THE MEIOTIC ORIGIN OF TEMPERATURE-INDUCED CROSSEOVERS IN DROSOPHILA MELANOGASTER FEMALES

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It has been known since the early work of Plough (1917, 1921) that alterations in temperature can increase recombination frequencies in females of Drosophila melanogaster. Two distinct, yet related, problems have arisen from such studies. First, does the additional frequency of crossing over represent gonial or meiotic events, or both? Second, does temperature act at the time of crossing over or before?

Initially, Plough proposed that the effect is a meiotic one, occurring in the earliest oocyte and most probably at the time of exchange. In a subsequent paper (1924) he expressed the somewhat altered view that the sensitive stage was the gonial cell and that induction occurred prior to the meiotic event. Whittinghill’s studies (1964) indicated to him that at least a component of heat-induced crossovers might be oogonial in origin, whereas Thompson (1964) not only favored a gonial origin, but also suggested that heat treatment permanently affects the female, leading to increased recombination frequencies among her progeny for the duration of her egg production. A permanent effect must have its origin in the stem cells.

The present experiments are directed toward answering only the first question, i.e., the origin of temperature-induced crossovers. Genetic tests are available which permit one to arrive at an unambiguous answer. Two such tests have been utilized here, one employing the phenomenon of secondary nondisjunction and the other nonrandom disjunction. Both are special types of meiotic segregation, the former occurring at anaphase I, the latter at anaphase II. In each case the frequency of segregation is directly related to the amount of meiotic exchange. When exchange is increased by temperature, one kind of segregation will result if meiotic exchange is involved; another kind if it is gonial exchange. Hence, the effect of increased exchange on meiotic segregation provides a means of discriminating between the two different types of origin. The results indicate an exclusively meiotic origin.

EXPERIMENT I—SECONDARY NONDISJUNCTION

Materials and Methods: Secondary nondisjunction is X nondisjunction in females that carry a Y chromosome. At anaphase I, the two X’s instead of segregating to opposite poles as is normally the case, both move to the same pole while the Y chromosome moves to the other pole. If the

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Figure 1.—The predicted consequences on the frequency of secondary nondisjunction and on the X-chromosome constitution of exceptional female progeny if temperature-induced crossing over is of the following origins: (b) gonial, (c) gonial and meiotic, (d) meiotic.
maternal and paternal X's are properly marked, the progeny that result from this XX ↔ Y segregation are recognizable as female exceptions (matroclinous females), which carry both maternal X's but no paternal X, and as male exceptions (patroclinous males), which carry only the paternal X.

If one uses progeny testing to examine the two maternal X's carried by exceptional females, one finds that they are virtually always noncrossover chromosomes (BRIDGES 1916; GEMHENSON 1935; FALK 1955). Furthermore, one can interfere with crossing over between the two maternal X's by introducing an inversion into one and in this way increase the number of noncrossover X-tetrads. One then finds that the frequency of secondary nondisjunction is greatly increased. Using a variety of heterozygous inversions which interfere with crossing over to different degrees, it has been possible to show that as crossing over decreases, secondary nondisjunction increases (STURTEVANT and BEADLE 1936; GRELL 1962). Conversely, if one alters two X's from a heterozygously inverted to an isosequential state, thus increasing the amount of crossing over, one notes a corresponding reduction in secondary nondisjunction.

Instead of using inversion heterozygosity vs. isosequentiality to alter recombination frequency, one may use temperature. For certain chromosome regions higher temperature has been shown to cause an increase in recombination (PLOUGH 1917; STERN 1926). If one is dealing here with an increase in meiotic exchange, then a decrease in noncrossover X-tetrads and a correlated decrease in secondary nondisjunction will be expected, as shown in Figure 1d. Alternatively, if temperature is creating gonial crossovers, these crossovers will be resolved at the next mitotic division. The resulting X chromosomes, although recombinant, will enter meiosis without chiasmata.

