STUDIES ON THE MECHANISM OF CROSSING OVER

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ONE of the major objections to the torsion theory of crossing over as proposed by DARLINGTON (1935) is that it is difficult to comprehend why a break in one chromatid should cause a break to occur in exactly the same position in a chromatid from the homologous chromosome. This objection holds for any mechanism which postulates that crossing over occurs after the duplication of the chromosome into chromatids. However, as will be discussed, the localization of the crossover at identical loci offers no difficulty if it is postulated that crossing over is the result of an exchange between the new chromatids during the process of their formation (BELLING 1931). Such crossing over, of course, will give rise to only the 2-strand type of multiple crossovers, and the hypothesis requires exchanges between sister chromatids to explain the 3-strand and 4-strand multiple crossovers as was pointed out by LINDEGREN and LINDEGREN (1937). In the face of strong objections to assuming the occurrence of sister-strand crossing over, BELLING modified his original theory, thereby weakening it considerably (BELLING 1933). However, in view of evidence which has recently been presented supporting crossing over between sister strands (SCHWARTZ 1953), we should reconsider the hypothesis that crossing over between homologous chromosomes involves only the newly formed or daughter chromatids.

On such a hypothesis, two crossovers between homologous chromosomes will give rise to a 2-strand type of double exchange. A double exchange associated with a sister-strand crossover in one of the chromosomes, in the region between the two non-sister crossovers, will be converted into a 3-strand double. A sister-strand crossover in each of the homologous chromosomes will result in four single exchange chromatids, a 4-strand double. This is shown diagrammatically in figure 1. If the frequency of sister-strand crossing over is high, as was shown to be the case from the study of ring-rod heterozygotes in maize (SCHWARTZ 1953), and there is an equal probability of an even or odd number of sister-strand exchanges occurring in the region between the two non-sister-strand exchanges, the four double crossover classes would appear in a 1:1:1:1 ratio, that which is also expected (in the absence of chromatid interference) as a consequence of crossing over which involves exchanges between any of the chromatids of the homologous chromosomes.

We are thus confronted with the problem of distinguishing between these two hypotheses, each of which yields the same end result as far as meiotic

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crossing over is concerned. This problem was attacked by a study of somatic crossing over in attached-X chromosomes of Drosophila melanogaster. Studies on the stability of ring chromosomes in Drosophila have shown that little or no sister-strand crossing over occurs under normal conditions in somatic tissue (BATTACHARYA 1950; BROWN and HANNAH 1952). The instability associated with such exchanges results from the formation of dicentric double-size rings. Thus, in the absence of sister-strand crossing over, only 2-strand double somatic crossover classes would be expected on the hypothesis that crossing over between homologous chromosomes is limited to the newly formed chromatids. Somatic exchanges are not frequent enough to render an experiment on double crossovers feasible. However, by employing attached-X chromosomes it is possible to determine from single crossovers which strands are involved. Specifically, it is possible to determine whether or not chromatids which are not attached to a common centromere, that is, an old and a new chromatid, are involved in crossing over.

**EXPERIMENTAL METHODS AND MATERIALS**

Flies carrying an attached-X chromosome of the constitution $y^{Hw} sn^+$. $sn^3 Hw^+ y^+$ were synthesized by DR. W. K. BAKER of this laboratory from a triploid stock furnished by DR. E. B. LEWIS. These flies were mated individually to Muller-5 males, and the female offspring were scored for abdominal twin spots. The scoring was limited to twin spots since this is the one class of spotting which can result only from somatic crossing over. Spots of yellow or singed tissue alone could also arise as a result of mutation or chromo-

