MEIOTIC BEHAVIOR OF ASYMMETRIC DYADS IN THE MALE DROSOPHILA

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ABSTRACT

Crossing over in the interstitial region of the heterozygous V₄ translocation in Drosophila melanogaster generates asymmetric dyads each consisting of a shorter and a longer chromatid. It was shown previously that in the female the shorter is recovered preferentially (ZIMMERING 1955); the present work suggests that the same occurs in the male as well. The mechanism in the female is envisioned as involving the non-random inclusion of the shorter chromatid into the functional egg (Novitski 1951); in the male, the two formal possibilities appear to be (1) that some proportion of sperm carrying the longer chromatid derived from an asymmetric dyad undergoes dysfunction, or (2) the longer chromatid is preferentially included into regularly-produced nonfunctional sperm, the nonfunctionality set up at the second division and distinguishing second division daughter cells.

SINCE only an outer product of the linear array of four products of oogenesis becomes the functional egg, the suggestion of nonrandom disjunction in the female Drosophila melanogaster (Novitski 1951) rests on a firm developmental basis; it requires only that of a longer and a shorter chromatid making up an asymmetric dyad, generated following exchange between members of a heteromorphic bivalent (Novitski 1951; ZIMMERING 1955) or in certain kinds of compound X’s (Novitski and Sandler 1956; LINDSLEY and Sandler 1965; Sandler and LINDSLEY 1967), the shorter chromatid is ultimately situated preferentially in an outer egg nucleus and the longer in an inner nucleus. In order to throw more light on the nature of meiosis in the male Drosophila, a genetic analysis was made of the meiotic behavior of asymmetric dyads induced by X-rays in primary spermatocytes. More direct comparison between results in the male and female was achieved through the use of the V₄ translocation [T(2;3)bw⁺]; evidence of nonrandom disjunction in the heterozygous V₄ female, as predicted by Novitski (1951), was reported earlier by ZIMMERING (1955).

MATERIALS AND METHODS

The V₄ translocation arose following a break in 2L at the bw⁺ locus, a break in 3L in the centromeric heterochromatin and appropriate reattachments to produce translocated chromosomes

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Figure 1.—The consequences of adjacent I disjunction following crossing over in the interstitial region in the heterozygous $V_4$ translocation. The numbers 2 and 3 stand for the centromere regions of the normal second and third chromosomes, respectively; 2' and 3' for the homologous regions of the translocated chromosomes; S denotes 2R; and L' represents 3L of the translocated chromosome. It is implied that the unmarked arms, 2R and 3R, as well as 3L of the normal chromosome, are always associated with the appropriate centromere.

of the types 2L:2R+3L and ·3L (the raised dot indicates the position of the centromere; Glass 1933). A purely diagrammatic representation of the pairing configuration in heterozygous $V_4$ males is shown in Figure 1. The numbers 2 and 3 designate the centromeres of the normal second and third chromosomes, respectively; 2' and 3' designates the homologous regions of the translocated chromosomes; S denotes 2R; and L' denotes 3L of the translocated chromosome. Now an exchange in the interstitial region gives rise to asymmetric dyads consisting of a shorter chromatid (S) and a longer (L'). Following adjacent I disjunction (or alternate disjunction if
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the positions of 2 and 2' are reversed after crossing over), four products are formed, two of which, 01 and 02, are orthoploid gametes and recoverable in crosses with non-translocation-bearing females. (A1 and A2 are aneuploid gametes and recoverable only in crosses with heterozygotes translocation females.) It should be noted that 01 carries the shorter crossover chromatid (S) and 02 the longer (L'). Now in the absence of an exchange in the interstitial region, and upon alternate disjunction, orthoploid gametes of chromosomal compositions identical with those of 01 and 02 are produced, but in this case the shorter (S) and longer (L') chromatids are derived from symmetric dyads.

In each of two experiments, prepupal males of the composition V4/b cn px bw sp were irradiated with 1000 r of X-rays, brooded for each of four days with 4-5 b cn px bw sp females and then discarded; the first brood was made 24 hours after eclosion of the male. It has been found that the vast majority of the most mature cells at the prepupal stage are at or just prior to the first division of meiosis (Khushin 1955). Experiments were also carried out with females identical in composition with the male, i.e., V4/b cn px bw sp, and in females of the composition b cn V4/px bw sp; the females were not irradiated since spontaneous exchange in the interstitial region is sufficient to generate an appreciable number of asymmetric dyads (Zimmering 1955). Since bw/bw and bw/V4 individuals are indistinguishable without further testing, progeny were scored on the basis of the presence or absence of one or more of the differential markers, b, cn, px, and sp. The positions of these markers on the genetic map are b48.5, cn-57.5, px-100.5, and sp-107.0; b is located to the left of the centromere, in 2L, and all others in 2R (Bridges and Brehme 1944). The b-cn region includes the centromeric heterochromatin and is expected to yield the majority of induced crossovers in the male by virtue of its well-known high susceptibility to X-ray breakage. The two regions for which crossovers were obtained in both males and females permitting direct comparison were b-cn and cn-px; in Table 1, single crossovers in the former region are referred to as SCO-1, in the latter as SCO-2 and double crossovers, one in each region, as DCO-1,2.