A second distinction between the consequences of gonial and meiotic exchange can be made. If temperature were inducing gonial exchange, some of the meiotic noncrossovers that become secondary exceptions would have undergone premeiotic exchange. In contrast to the normal situation, where secondary exceptions carry noncrossover X's, some of the female exceptions would now be expected to carry crossover X's (Figure 1b). A fraction of such crossovers would be detected immediately by homozygosis of markers carried by either X; the remainder could be detected by progeny tests.

For the present studies XXX females were prepared that carried one X chromosome with the inversion, In(1)65 (LINDSLEY, EDINGTON and von Halle 1960; GRELL 1962), one X in normal sequence, and a Canton-S Y chromosome. The inverted X carried the recessive markers yellow\(^1\) (y\(^1\), 0.0) and forked (f, 56.7); the normal X carried yellow-2 (y\(^2\), 0.0), acute (sc, 0.0), vermilion (v, 33.0), cararnation (car, 62.5), and Dp(1;1)vsc\(^1\), y\(^+\) to the right of the centromere. Earlier work of STERN (1926) has shown that crossing over in the proximal region of X is increased with higher temperature. Crossing over was measured for the three proximal regions, u-f, f-car, and car-centromere. The region between car and the spindle fiber attachment was studied by utilizing the pericentric inversion Inp(1)vsc\(^1\) to place y\(^+\) to the right of the centromere and by replacing the deficient left arm of X with a normal X chromosome carrying y\(^2\), sc, v, and car.

Disjunction of the X chromosomes was followed by the presence of Bar (B, 57.0) in the attached-XY (LINDSLEY and Novitski 1950) chromosome of the father. Regular males and females are B\(^+\) and B, respectively, whereas exceptional males and females are B and B\(^+\), respectively. The experimental procedure was as follows: Virgin females of the genotype shown in the following cross, Cross No. 1—In(1)65, y\(^1/y\(^2\) se v car y\(^+\)/Yc-\(\ast\) females × attached-XY, y B/Y males—were collected within 6 hours after eclosion. One group, in bottles, was placed in an incubator at 35 ± 1.0°C for 8 hours and then kept at 25 ± 1.0°C for 16 hours. A control group was kept at 25 ± 1.0°C for the entire 24-hour period. At the end of this time both groups were mated in bottles (5 females and 8 males per bottle) to attached-XY, y B/Y males. The parents were transferred to fresh bottles daily for 14 days, and kept at 25 ± 1.0°C throughout. The period of egg laying comprising the first 24 hours was designated day 1 and represented eggs laid 16 to 40 hours after heat treatment, or a mean of 28 hours; the next 24-hour period was designated day 2, etc. Brooding in this manner permits sampling of successively earlier stages of oogenesis at the time of treatment. Although it has been shown (GRELL and CHANDLEY 1965)
that the time elapsing between DNA replication and egg laying is not uniform for all oocytes, and that this leads to some heterogeneity in each day's sample, nevertheless the majority sampled in the day-2 brood represent oocytes treated at an earlier stage than the majority sampled on day 1, etc.

Progeny tests of exceptional females were carried out in order to determine whether such females carried only noncrossover X chromosomes, as expected with exclusively meiotic exchange, or whether they also carried some crossover X's, which would represent gonia1 exchanges contributed to the pool of meiotic noncrossover X-tetrads available for secondary nondisjunction. In the broods from days 7 to 10, 74 exceptional virgin daughters were collected from heat-treated mothers and 72 exceptional virgin daughters from the control mothers. Each female was individually mated in a vial to three attached-XY, \( Y^B/Y \) males. Her male progeny were later examined in order to analyze the genotype of her X chromosomes.

In addition, 134 nonvirgin exceptional females from broods representing days 7 to 14 of heat-treated mothers were progeny-tested. Progeny of nonvirgin XXY females provide somewhat less conclusive evidence of the X chromosome constitution of the mother since she could have mated with a brother carrying a noncrossover maternal X. In that case, exceptional male progeny would carry a noncrossover X of paternal origin, and such males would be indistinguishable from regular males carrying a noncrossover maternal X. Nevertheless, the two major classes of sons should represent the two maternal X's since male exceptions are less frequent than either type of regular noncrossover-X son.

