TABLE 1

*Crossover percentages in the centromere region of chromosome 3 in each treatment*

Region†	X-chromosome constitution* and treatment				
	1 RM/0 0 rad	2 RM/0 4000 rad	3 RA/0	4 RM/0 0.7N NaCl	5 RM/0 actinomycin D
Brood I (1-5 days post-treatment)					
Total progeny	2,718	2,038	3,281	1,736	1,078
1	2.28	2.69	8.96	1.55	1.48
2	0.25	0.04	0.91	0.05	0.0
3	1.17	2.50	10.72	0.92	0.55
Brood II (6-10 days post-treatment)					
Total progeny	3,738	3,927	1,161	1,847	1,191
1	1.09	2.34	7.23	0.64	2.09
2	0.10	0.10	0.94	0.0	0.25
3	0.53	3.15	11.19	0.43	2.01

\* RM=reversed metacentric compound-X chromosome. RA=reversed acrocentric compound-X chromosome.

† Regions are: *st*-(1)-*in*-(2)-*ri*-(3)-*p<sup>p</sup>*.

The ratios obtained by dividing experimental values ( $p_1$ ) by control values ( $p_0$ ) for each region for each treatment series are plotted with their 95 percent confidence limits in Figure 1. Any ratio and its confidence limits spanning the value of 1.0 indicates that the two values being compared are not significantly different. The method used for calculating the confidence limits has been outlined previously (SUZUKI 1962a). In spite of the large confidence limits which are the result of the small intervals being compared, the pattern of effect may be indicative.

It is obvious that the RA has an effect on crossing over which is quantitatively greater than that of either the actinomycin D or radiation treatment (Figure 1). The RA/0 condition significantly increases crossing over even in region 2. Note that region 3, which is half the length of region 1 in the controls, exhibits a much greater induced increase than does region 1 in the presence of the RA chromosome.

Both actinomycin D and radiation significantly increase crossing over in regions 1 and 3 but not in region 2 (Figure 1). There is an indication that the crossover frequency is again increased to a greater extent in region 3 than in region 1.

Table 2 lists the number of offspring of each crossover type in all of the experiments. The one double crossover obtained in over 10,000 offspring in both broods of the control series could actually represent a cell derived from a gonial exchange followed by a single crossover during meiosis. Thus, interference is virtually complete in the *st-p<sup>h</sup>* region under standard conditions. In the presence of the RA however, interference is reduced or even negative (Table 2) as the total genetic distance between *st* and *p<sup>h</sup>* is increased five to tenfold (Table 1).

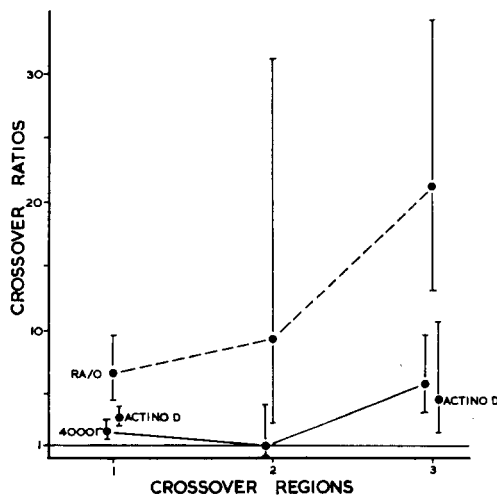


FIGURE 1.—Ratio of crossover values in each region of the treated series ( $p_1$ ) to their respective control ( $p_0$ ) values in Brood II (5 to 10 days after treatment).

