THE EFFECT OF X CHROMOSOME INVERSIONS ON CROSSING-OVER IN THE THIRD CHROMOSOME OF DROSOPHILA MELANOGASTER

- by -

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INTRODUCTION

The term "crossing-over" is used to denote the exchange of pieces or segments between homologous chromosomes. The mechanism by which this exchange is brought about has constituted one of the major fields of genetical and cytological research for many years. The early work on crossing-over was done by genetical methods which provided the means of analysis of the organization of linkage-groups—an analysis which was fundamental to the chromosome theory of heredity. Among the important discoveries resulting from the study of crossing-over were: (1) the linear arrangement of the genes (Sturtevant, 1913a), (2) the occurrence of interference between the crossing-over of nearby segments (Muller, 1916), and (3) proof that crossing-over occurs in the "4-strand stage" (Bridges, 1916; Bridges and Anderson, 1925; etc.). It was not until 1931, however, that Stern (1931), working with *Drosophila*, and Creighton and McClintock (1931) and Brink and Cooper (1935), working with *Zea mays*, demonstrated that crossing-over actually is an interchange of parts between homologous chromosome.

Percentage crossing-over may be affected by several environmental factors. Among these are:

(1) Temperature. That temperature affects crossing-over in *Drosophila* has been demonstrated by Plough (1917, 1921) who found that an increase in temperature decreased crossing-over in chromosome II, increased it in chromosome III, and had no effect on chromosome I. Stern (1926), however, reported that increase in temperature did produce an effect in chromosome I. Schwab (1935) confirmed Plough's results for chromosome III. Both Stern and Schwab found that the increase was greatest in that region of the chromosome which was associated with the spindle fibre attachment.

(2) Age of female. Bridges (1915) found that crossing-over in chromosome II decreased as the age of the female increased. There was no change in chromosome I, and a slight rise in chromosome III, which, he said, might not be significant. Plough (1917, 1921) confirmed these results. Bridges (1927) studied the effect on chromosome III in more detail, and found that the recombination percentage showed an initial high value, decreasing to a minimum value at about the eleventh day, and then rising again. The greatest effect (as in the case of the temperature-effect) was found to be in the region of the spindle fibre attachment. Stern (1926) found a slight, but significant, age-effect in chromosome I, crossing-over reaching a minimum on the fourth day and then rising to normal. Again the most susceptible region seemed to be that of the spindle-fibre attachment.

(3) Culture conditions. Neel (1941) showed that partial starvation of larvae caused a significant increase in crossing-over in all the regions which he studied in the third chromosome. The region of the spindle fibre attachment was, however, no more sensitive than the other regions.

The first evidence of a cross-over suppressor was found in *Drosophila melanogaster* by Sturtevant (1913b), although he did not recognize the fact at the time. In 1921, Sturtevant (1921) found the first definite evidence of an inversion when he showed that several third chromosome genes in *Drosophila melanogaster* were arranged in an inverted order with respect to similar genes in the third chromosome of *D. similans*. He suggested that this was due to a portion of the normal chromosome actually being inverted with respect to the rest of the chromosome. At this time he predicted that a heterozygous inversion would suppress crossing-over within the chromosome in which it occurred, but it was not until 1926 that he actually demonstrated a case of suppression of crossing-over due to an inversion (Sturtevant, 1926).

Until recently the only effect on crossing-over ascribed to inversions was that crossing-over was reduced in the chromosome which was heterozygous for the inversion. Even as long ago as 1919, however, Sturtevant (1919) found a third chromosome "gene" (really an inversion) which reduced crossing-over in the third chromosome and increased crossing-over in chromosome II. Evidence of an interchromosomal effect due to an inversion was presented by Ward (1923), who came to the conclusion that the curly inversion (in chromosome II) probably affected crossing-over in chromosomes I and III. Here the problem rested until 1932 when Schultz and Redfield (Morgan, Bridges and Schultz, 1932) showed that inversions in chromosomes I and II increased crossing-over in chromosome III, and later (Morgan, Bridges and Schultz, 1933) demonstrated that inversions in chromosomes I and III increased crossing-over in chromosome II. The increase in crossing-over in both cases was found to be due to an increase in multiple cross-overs. A similar interchromosomal effect was reported by MacKnight (1937) in Drosophila pseudoobscura.