RESULTS

Secondary nondisjunction frequencies: The frequencies of both crossing over and secondary nondisjunction for the 14 daily broods of heat-treated and control females are shown in Table 1 and Figure 2. The two sets showed no significant differences.

**TABLE 1**

<p>| Crossing over and secondary nondisjunction in 14 daily broods from heat-treated and control females of the genotype ( In(1)^{65}, Y f/y^2 sc v car\cdot y^+ + Y^Q^O-8 ) females |
|---------------------------------|--------|--------|--------|--------|--------|--------|</p>
<table>
<thead>
<tr>
<th>Daily brood</th>
<th>Control</th>
<th>Heat-treated</th>
<th>Percent crossing over v to centromere</th>
<th>Control</th>
<th>Heat-treated</th>
<th>Percent nondisjunction</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>1586</td>
<td>1155</td>
<td>19.3</td>
<td>19.7</td>
<td>2170</td>
<td>1535</td>
</tr>
<tr>
<td>2</td>
<td>1840</td>
<td>1680</td>
<td>15.6</td>
<td>18.5</td>
<td>2496</td>
<td>2261</td>
</tr>
<tr>
<td>3</td>
<td>890</td>
<td>1017</td>
<td>12.7</td>
<td>15.1</td>
<td>1190</td>
<td>1380</td>
</tr>
<tr>
<td>4</td>
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<td>664</td>
<td>13.5</td>
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<td>870</td>
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<tr>
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<td>492</td>
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<td>8.9</td>
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<td>662</td>
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<tr>
<td>6</td>
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<td>472</td>
<td>9.3</td>
<td>8.3</td>
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<tr>
<td>7</td>
<td>516</td>
<td>414</td>
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<td>8.0</td>
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<td>564</td>
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<tr>
<td>8</td>
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<td>463</td>
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<td>12.8</td>
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<td>15.9</td>
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<td>364</td>
<td>8.6</td>
<td>17.0</td>
<td>543</td>
<td>486</td>
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<tr>
<td>11</td>
<td>341</td>
<td>304</td>
<td>8.5</td>
<td>14.5</td>
<td>453</td>
<td>400</td>
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<tr>
<td>12</td>
<td>243</td>
<td>348</td>
<td>9.9</td>
<td>12.9</td>
<td>337</td>
<td>464</td>
</tr>
<tr>
<td>13</td>
<td>268</td>
<td>326</td>
<td>11.2</td>
<td>15.6</td>
<td>358</td>
<td>437</td>
</tr>
<tr>
<td>14</td>
<td>270</td>
<td>276</td>
<td>11.1</td>
<td>13.4</td>
<td>360</td>
<td>374</td>
</tr>
</tbody>
</table>

* Crossing over calculated from all males and one half of females (i.e. non-\( y^+ \) regular females and observed exceptional females).

† Nondisjunction frequencies corrected for lethality of one half of exceptional progeny.
difference in crossover values for the first 8 days. On days 9 and 10 the heat-
treated group showed significant increases over control values. The peak occurred 
on day 10, and subsequent days showed no significant difference between groups.

Nondisjunction values for heat-treated females were also similar to those for 
controls for the first 8 days. As expected, the maternal-age effect on crossing over, 
reflected in the decreasing crossover values between days 1 and 6, had its counter-
part in a correlated increase in secondary nondisjunction between days 1 and 6. 
On day 9 a marked decrease in secondary nondisjunction as compared to the 
control was observed; on day 10 it reached a minimum corresponding to the 
maximum crossing-over value on day 10.

If heat-induced crossovers were gonial in origin, nondisjunction values should 
have remained unaltered despite increased recombination frequencies. If heat-
induced crossovers were totally or partially meiotic in origin, a reduction in non-
disjunction values would be expected. The results are in accord with the latter 
expectation, indicating that heat-induced crossing over occurs in the oocyte.