2 The period indicates the position of the centromere. The singed mutant used throughout this study was $sn^3$; the superscript is omitted in the rest of the paper.
some breakage and loss. The genotype of each fly used in a cross was determined from the phenotypes of the offspring which it produced. Only non-yellow, Hairy wing, non-singed females were employed in these crosses. Since crossing over between \( y \) and \( Hw \) is practically nonexistent, selection of flies heterozygous for yellow was assured. Females which failed to yield singed offspring were discarded, since they obviously did not carry the mutant singed gene. Flies which produced \( F_1 \) yellow-singed females were classified as having both mutants yellow and singed on the same chromosome arm, \( y sn \cdot sn^+ y^+ \). Thus only flies which produced singed and yellow but not yellow-singed offspring were used in these studies. Furthermore, only the Hairy wing, non-yellow, non-singed offspring were scored for twin spots. By this method it was possible to limit the scoring, for the most part, to flies of the desired genotype, \( y sn^+ \cdot sn y^+ \). However, this screening method does not eliminate \( F_1 \) flies of the constitutions \( y sn^+ \cdot sn^+ y^+ \) and \( y sn \cdot sn^+ y^+ \). A correction factor is required to account for the frequency of flies of these genotypes in the population of offspring scored since these flies will not give rise to twin spots by somatic crossing over. The frequency of flies of these undesired genotypes in the scored population was determined from table 2 in the Beadle and Emerson (1935) paper. Beadle and Emerson used the markers scute (\( sc \)) and cut (\( ct \)) in their study. These markers are in approximately the same positions as yellow and singed (scute and yellow are both at 0.0, cut is at 20.0 while singed is at 21.0). Thus the frequencies of the genotypes \( sc ct^+ \cdot ct sc^+ \), \( sc ct^+ \cdot ct^+ sc^+ \), and \( sc ct \cdot ct^+ sc^+ \) in the \( F_1 \) population from a \( sc ct^+ \cdot ct sc^+ \) mother will essentially be the same as the frequencies of the genotypes \( y sn^+ \cdot sn y^+ \), \( y sn^+ \cdot sn^+ y^+ \), and \( y sn \cdot sn^+ y^+ \) in the \( F_1 \) population from a \( y sn^+ \cdot sn y^+ \) mother. From this table it was calculated that 76.5 percent of the \( F_1 \) daughters scored should be of the desired constitution and 23.5 percent of the type which would not give twin spots. The correction was made by multiplying the number of flies scored by a factor of 0.765.

RESULTS AND DISCUSSION

The discussion will be limited to crossovers between singed and the centromere since that is where somatic crossing over is most prevalent (Stern 1936), and only crossovers in that region give rise to twin spots of yellow and singed tissue. As is shown in figure 2, somatic crossing over between attached-X's will give rise to a twin spot when the crossover involves chromatids which are not attached to a common centromere. A crossover which involves chromatids attached to the same centromere, i.e., the two newly formed chromatids (and also, but more unlikely the two old chromatids) will give rise only to wild-type tissue. Thus, on the hypothesis that crossing over between homologous chromosomes is limited to the new chromatids, few or no twin spots would be expected. On the other hand, according to the hypothesis that crossing over can occur between any of the chromatids, twin spotting should be at least as frequent and perhaps even more so than is found when unattached-X chromosomes are employed since attachment of the chromosomes at one end could make for closer somatic pairing. On the latter hypothesis only half of
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Figure 2.—Diagrammatic representation of the results of somatic crossing over in attached-X chromosomes involving (a) chromatids attached to a common centromere, and (b) attached to different centromeres. Broken lines represent new chromatids.

The crossovers between attached-X chromosomes would yield twin spots. In the case of unattached-X chromosomes all the crossovers can give twin spots, however, as Stern has shown, only half of the segregations are of the type which will give this result.

The results are shown in table 1. Line 1 in this table gives the results of the experiment without Minute in the cross. Line 2 shows the frequency of twin spots obtained when the spotting was increased by using the autosomal Minute, M(3)y. The Minutes have been shown to be responsible for a marked increase in the frequency of somatic crossing over (see Stern 1936). It is readily apparent that the frequency of twin spots is extremely low as compared to that obtained when the same markers were used in unattached-X chromosomes (line 4). This indicates that crossing over between attached-X chromo-

Table 1

<table>
<thead>
<tr>
<th>Total flies scored</th>
<th>Corrected* total</th>
<th>Number twin spots</th>
<th>Percentage twin spots</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Attached-X (not aged)</td>
<td>945</td>
<td>723</td>
<td>1</td>
</tr>
<tr>
<td>2. Attached-X (not aged)</td>
<td>1360</td>
<td>1041</td>
<td>8</td>
</tr>
<tr>
<td>3. Attached-X (aged)</td>
<td>926</td>
<td>709</td>
<td>48</td>
</tr>
<tr>
<td>4. Unattached-X (not aged)</td>
<td>180</td>
<td>27</td>
<td>15.00</td>
</tr>
<tr>
<td>5. Unattached-X (aged)</td>
<td>196</td>
<td>26</td>
<td>13.27</td>
</tr>
</tbody>
</table>

*See text.
†All experiments except those listed in line 1 were segregating M(3)y.
somes is limited to the newly formed chromatids. The few cases of twin spots observed in the attached-X females are discussed in a later section.