Steinberg (1936) investigated the effect of inversions in chromosomes II and III on crossing-over in chromosome I. He found that each inversion (Curly in II and Payne in III) increased crossing-over in chromosome I, that the effect of the combined inversions was greater than the sum of the effects of each inversion taken separately, and that the increase was again due to an increase in multiple cross-overs. Further study (Steinberg, 1937) showed that the effect of an inversion on crossing-over in a non-homologous chromosome is directly proportional to the length of the chromosome affected.

The present study was undertaken to determine whether there is also a relationship between the effect on crossing-over in a non-homologous chromosome and the length and position of the inversion by which it is affected.

MATERIALS AND METHODS

Environmental conditions.

During the experiments all environmental conditions which might affect crossing-over were kept as constant as possible. All bottles were kept in an incubator at a temperature of 25-0.2°C. Hence no variation in crossing-over could have been due to temperature variations.

Three pairs of flies were used for each cross, so that there would be about the same number of offspring in each bottle, and the variation in crossing-over due to the crowding-effect would be negligible. The following food medium was used for most of the crosses: 15 g. agar, 110 g. corn meal, 15 g. dry brewer's yeast, 1 g. Moldex dissolved in 5 cc. alcohol, 125 cc. corn syrup, 750 cc. water (Sinnott and Dunn, p.398). Bottles were yeasted with a fresh yeast-suspension before being used. At one point in the experiment it was necessary to modify this food medium, but it was established that this modification caused no significant variation in crossing-over.

Inversions used.

Three X chromosome inversions of varying lengths were used (See fig. 1). The following information concerning them was taken from Sturtevant and Beadle (1936), and D.I.S. 9:

(1) Inversion yellow-4. This inversion is inseparably associated with a mutation of the yellow gene to an allelomorph very closely resembling the original yellow-1. The left-most break is located very near to or at the yellow locus, and the right between the genes fu and da. Its standard map length is about 61 units.

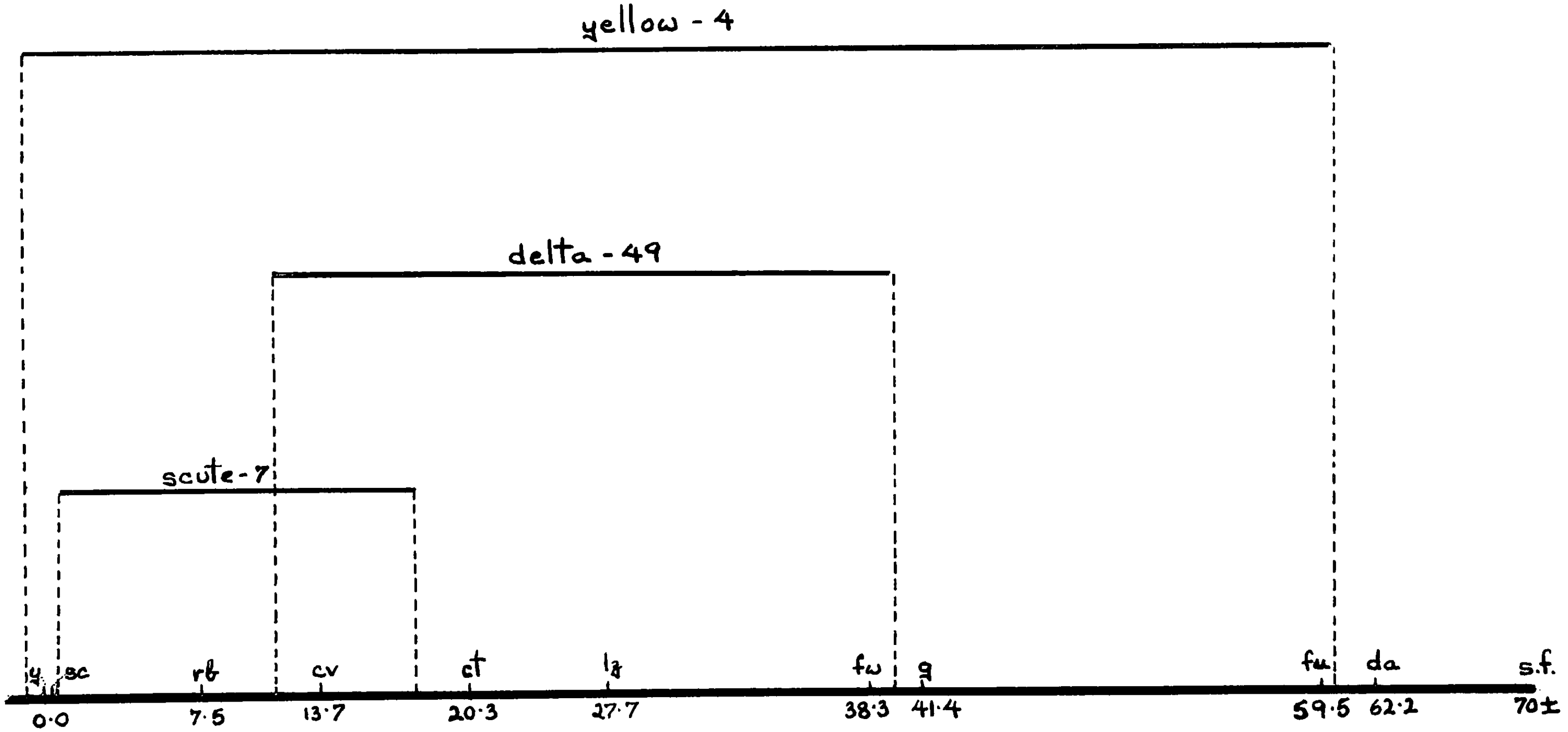
Females heterozygous for this inversion show among their regular off-spring about 2.7 per cent of double cross-overs within the inversion, as compared with a normal value of 4.6 per cent (Steinberg 1936). Crossing-over is markedly reduced to the right of the inversion.