Constitution of X chromosomes of female exceptions: Although the above 
results demonstrate temperature-induced crossing over occurs in the oocyte, they 
do not preclude the possibility that a small but measurable component of heat-
induced recombination occurs in gonia. To determine whether this is so, the 
phenotypes of all of the exceptional female progeny were examined. Gonial ex-
change followed by equational separation of sister chromatids with equal proba-
bility of the recovery or nonrecovery of the two exchange products in the same
daughter cell is expected to lead to homozygosis for the recessive markers carried by the X chromosome 25 to 50% of the time, depending on the location of the crossover. Thus, a crossover between \( v \) and \( f \) will lead to homozygosis for \( sc \) and \( v \) 25% and to a wild phenotype 75%; a crossover between \( f \) and \( car \) will lead to homozygosis for \( f \) 25%, homozygosis for \( sc \) and \( v \) 25%, and to a wild phenotype 50%; a crossover between \( car \) and the centromere will lead to homozygosis for \( f \) 25%, homozygosis for \( sc \), \( v \), and \( car \) 25% and to a wild type 50%. On the other hand, directed segregation of the two crossover products to opposite poles should lead to 50% homozygosis for a crossover between \( v \) and \( f \) and to 100% homozygosis for a crossover in the other two regions. No single instance of homozygosis for X chromosome markers was detected among 1954 exceptional females.

To uncover possible X chromosome recombinants that were not directly observable, progeny tests of 74 virgin exceptional females recovered in the 7-10 day broods from heat-treated mothers and of 72 virgin exceptional females from comparable broods of control mothers that were carried out. The tests of the 74 females from heat-treated mothers revealed that 72 carried noncrossover X chromosomes; the remaining two were sterile. Similarly, the tests of the 72 females from control mothers disclosed that the 70 fertile females carried noncrossover X chromosomes.

Finally, among the 134 nonvirgin exceptional females collected from the day 7-14 broods of heat-treated mothers, male progeny carrying both the \( \text{In}(1)_{65}, y f \) and the \( \text{y}^{2} \text{sc v car} \cdot y^{+} \) chromosomes were recovered in every case. As noted above, one such class of noncrossover X could represent exceptional males carrying a paternal rather than a maternal X. Although a smaller number of exceptional males than either class of noncrossover regular male is expected, the number of progeny was in many instances too few to permit a conclusion.

The absence of homozygosis for any of the recessive markers in the exceptional females, as well as the failure to uncover a single crossover X from progeny tests of exceptional virgins, indicates that temperature exerts its effect by creating additional meiotic crossovers. If a gonial component is present, it is so small that much larger numbers of exceptional females are required for examination and progeny testing in order to detect it.

EXPERIMENT II—NONRANDOM DISJUNCTION

**Materials and Methods:** Two homologues of unequal length often fail to be recovered in a 1:1 ratio from Drosophila females. The reason for this departure from equality was first understood by Novitski (1951), who gave it the name “non-random disjunction.” Its application to translocation heterozygotes was demonstrated by Zimmering (1955). Novitski was able to show that this phenomenon depends on the occurrence of a crossover somewhere between the homologous centromeres and the tip of the shorter homologue, leading to the formation of the two unequal dyads at anaphase I and the preferential inclusion of the shorter of the two chromatids into the egg nucleus at anaphase II. The extent of preferential inclusion is called the coefficient of nonrandomness, or \( c \) value, and apparently depends on the degree of asymmetry between the two components of the dyad. It is constant for any particular genotype.