Brown and Hannah (1952) have reported that the stability of the ring chromosome in Drosophila is strongly influenced by certain environmental conditions. For example, aging of the female before mating was responsible for a high degree of mosaicism in the offspring. One of the hypotheses proposed to explain this effect was that aging increased the frequency of sister-strand crossing over. On the basis of this report the effect of aging on twin spotting was studied.

As was mentioned previously, somatic crossing over was chosen for these studies since sister-strand crossing over does not normally occur with an appro-

![Diagram of somatic crossing over between the newly formed chromatids not associated (upper) and associated with sister-strand exchange (lower). Broken lines represent newly formed chromatids.](image)

preciable frequency in somatic tissue. Under these conditions an extremely low frequency of twin spots is obtained. However, the frequency of twin spots should be greatly increased if, in addition to the exchanges between homologous chromosomes, sister-strand exchanges occurred. This is diagramed in figure 3. Crossovers between the newly formed chromatids yield only wild-type tissue in the absence of sister-strand exchanges. When the crossover is associated with a sister-strand exchange in the region of the centromere, a twin spot will result. On the hypothesis that crossing over occurs at random between the new and old chromatids, the frequency of twin spots should remain unaffected by aging. A sister-strand exchange will cause a twin spot to be formed when associated with a crossover between chromatids which are attached to a common centromere, but conversely, only wild-type tissue will result when the sister-strand exchange is associated with a crossover.
between chromatids attached to different centromeres (without the sister-strand exchange such crossovers would give a twin spot). The results of these experiments are given in line 3 of table 1. An 8.7-fold increase in twin spotting was obtained by aging the attached-X mothers as virgins for approximately 13 days before mating.

An alternative explanation for the effect of aging on the frequency of twin spotting is that aging causes an over-all increase in the frequency of somatic crossing over. It was possible to rule out this alternative by comparing the frequency of twin spots obtained in the progeny of aged and unaged females which carried unattached-X chromosomes. If aging increases somatic crossing over, the aged females would be expected to show the ninefold increase in twin spotting which was obtained with attached-X's. However, if the effect of aging is limited to sister-strand crossing over no difference would be expected between the progeny of aged and unaged females. The frequency of twin spots in unattached-X flies should not be increased by sister-strand crossing over.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Effect of sister-strand exchange on somatic crossing over between free-X chromosomes; without (upper) and with sister-strand exchange (lower).}
\end{figure}

This is diagrammatically shown in figure 4. Sister-strand crossing over would merely cause twin spots to result from those segregations which normally would yield only wild type tissue, and conversely, produce wild-type tissues from those segregations which would form twin spots in the absence of sister-strand crossing over. The results are shown in lines 4 and 5 of table 1. Unaged females gave 15.0 percent twin spots in their offspring while the aged females gave 13.3 percent.

Brown and Hannah (1952) propose two alternative explanations for the effect of environment on the stability of ring chromosomes: (1) sister-strand crossing over, and (2) twists in the plane of reproduction. The results herein reported point to the first of these as the correct hypothesis since twists in the plane of reproduction, such as were suggested by Braver and Blount (1950) to give interlocked rings, would not increase the frequency of twin spotting resulting from crossing over between attached-X chromosomes where the ends are free.

During the course of these experiments it was noted that a considerable
number of singed spots not associated with a yellow twin were found. Could these spots be considered as consequences of somatic crossing over? Stern explains the singed spots in flies of the constitution \( y^+ sn+ / y+ sn \) as resulting from two possible events. First, they could arise, but with a very low frequency, from double crossovers occurring on each side of the singed locus. The other possibility is that the singed spots are actually twin spots in which the tissues arising from the cells which were homozygous yellow did not form hypodermal tissue which would give rise to setae, and thus would not be recognizable. We have tested the latter explanation in experiments on the spotting produced in flies which had both recessive genes on the same chromosome arm, \( y^+ sn. sn+ y^+ \). If the singed spots found in the \( y^+ sn+ . sn y^+ \) flies were actually twins where the yellow spot was undetected, they should not arise in \( y^+ sn. sn+ y+ \) flies since here somatic crossing over will produce spots in which the same tissue is both yellow and singed. It was found that the same frequency of singed spots were found in flies of both genotypes, 10.0 and 10.3 percent. This rules out somatic crossing over as the agent responsible for most of the spots which were singed alone, and suggests that they represent cases where the singed phenotype was expressed in the heterozygote. The distribution of these singed spots was not at random over the abdomen and very frequently involved the same bristle in many flies.