(2) Inversion delta-49. The left break of this inversion lies between rb and cm, the right between fw and g . Its approximate map length is 31 units.

Females heterozygous for inversion delta-49 give few or no cross-overs within the inversion among their offspring, but do give recoverable cross-overs outside the limits of the inversion, those to the left occurring with considerably lower frequency than those to the right of the inversion.

(3) Inversion scute-7. The left break in this inversion is between sc and svr, the right between cv and ct. Its approximate map length is 16 units.

Females heterozygous for scute-7 have shown no crossing-over for any loci to the left of ct and a reduction of crossing over from ct to lz (see fig. 1), but to the right of lz percentage crossing-over is substantially normal.



Text fig. 1

Diagram showing relative lengths and positions of the inversions used in the present study.

Markers used.

The following mutants are used as markers to test crossing-over in chromosome III:

scarlet (st) at 44.5
stripe (sr) at 62.0
sooty (e^s) at 70.7
rough (ro) at 91.1
claret (ca) at 100.7

Outline of crosses.

Control cross

(1) $\frac{+}{+}\ ;\ \frac{\mathrm{st\ sr\ e^s\ ro\ ca}}{\mathrm{st\ sr\ e^s\ ro\ ca}}\ \mathrm{x}\ +\ ;\ \frac{+}{+}$ (Oregon-R)

(2) $\frac{+}{+}\ ;\ \frac{\mathrm{st\ sr\ e^s\ ro\ ca}}{+\ +\ +\ +\ +}\ \mathrm{x}\ +\ ;\ \frac{\mathrm{st\ sr\ e^s\ ro\ ca}}{\mathrm{st\ sr\ e^s\ ro\ ca}}$

Inversion yellow-4

(1) $\frac{\mathrm{y^4}}{\mathrm{y^4}};\ \frac{+}{+}\ \mathrm{x}\ +\ ;\ \frac{\mathrm{st\ sr\ e^s\ ro\ ca}}{\mathrm{st\ sr\ e^s\ ro\ ca}}$

(2) $\frac{\mathrm{y^4}}{+};\ \frac{\mathrm{st\ sr\ e^s\ ro\ ca}}{+\ +\ +\ +\ +}\ \mathrm{x}\ +\ ;\ \frac{\mathrm{st\ sr\ e^s\ ro\ ca}}{\mathrm{st\ sr\ e^s\ ro\ ca}}$

Inversion delta-49

(1) $\frac{+}{+}\ ;\ \frac{\mathrm{st\ sr\ e^s\ ro\ ca}}{\mathrm{st\ sr\ e^s\ ro\ ca}}\ \mathrm{x}\ \ \mathrm{dl\text{-}49,y\ Hw\ m^2\ g^4};$

(2) $\frac{\mathrm{dl\text{-}49,y\ Hw\ m^2\ g^4}}{+\ \ +\ +\ +\ +};\ \frac{\mathrm{st\ sr\ e^s\ ro\ ca}}{+\ +\ +\ +\ +}\ \mathrm{x}\ +\ ;\ \frac{\mathrm{st\ sr\ e^s\ ro\ ca}}{\mathrm{st\ sr\ e^s ro\ \ ca}}$