In practice, nonrandom disjunction is recognized by studying the reciprocal crossover classes of progeny for the region involved and determining their departure from a 1:1 ratio. When
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a. A GONIAL CROSSOVER (NO MEIOTIC CROSSOVER)

GONIA

MEIOSIS

INITIATION

REPLICATION AND CROSSING OVER

ANAPHASE I

RESULT

1. GONIAL CROSSOVER RESOLVED AT MITOTIC DIVISION NEVER LEADS TO ASYMMETRIC DYADS. SEGREGATION I NEVER LEADS TO ASYMMETRIC DYADS. SEGREGATION II LEADS TO ASYMMETRIC DYADS. THE COEFFICIENT OF NON-RANDOMNESS REDUCED TO THE EXTENT GONIAL CROSSING-OVER OCCURS.

b. A MEIOTIC CROSSOVER (NO GONIAL CROSSOVER)

FIGURE 3.—The predicted consequences of (a) a gonial and (b) a meiotic crossover between B and y+ on asymmetric dyad formation and on the coefficient of nonrandomness among the crossover progeny from females T(1;4)B5, B y+/y2 sc f in genotype.

temperature is used to increase exchange, if the increase occurs in the oocytes there should be an increase in the frequency of asymmetric dyads but no alteration in their coefficient of nonrandomness. Alternatively, if temperature acts to produce gonial exchanges, such recombinants will be resolved at the next mitotic division. In fact, the segregation of the tetrads at this division should frequently and perhaps usually lead to structural homoygosis for the translocation and for the normal chromosomes, and thus even preclude the formation of asymmetric dyads from a subsequent meiotic crossover (See Figure 3, segregation I). Hence, gonial crossovers will not form asymmetric dyads at anaphase I, they will not participate in nonrandom disjunction at anaphase II, and the two products of gonial exchange are expected to be recovered in a 1:1 ratio.

Since the determination of the c value is based upon the total observed crossover progeny, c will be reduced by the occurrence of gonial crossovers. Moreover, the extent of reduction of c becomes a measure of the amount of gonial exchange. In fact, whether the c value is reduced or not constitutes a highly sensitive test for the occurrence of gonial exchange since a relatively small contribution of gonial exchanges should cause a detectible reduction in the c value.

For the present studies, females were prepared that carried the reciprocal translocation (1;4) Bar-Stone [T(1;4)B5] heterozygously. In this translocation both the X and fourth chromosomes are broken into two parts. The break in X lies between f and B at 16A1; the break in four is distal to all fourth chromosome markers. The small tip of four is translocated to the proximal part of X close to the B locus, and the large distal part of X is translocated to the main body of four. The proximal piece of X carries the inseparable marker Bar (B, 57.0) at its distal end, and
at its other end, to the right of the centromere, the inseparable marker \(y^+\), again introduced by utilization of the pericentric inversion \(Inp(1)sc^V1\). The distal piece of \(X\) carries the marker yellow-2 (\(y^2\), 0.0). The normal \(X\) chromosome is marked with yellow-2 (\(y^2\), 0.0), scute (\(sc\), 0.0), and forked (\(f\), 56.7). The male parent carried \(y^2\), sc, and \(f\) in his \(X\) chromosome. The genotypes of the parents in Cross No. 2 are \(T(1;4)B^8\), \(B\ y^+/y^2\ sc\ f\) female \(\times y^2\ sc\ f\) male.

In order that asymmetric dyads be formed at meiosis I, crossing over must take place between the proximal piece of \(X\) and the normal \(X\). All single crossovers in this region will lead to a realignment of the parental markers \(B\) and \(y^+\) as shown in Figure 3. The presence of \(f\) in the normal \(X\) chromosome of the mother as well as in the paternal \(X\) permits a distinction to be made between progeny carrying both parts of the translocation, which are \(y^+ f^+ B\) in phenotype, and progeny carrying only the proximal part of \(X\) as a duplication, which are \(y^+ f B\) in phenotype.

For this test virgin females of the genotype shown in Cross No. 2 were collected within 6 hours after eclosion. One group, in bottles, was placed in an incubator at \(35 \pm 1^\circ C\) for 24 hours; the control group was kept at \(25 \pm 1^\circ C\) for the first 24 hours. At the end of this time both groups were mated in bottles to \(y^2 sc v\) males (four females and four males per bottle); the offspring were kept at \(25 \pm 1^\circ C\) throughout development. Beginning on day 6 and daily thereafter through day 11, the parents of both groups were transferred to fresh bottles. At the end of the 11th day the parents were discarded.