TABLE 2  
*Numbers of each crossover type for each treatment*

Regions with crossing over*	X chromosome constitution and treatment				
	RM/0 0 rad	RM/0 4000 rad	RA/0	RM/0 saline	RM/0 actinomycin D
<b>Brood I</b>					
Noncrossovers	2,617	1,931	2,643	1,692	1,056
1	62	55	259	27	16
2	7	1	20	1	0
3	32	51	321	16	6
1, 2	0	0	7 (2.62)†	0	0
1, 3	0	0	28 (0.89)	0	0
2, 3	0	0	3 (0.94)	0	0
Total	2,718	2,038	3,281	1,736	1,078
<b>Brood II</b>					
Noncrossovers	3,674	3,711	946	1,827	1,140
1	40	88	77	12	24
2	4	4	7	0	3
3	19	120	121	8	23
1, 2	0	0	1 (2.27)	0	0
1, 3	1	4	6 (0.64)	0	1
2, 3	0	0	3 (2.46)	0	0
Total	3,738	3,927	1,161	1,847	1,191

\* Regions are: *st*-(1)-*in*-(2)-*ri*-(3)-*p*<sup>p</sup>.

† Coincidence values are given in parentheses.

#### DISCUSSION

The increased crossover values induced in the *st-p*<sup>p</sup> region by the reversed acrocentric chromosome are several times greater than the increases induced by radiation and actinomycin D and are in better accord with the known cytological lengths of that region. Since SUZUKI (1962a) has shown that the RA-effect on the *st-p*<sup>p</sup> region is two to three times greater than the effects of the chromosomes used to generate the reversed acrocentrics (*sc*<sup>s</sup>/+ and *B*<sup>s</sup> *sc*<sup>s</sup>/+), RA-effects appear to be an intrinsic property of such compound chromosomes. SANDLER (1954) demonstrated that exchange within the RA is characterized by an absence of single-exchange tetrads and equal frequencies of tetrads of rank 0 and 2. SUZUKI (1962b) has suggested that the high frequency of no-exchange tetrads is indicative of a failure of the RA arms to pair and that this asynapsis is, in turn, responsible for its interchromosomal effects. An equally plausible explanation for its action is that the presence of the RA *per se* results in an increase in multiple-exchange tetrads in all chromosomes. Both of these possibilities are currently being tested.

A regional difference in the magnitude of response to various treatments is demonstrated (Figure 1). Region 3 has a greater increase relative to its control than does region 1 even though region 3 is genetically and cytologically the smaller. Such an effect would be expected if a gradient of sensitivity exists such

that the closer a region is to the centromere or heterochromatin, the higher is its response to the induction of crossing over. Unfortunately, the salivary chromosome positions of *in* and *ri* are not known. If crossing over does not occur in heterochromatin as BAKER (1958) has suggested, then *in* and *ri* must lie immediately adjacent to or in heterochromatin.

Actinomycin D and radiation both increase crossing over in region 3 to a greater degree than region 1, an effect qualitatively similar to that of the reversed acrocentric (Figure 1). Since the primary biological effects of the three experimental conditions are different from each other, such a qualitative similarity might not be expected if the changes in crossover values were the direct result of the treatments. These results suggest that conditions resulting in increased crossover frequencies do not directly influence the process of crossing over. SUZUKI (1963a) has suggested that inhibition of dm-RNA synthesis by actinomycin D alters the structure of the chromosome so that crossing over can occur in these regions. We suggest that actinomycin D, radiation and a reversed acrocentric chromosome as well as other treatments which are known to increase crossing over near the centromere may do so by inhibiting the functioning of that region, thereby rendering exchange in that area more probable.

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#### SUMMARY

Crossing over was measured in the *st-in-ri-p<sup>p</sup>* regions in chromosome 3 in two consecutive five-day broods. The centromere is presumed to lie between *in* and *ri*. The presence of a reversed acrocentric compound-X chromosome with no free Y chromosome results in a five to tenfold increase in the total crossover distance between *st* and *p<sup>p</sup>* in both broods. Gamma rays (4000 rads) and actinomycin D (50  $\mu$ g/ml) increase crossing over in the *st-in* and *ri-p<sup>p</sup>* regions; the *ri-p<sup>p</sup>* region shows a greater increase relative to the control. It is suggested that the qualitative similarity of effect of three such diverse conditions indicates that these agents are not directly influencing the process of crossing over.

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