Inversion scute-7

(1) $\frac{\mathrm{sc^7\ w^a\ fa^2\ sn^3\ v}}{\mathrm{sc^7\ w^a\ fa^2\ sn^3\ v}};\ \frac{+}{+}\ \mathrm{x}\ +\ ;\ \frac{\mathrm{st\ sr\ e^s\ ro\ ca}}{\mathrm{st\ sr\ e^s\ ro\ ca}}$

(2) $\frac{\mathrm{sc^7\ w^a\ fa^2\ sn^3\ v}}{+\ \ +\ \ +\ \ +\ \ +};\ \frac{\mathrm{st\ sr\ e^s\ ro\ ca}}{+\ +\ +\ +\ +}\ \mathrm{x}\ +\ ;\ \frac{\mathrm{st\ sr\ es\ ro\ ca}}{\mathrm{st\ sr\ e^s\ ro\ ca}}$

Cross-over classes.

The following are the possible cross-over classes and the numbers by which they are classified:

Non-cross-overs

0	st sr e^s ro ca	+ + + + +

Singles

1	+ sr e^s ro ca	st + + + +
2	+ + e^s ro ca	st sr + + +
3	+ + + ro ca	st sr e^s + +
4	+ + + + ca	st sr e^s ro +

Doubles

1,2	st + e^s ro ca	+ sr + + +
1,3	st + + ro ca	+ sr e^s + +
1,4	st + + + ca	+ sr e^s ro +
2,3	st sr + ro ca	+ + e^s + +
2,4	st sr + + ca	+ + e^s ro +
3,4	st sr e^s + ca	+ + + ro +

Triples

1,2,3	st + e^s + +	+ sr + ro ca
1,2,4	st + e^s ro +	+ sr + + ca
1,3,4	st + + ro +	+ sr e^s + ca
2,3,4	st sr + ro +	+ + e^s + ca

Quadruples

1,2,3,4	st + e^s + ca	+ sr + ro +

RESULTS

Cross-over values obtained in the various regions for each experiment are presented in table 1, and a strand analysis showing the relative proportion of non-cross-over, single, double, and triple cross-over strands is presented in table 2. These values are calculated from the raw data which has been summarized in table 3. Both females and males were counted in the control cross, but only females were counted in the other crosses, since in each case X chromosome mutants were involved which affected eye-colour or body colour, making classification of the third chromosome mutants difficult. As a check, however, counts were made of the total number of males which hatched during each count, and no significant deviation in the sex-ratio was found. Also there was no significant difference in cross-over values for males and females in the control cross.

Table 1

Cross-over values obtained in chromosome III from backcrosses involving the various inversions.

Region	1	2	3	4	Map length	Total number of flies
Standard map length	18.0	8.7	20.4	9.6	56.7	
Control	21.3	8.9	25.6	14.0	69.8	2430
In sc^7	23.5	10.8	23.9	13.1	71.3	1277
In dl-49	29.9	11.6	22.1	14.8	78.4	1619
In y^4	30.8	11.8	23.6	15.3	81.5	1249

Table 2

Classification of strands into cross-over classes.

	Control %	In sc^7 %	In dl-49 %	In y^4 %
non-crossovers	41.4	40.7	37.9	35.5
singles	47.8	47.0	47.2	48.2
doubles	10.7	11.8	14.3	15.6
triples	0.2	0.2	0.6	0.8

Table 3

Showing numbers and types of offspring from backcrosses described above.

Regions	Control	In sc^7	In dl-49	In y^4
0	1007	522	614	444
1	301	186	282	226
2	157	93	125	87
3	478	229	221	185
4	222	94	136	104
1,2	20	13	17	19
1,3	110	56	101	81
1,4	82	43	63	50
2,3	17	10	18	15
2,4	19	20	21	23
3,4	12	9	11	7
1,2,3	0	1	1	1
1,2,4	2	1	4	3
1,3,4	2	0	4	5
2,3,4	1	0	1	1
1,2,3,4	0	0	0	0