Crossing over between \(B\) and \(y^+\) was measured for the six broods laid between days 6 and 11, i.e., those days when the progeny were expected to show the effect of the heat treatment (Grell and Chandley 1965). The coefficient of nonrandomness among the \(y^2 B\) and \(y^+ B^+\) crossovers was calculated for the heat-treated and control groups by use of the simplified formula

\[
c = \frac{[\text{shorter chromatid crossovers} (y^2 B)]}{[\text{total crossovers} (y^2 B \text{ plus } y^+ B^+) ]}
\]

where a value of 0.5 represents random disjunction and 1.0 represents exclusive recovery of the shorter chromatid. (This formula is applicable in the present case since crossovers of a rank higher than \(E_1\) are negligible.)

**RESULTS**

Crossover values for the progeny of the heat-treated and control females are shown in Table 2. Only the total crossover data for the six-day period are presented, since the numbers for each daily brood were too small for significance. The amount of crossing over in the progeny of the heat-treated females (7.9 \(\pm\) 0.5\%) showed more than a threefold increase over control values (2.3 \(\pm\) 0.2\%). Despite this difference in exchange, the \(c\) values for the two groups (Table 1) are alike 78.9 \(\pm\) 2.4\% for progeny of heat-treated females and 78.6 \(\pm\) 4.0\% for the control.

The \(c\) value would have been reduced if some or all of the temperature-induced increase in crossing over had been due to gonial crossovers, whereas no change in

<table>
<thead>
<tr>
<th>Reciprocal crossovers between (B) and (y^+)</th>
<th>Total progeny</th>
<th>Crossing over between (B) and (y^+)</th>
<th>Coefficient of nonrandomness</th>
</tr>
</thead>
<tbody>
<tr>
<td>(y^2 B^+)</td>
<td>(y^2 B)</td>
<td>Total</td>
<td>4448</td>
</tr>
<tr>
<td>Heat-treated</td>
<td>60</td>
<td>227</td>
<td>287</td>
</tr>
</tbody>
</table>

**TABLE 2**

Crossing over and nonrandom disjunction in day 6–11 broods of heat-treated and control females of the genotype \(T(1;4)B^8\), \(B\ y^+/y^2\ sc\ f\) mated to \(y^2\ sc\ f\) males
the $c$ value is anticipated if the increase involves only meiotic crossovers. The unaltered $c$ value indicates that temperature increases meiotic exchange exclusively.

**DISCUSSION**

Alterations in the frequency of crossing over by temperature have been reported for a variety of organisms including Drosophila (Plough 1917; Stern 1926), Neurospora (Rifaat 1959; McNelly and Frost 1963; Towe and Stadler 1964), Ascobolus (Lissouba 1961), and Sphaerocarpus (Abel 1965). Whether temperature acts to bring about its effect directly, at the time of exchange, or indirectly, i.e., at some earlier time, is unknown. That the mechanism is not a simple one, as for example an alteration in the rate of a chemical reaction, is suggested by the fact that departures from a certain normal temperature in either direction will increase recombination; although here it might be postulated that the kinetics of some crossover-repressor substance is being affected.

In Drosophila the site for the induction of the temperature effect has been variously ascribed to the stem cells (Thompson 1964), to oogonial cells (Plough 1924; Stern 1926), and the oocyte (Plough 1917). The possibility of stem-cell induction, either directly or indirectly, seems to be ruled out by two types of experiments. First, a number of studies (Plough 1917; Stern 1926; Grell and Chandley 1965) have demonstrated that the effect is limited in duration and does not affect the entire subsequent egg production of the female. Second, heat treatment of stem cells during early development, when oogonia and oocytes are absent, does not produce the increase in crossing-over associated with heat treatment of later stages (Plough 1917; Grell unpublished).