A critical analysis of the data of Stern (1936) clearly shows that it is the presence of the \( Y \) chromosome which is responsible for the frequent expression of the singed phenotype in the heterozygous females. Stern found that the frequency of spotting was greatly increased (sixfold) by the presence of a \( Y \) chromosome. In one experiment 11.6 percent spots were found in the XX females and 72.8 percent in the XXY females. Furthermore, Stern states, "The influence of the \( Y \) is of course even greater in this experiment than the number 72.8 percent indicates, for this represents all individuals of experiment 2, about half of which were of XX constitution." However, a breakdown of the types of spots shows no significant difference between XX and XXY females in the frequency of yellow spots and yellow-singed twin spots. The high rise in the frequency of spotting is due almost entirely to a tremendous increase in the frequency of singed spots; 1.4 percent (15/1040) in the XX females as compared to 56 percent (195/349) in the XXY + XX females. On the basis of these experiments it is possible to explain the high frequency of singed spots in our study since all of the attached-X females carried a \( Y \) chromosome. These experiments are of further importance in that they establish that the \( Y \) does not reduce the frequency of somatic crossing over between the X chromosomes in an XXY female.

CONCLUSIONS

The results of the experiments reported in this paper suggest that crossing over is a result of two events: (1) exchanges between the newly formed chromatids of homologous chromosomes, and (2) exchanges between sister chromatids. These two types of exchanges are entirely different phenomena which may occur at different times.
The experiments further indicate that chromosome duplication involves the formation of a new chromatid on a template provided by the old chromosome. If duplication involved the splitting of a double-size chromosome into two equal chromatids, both of these chromatids should be equally capable of participating in non-sister-strand crossing over. Somatic crossing over in Drosophila is for the most part limited to exchanges between the new chromatids. In view of this evidence it is proposed that meiotic crossing over involves both exchanges between the new chromatids of homologous chromosomes and between sister chromatids. The few cases of twin spots observed in attached-X daughters from unaged females could be due either to a low frequency of sister-strand crossing over, or to a breakdown of the attached-X chromosome followed by somatic crossing over.

If, as this work suggests, crossing over occurs during the process of the formation of the new chromatids, the time of chromosome duplication in meiosis can be approximated from the time at which crossing over takes place. It has been generally accepted that chromosome pairing precedes crossing over. It is therefore possible to fix the time of chromosome duplication as occurring after chromosome pairing, i.e., zygotene or early pachytene. However, the replication of the genic material (deoxyribonucleic acid) which, in part at least, constitutes the chromosome, occurs much earlier, in interphase, as determined from studies with P\textsuperscript{32} incorporation (see Taylor 1953; Swift 1953). The discrepancy between the time of gene replication and chromosome duplication is large, and leads to the obvious conclusion that they do not represent one and the same process. It is therefore proposed that chromosome duplication involves simply the joining together of the genic material which had previously been synthesized on the chromosome. It should be pointed out that this is the conclusion which Belling (1931) had reached from his cytological studies.

It is further proposed that chromosome duplication is initiated at a number of loci forming partial replicas along the length of the chromosome. There need not be any correlation between the loci where duplication is initiated on the homologous paired chromosomes. In the absence of crossing over, the partial replicas laid down on one chromosome meet and join to form a new chromatid. Crossing over occurs when the homologous parental chromosomes are closely paired and union occurs between the end of a replica from one chromosome with the end of a replica from the other. The parental chromosomes acting as templates ensure that only equal crossing over will occur, i.e., since on the parental chromosome \(a\) is next to \(b\), a replica with \(a\) at its end can unite only with the \(b\) end of another replica. This is in essence only a modification of the hypothesis of crossing over which Belling proposed in his 1931 paper.

It has long been recognized that the problem of crossing over is basic to the study of genetics. Following a very active period in the early '30's it has not been the subject of much intensive research. More work is needed in this field, and it is hoped that the new findings on recombination in microorganisms will stimulate further studies on this important but elusive problem.
SUMMARY

The low frequency of twin spots resulting from somatic crossing over between attached-X chromosomes of *Drosophila melanogaster* suggests that such exchanges are normally limited to the newly formed chromatids. Aging of the mothers as virgins before mating results in a nine-fold increase in the frequency of twin spots recovered in the offspring. This increase is interpreted as being due to sister-strand exchanges. Somatic crossing over between the new chromatids in attached-X's will give rise to only wild type tissue unless associated with sister-strand exchanges. The results herein reported support the BELLING hypothesis of crossing over.

ACKNOWLEDGMENT

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LITERATURE CITED


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