DISCUSSION

It will be noticed that the cross-over values in the control cross are higher than the corresponding map distances, particularly in regions 3 and 4. The reason for this is not known, although it may have been due to an undetected inversion in chromosome I or II of the st sr e^sro ca stock. (The Oregon-R stock has been tested both cytologically and genetically in the laboratories of Columbia University, and almost certainly contain no inversions). With regard to this problem it is interesting to note a paper by Gowen (1933) who was investigating a cross-over suppressor, c III G, in the third chromosome, which when homozygous almost completely inhibits crossing over in all the chromosomes. Gowen, to test the effect of the gene in its heterozygous state on crossing-over in the third chromosome, used the same markers as have been used in this experiment (st sr e^s ro ca), and got cross-over values which were higher than the standard values, and which very much resembled the values obtained in the control cross of this experiment. He attributes this increase to the presence of the heterozygous c III G gene, but, as he ran no control cross with the c III G gene completely absent, it is not certain that this is the case. There may have been some other factor in his st sr e^s ro ca stock, perhaps the same one that seems to be present in the stock used in the present experiment.

Cross-over values in the experimental crosses also show irregular values for regions 3 and 4 (see table 1). Again the reason for the discrepancy is not known. It might be due to some abnormality such as a cross-over-suppressing gene or an undetected inversion which reduced crossing-over in those regions. Since the discrepancy was in the same region in all three crosses, it is not likely that the abnormal chromosomes came from the stocks carrying the inversions, but rather from the st sr e^s ro ca stock which was involved in all three crosses.

In any case, whatever the cause of the discrepancy, it is apparent that these regions (3 and 4) are definitely abnormal, and that no conclusions concerning the effects of the inversions can be drawn from them. Hence the discussion will be limited to the cross-over values obtained in regions 1 and 2, and to the results obtained from the analysis of strands into cross-over classes.

The significances of the observed increases were calculated according to the formula $\sigma_D = \sqrt{pq\ (\frac{1}{N_1}\quad \frac{1}{N_2})}$ taken from Arkin and Colton, p.122.

In region 1 the scute-7 inversion has no significant effect on crossing-over. The effects of both delta-49 and yellow-4 show a significant increase over that of scute-7, but the increase caused by yellow-4 over that by delta-49 is not significant.

In region 2 the value for sc^7 is not significantly greater than the control value, although it is very nearly so. (The deviation has 6 chances out of 100 of arising by chance).

Values for delta-49 and yellow-4 show a significant increase over the control values, but not over the scute-7 value, and the increase of the yellow-4 value over the delta-49 value is not significant.

With regard to map length, the scute-7 inversion again causes no significant increase over the control value, but the delta-49 value shows a significant increase over that of scute-7, and the yellow-4 value is significantly greater than that of delta-49.

From these data, therefore, it seems that inversion scute-7 has least interchromosomal effect, while both delta-49 and yellow-4 have a significantly greater effect. Although the effect of the yellow-4 inversion is not significantly greater than that of delta-49 in regions 1 and 2, the facts that: (1) the increase is in the same direction in both these regions (and in regions 3 and 4); and (2) that the increase in map-length caused by the yellow-4 inversion is significantly greater than that caused by delta-49, indicate that yellow-4 does have a greater effect than delta-49.

This impression is confirmed by the analysis of strands into cross-over classes (table 2). Here it may be seen that in the crosses involving the inversions non-cross-over strands are reduced, singles remain approximately constant, doubles show a decided increase, and triples show a slight increase, the effect in each case being least in the case of scute-7, greater in the case of delta-49, and greatest in the case of yellow-4. Hence it seems that the longer the inversion the greater its interchromosomal effect, although the effects of the inversions are not directly proportional to their lengths, which are in the approximate ratio of 1:2:4.

Sturtevant and Beadle (1936) have described the effects which these inversions have on crossing-over in the X chromosome (see the description of inversions in the section on materials and methods, p. 7 above). From this description it can be seen that inversion delta-49 causes the greatest reduction in crossing-over in chromosome I. The scute-7 inversion causes the least reduction in crossing-over, and inversion yellow-4 is intermediate between the other two. Hence it can be seen that, according to the results presented above, the interchromosomal effect of an inversion is not correlated with the amount of reduction in crossing-over which it causes in the chromosome pair which is heterozygous for it. The significance of this conclusion will be discussed below.

There have been several theories advanced to explain the interchromosomal effect of inversions on crossing-over. Darlington (1933), Mather and Lamm (1935), and Mather (1936) found a negative correlation between the frequencies of chiasma-formation in different bivalents of the same nucleus in a number of organisms.