RESULTS AND DISCUSSION

Results of the two experiments of irradiated V4/b cn px bw sp males, and the experiments involving V4/b cn px bw sp and b cn V4/px bw sp females are shown in Table 1. The former type of female is designated Female I and the latter Female II. It should be noted that in Female II, the genetic and chromosomal makeup of the SCO-2 class corresponds to the NCO class in both males and Female I, the DCO-1,2 class to SCO-1, NCO to SCO-2, and SCO-1 to DCO-1,2. Examination of the data reveals the following points. As approximate equality of reciprocal classes for SCO-1's was found in each irradiated male experiment, cn px sp/b = 63/73 and 121/111 respectively; for SCO-2's, an inequality was found, px sp/b cn = 32/17 and 53/30. Attention is directed to SCO-2's since (virtually) all are derived from asymmetric dyads, the px sp class representing the recovery of the shorter crossover chromatid (S) and b cn the longer (L'). The c value of (S), i.e., the coefficient of nonrandom recovery, computed as the number of (S) chromatids/number of (S) + (L') chromatids (Novrremark 1951) is 0.64 (85/132) in the male based on the sum of the classes of the two experiments, and 0.65 in Female I (965/1481). The deviation from equality in the male is highly significant (P = .001) and in the female more so. Deviations of this magnitude cannot be accounted for either on the basis of (a) the chromosomal makeup of the gametes since gametes bearing chromosome types identical with these but recovered as NCO's in the male and Female I exhibit no such inequality (c of (S) = 0.47), or (b) differential viability since individuals of identical phenotype, px sp and
TABLE 1

Results from test crosses of V_{y}/b cn px bw sp males irradiated at the prepupal stage with 1000 r with b cn px bw sp females, and of unirradiated V_{y}/b cn px bw sp and b cn V_{y}/px bw sp females, Female I and Female II, respectively, with b cn px bw sp males

(See text for further explanation)

<table>
<thead>
<tr>
<th>Crossover classes for Male and Female I</th>
<th>Chromosomal makeup of gametes</th>
<th>Shorter (S) or Longer (L') chromatin</th>
<th>Genetic makeup of gametes</th>
<th>Irradiated male</th>
<th>Female I</th>
<th>Female II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Experiment 1</td>
<td>Experiment 2</td>
<td>Totals</td>
</tr>
<tr>
<td>NCO (a) 2L'2R/3L'3R S b cn px sp</td>
<td></td>
<td></td>
<td></td>
<td>19,545</td>
<td>29,221</td>
<td>48,766</td>
</tr>
<tr>
<td>(b) 2L'2R+3L'/3R L' + + + +</td>
<td></td>
<td></td>
<td></td>
<td>22,617</td>
<td>32,293</td>
<td>54,910</td>
</tr>
<tr>
<td>SCO-1 same as (a) S + cn px sp</td>
<td></td>
<td></td>
<td></td>
<td>63</td>
<td>121</td>
<td>184</td>
</tr>
<tr>
<td>same as (b) L' b + + + +</td>
<td></td>
<td></td>
<td></td>
<td>73</td>
<td>111</td>
<td>184</td>
</tr>
<tr>
<td>SCO-2 same as (a) S + + px sp</td>
<td></td>
<td></td>
<td></td>
<td>32</td>
<td>53</td>
<td>85</td>
</tr>
<tr>
<td>same as (b) L' b cn + +</td>
<td></td>
<td></td>
<td></td>
<td>17</td>
<td>30</td>
<td>47</td>
</tr>
<tr>
<td>DCO-1,2 same as (a) S b + px sp</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>same as (b) L' + cn + +</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>9</td>
<td>15</td>
</tr>
</tbody>
</table>
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are recovered with equal frequency as NCO's in Female II (c of (S) = 0.51). Furthermore, the same phenotypes recovered as NCO's in the male and Female I but as SCO-2's in Female II give clear indications in the latter of a departure from randomness (c of (S) = 0.62).

In both Females I and II, c of (S) for SCO-1's is 0.54. This result can most simply be explained if (1) symmetric dyads do not result in nonrandom recovery and (2) the majority of crossovers occur in the b-centromere rather than the centromere-cn region; the former give rise to symmetric dyads so that c of (S) is expected to be about 0.5, and the latter to asymmetric dyads to give a c of (S) of about 0.65. These arguments are supported from a consideration of genetic map distances where b at 48.5 is some 6.5 crossover units and cn at 57.7 some 2.5 crossover units from the centromere (around 55.0). In the male, c of (S) for SCO-1's, 0.50, is similar to that in the females, but significantly lower than the expected 0.57 if induced crossing over occurs equally frequently in 2L and 2R heterochromatin, i.e., in the heterochromatin contained within the b-centromere and the centromere-cn regions, respectively. This discrepancy can be explained simply, however, as resulting from the inclusion of individuals in the b class arising through "mutation" of b+ to b rather than through crossing over and/or from induced crossing over's occurring somewhat more frequently in the b-centromere than in the centromere-cn region.