A number of alternatives remain: (1) Temperature may be affecting gonia directly or indirectly to cause gonial crossing over. (2) It may be affecting gonia indirectly to cause meiotic crossing over. (3) It may be affecting the oocyte directly or indirectly to cause meiotic crossing over. (4) It may be causing both gonial and meiotic crossing over by direct or indirect means.

The present experiments are concerned with determining the origin of the crossovers and not the mechanism involved. Thus, the purpose is to distinguish between 1, 2–3, and 4—i.e., a gonial origin, a meiotic origin, or a mixed origin. The results of both of the tests indicate that temperature is creating only additional meiotic crossovers.

Whittinghill (1955) has presented a comprehensive review of the subject of gonial exchange. The analysis of the secondary exceptions provides some information concerning the possible occurrence of a small amount of gonial crossing over under normal conditions. Inspection of 924 exceptional females from control mothers did not reveal a single case of homozygosis for the recessive markers $sc$, $v$, $f$, or $car$. Successful progeny tests of 70 virgins from this group disclosed that all 70 carried noncrossover X chromosomes. These results suggest that gonial crossing over does not normally occur in females, or that it occurs so rarely as to make its contribution to the total amount of crossing over negligible.

Recent work (Grell and Chandley 1965) has suggested that the times of
DNA replication and of crossing over in the Drosophila oocyte are roughly co-incident. This conclusion hinges on the assumptions that the temperature effect on crossing over is a direct one and that temperature-induced crossovers are predominantly or exclusively meiotic in origin. The latter assumption has now been verified. Should it become possible to demonstrate that the former assumption is also correct, a very strong case for the interdependence of DNA synthesis and crossing over could be made. This would in no way imply that crossing over need involve a copy-choice mechanism; only that such a mechanism remains a possibility.

**SUMMARY**

Two tests were performed in order to discriminate between the alternatives of a meiotic, a gonial, or both a meiotic and gonial origin for temperature-induced recombinants. In the first test the phenomenon of secondary nondisjunction was utilized. Newly eclosed females of the genotype $In(1)65$, $y F/y^2$ sc v car$y^+$/Yc-s were treated with a temperature of $35 \pm 1^\circ C$ for 8 hours, and the frequencies of crossing over and secondary nondisjunction among their progeny were compared with control values. In this test, (1) a reduction in secondary nondisjunction and the occurrence of female exceptions carrying exclusively noncrossover X chromosomes would indicate a meiotic origin; (2) no reduction in secondary nondisjunction and the occurrence of some female exceptions carrying crossover X chromosomes would indicate a gonial origin; (3) a reduction in secondary nondisjunction and the occurrence of some female exceptions carrying crossover X chromosomes would indicate a mixed origin. The results showed that a reduction in secondary nondisjunction accompanied the increase in crossing over. No evidence was obtained for X chromosome crossovers among female exceptions as judged by homozygosis for X chromosome markers and by progeny tests. These results are consistent with an exclusively meiotic origin for temperature-induced recombinants.—In the second test, the phenomenon of nonrandom disjunction was utilized. Newly eclosed females of the genotype $T(1;4)B^8$, $B y^+ / y^2$ sc f were treated with a temperature of $35 \pm 1^\circ C$ for 24 hours, and crossovers between $B$ and $y^+$ were scored among the progeny. Crossing over in this region leads to the formation of asymmetric dyads at anaphase I and the preferential inclusion of the shorter of the two chromatids into the egg nucleus at the next division. In this test, (1) no change in the “$c$” value (i.e., the coefficient of nonrandomness describing the degree of preference) would indicate a meiotic origin for temperature-induced crossovers; (2) a reduction in the $c$ value would indicate a gonial or mixed origin. The observed $c$ values obtained were $78.9 \pm 2.4\%$ and $78.6 \pm 4.0\%$ for the progeny of heat-treated and control females, respectively, while appropriate crossover values were $7.9 \pm 0.5\%$ and $2.3 \pm 0.2\%$. Again the results indicate an exclusively meiotic origin for temperature-induced crossovers.
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LITERATURE CITED