To explain this negative correlation Mather (1939) advanced the theory that there was an upper limit to the number of chiasmata possible in a nucleus. According to this theory, the bivalents apparently "compete" for a limited number of chiasmata. If one chromosome gets more than its share of chiasmata, then there will be a corresponding reduction in chiasma frequency in the other chromosomes of the cell. It follows from this that a heterozygous inversion which reduces chiasma formation (and therefore crossing-over) in one chromosome pair will cause an increase in chiasma formation and crossing-over in the other chromosomes of the set.

However, if such is the case, this interchromosomal effect should be proportional to the decrease in crossing-over caused by the inversion in the chromosome pair which is carrying it. The data presented in this paper indicate that the interchromosomal effect is proportional to the length of the inversion, and not to its cross-over-suppressing effect on the chromosome pair which is heterozygous for it. These data, therefore, are not in agreement with Mather's theory.

Steinberg (Steinberg and White, 1939) has suggested that the interchromosomal effect of inversions on crossing-over were due to some physiological change caused by the chromosome breakages which gave rise to the inversion. If such is the case, the interchromosomal effect should not be proportional either to the length of the inversion or to the amount of reduction in crossing-over in the chromosome pair which carries it, since it should depend only on where the original inversion-breaks occurred.

However it must be remembered that there were only three inversions used in the present experiment, and that if the order of magnitude of the effects of the inversions was decided on a random basis, there would still be one chance in six that this order would correspond with the order of magnitude of the lengths of the inversions. Therefore no conclusions can be reached regarding this theory until the effects of several more inversions have been studied.

Schultz (Morgan, Bridges and Schultz, 1935) has attempted to explain the interchromosomal effects of inversions on a purely mechanical basis. He makes the assumptions that: (1) Crossing-over is a function of the twisting of chromosomes about each other. (2) At the last gonial telophase the homologous chromosomes tend to be associated due to somatic pairing. If this is the case, homologous chromosomes will go into interkinesis while still associated, and pairing will take place in the first meiotic prophase with a minimum of interference between chromosome pairs.

The presence of an inversion, however, will interfere with somatic pairing in the chromosome pair which is heterozygous for it, and may result in the two homologues of this pair being widely separated at interkinesis. At the first meiotic prophase, then, the efforts of these homologues to pair will set up a disturbance which will result in an increased frequency of twists or overlaps in the normally-pairing chromosomes, and hence to a rise in crossing-over in these chromosomes. In corroboration of this theory Schultz presents data showing that the exceptional daughters of XXY females, in which the X-chromosomes do not pair(and should therefore cause a disturbance in the cell), show an increase in crossing-over in the autosomes. Schultz also shows that the presence of the supernumerary Y chromosome in XXY females causes an increase in autosomal crossing-over over their normal XX sisters, as would be expected on the basis of this theory. However the control cross-over values in his experiments show a considerable decrease below the standard value, so his data may not be altogether reliable.

On the basis of Schultz's theory, the longer the inversion the more interference there will be. Hence the interchromosomal effect should be proportional to the length of the inversion. The data presented in this paper are therefore in agreement with this theory, but no definite conclusion should be drawn from them until further work has been done.

It is suggested that further investigation should be undertaken along the following lines.

1. The experiments described in this paper should be repeated, but should be arranged so that all the chromosomes involved except the one carrying the inversion and the marker chromosome (III) should come from one pure stock such as Oregon-R. Also the st sr e^s ro ca stock should be examined cytologically for a third-chromosome inversion. This would eliminate the possibility of

undetected inversions causing a discrepancy in the results.

2. The effects of more inversions should be studied.
3. The interchromosomal effects of translocations should be studied.
4. The interchromosomal effects of inversions on crossing-over should be thoroughly studied in some organism such as Zea mays which contains known inversions, and where both chiasma frequencies and genetical crossing-over can be measured.

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SUMMARY

1. It has been demonstrated in Drosophila melanogaster that the presence of a heterozygous inversion in one chromosome pair causes an increase in crossing-over in non-homologous chromosome pairs.

2. Three X chromosome inversions (sc^7, dl-49, y^4) were tested for their effect on crossing-over in the right arm of chromosome III, using the markers st sr e^s ro ca. Results indicated that the interchromosomal effects of the inversions were proportional to their lengths.

3. These results do not agree with those expected on the basis of Mather's theory of "competitive pairing", but can be explained on the basis of Schultz's theory of mechanical interference. Their significance with regard to Steinberg's hypothesis of a physiological effect cannot be determined until more inversions have been studied.

4. Suggestions are made for further investigation of this problem.

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