DCO-1.2's in both females yield a high c of (S), 0.62, close to or identical with SCO-2's. These results can be explained along the same lines accounting for the c of (S) in SCO-1's (see above); that is, the vast majority of crossovers occur in the b-centromere rather than the centromere-cn region. This is so because all double exchanges involving the b-centromere and cn-px regions give rise to asymmetric dyads in 2R and consequently the c for the recovery of shorter chromatid, b px sp, should be equal to that of px sp from SCO-2's. Some diminution in c would come about from double exchanges involving the centromere-cn and cn-ps regions since in these cases, only 1/3 of all DCO's are derived from asymmetric dyads.

In the male, c of (S) for DCO's, 0.25, is significantly out of line with the female, although not a inordinately high "mutation rate" of cn+ to cn is necessary to account for this discrepancy. For example, if c were in fact identical to that in the female, 0.62, then relative numbers of b px sp and cn of, 5 and 3, respectively, would require an induced "mutation rate" of about $22 \times 10^{-5}$ or about 1/5000 at 1000r to primary spermatocytes [the rate is based on 12 cn's (15–3) among some 55,000 (L')-bearing offspring]. It is obvious, however, that the numbers involved here are very small so that estimates of this kind are subject to considerable error.

Two comments may be made concerning evidence favoring the meiotic origin of the crossovers recovered in the male: (a) Analysis of the distribution of crossovers among the progeny of the irradiated males shows, for example, for SCO-2's, the most critical class in the experiment, that of the 84 px sp (S) offspring, 74 were recovered as singles, six as clusters of two from each of three males, and three as a cluster of three from one male; of the 47 b cn (L') offspring, 45 were
recovered as singles, and the remaining two as a cluster from one male. (b) It may be seen that if a spermatogonial crossover occurs in the interstitial region, two possible kinds of primary spermatocytes may be produced—one type carrying \(2S/2L'\), both noncrossovers and identical with the top configuration in Figure 1, and the other, \(2L'/2S\), both crossovers. From the former, \((S)\) and \((L')\) gametes will be recovered as NCO's with equal frequency, and from the latter, being derived, as those above, from symmetric dyads, \((S)\) and \((L')\) crossovers should similarly be recovered with equal frequency. This would mean that crossovers recovered from gonia1 interchange in this region would have the effect of reducing the size of the discrepancy between the \((S)\) and \((L')\) crossover classes for SCO-2's; despite this possibility, the data clearly suggest an excess of recovery of \((S)\) as compared with \((L')\) crossovers.

As indicated above, the regular establishment of an ordered array of the four products of oogenesis provides a basis for the suggestion of nonrandom disjunction to account for the results from the \(V_4\) female (Novitski 1951; Zimmering 1955). In the male, however, cytological studies have failed to reveal an ordered arrangement of the four meiotic products, as occurs in the female (see, for example, Cooper 1951). Furthermore, evidence for the possibility that two of the products of male meiosis are regularly nonfunctional and that the nonfunctionality is set up at the first division of meiosis (the “functional-nonfunctional poles” hypothesis of Peacock and Erickson 1965) has been brought into serious question (Zimmering and Fowler 1968; Hartl 1969), and its inapplicability to cases of meiotic drive in the male, i.e., segregation-distorter (Sandler, Hiraizumi and Sandler 1959) and “sex-ratio” (Gershenson 1928), recently demonstrated through electron micrography by Tokuyasu, Peacock and Hardy (1972) and Policansky and Ellison (1970), respectively. An interpretation of the data from the \(V_4\) male on the basis of regularly-produced nonfunctional sperm would require the establishment at the second division of meiosis two daughter cells, one of which will become a functional, the other a nonfunctional sperm, and the nonrandom inclusion of the longer crossover chromatid into the cell destined to become nonfunctional.

The alternative explanation is that for some reason about half the time a sperm carries the longer chromatid of an asymmetric dyad, but not one of equivalent composition from a symmetric dyad, and it dysfunctions. The term “gametic dysfunction” was coined by Lindsley and Sandler (1958) to describe some unique property of sperm representing one genetic alternative conferred upon it by reason of being heterozygous with another at meiosis. In the present case, heterozygosity would be most simply imagined as being between members of the asymmetric dyad rather than members of a heteromorphic bivalent as in the Bar-Stone case (Novitski and I. Sandler 1957; Novitski 1970), although the two may be related in some way. It should be noted that Mukherjee and Das (1971) have reported highly unequal recovery of reciprocal crossover products following spontaneous crossing over in the \(D. ananassae\) male, paralleling results in the female, but not involving asymmetric dyads. Since spontaneous crossing
over in the *D. ananassae* male may reach frequencies similar to those in the female, electron micrography could prove a useful tool in providing a basis for a decision between the alternatives suggested above; the V<sub>4</sub> case in *D. melanogaster* does not lend itself to an analysis of this kind since the critical event is recovered with a frequency of only 1% or so following a dose of 1000r. On the other hand, genetic approaches involving V<sub>4</sub> in combination with other systems of meiotic drive are expected to prove very useful in this regard (see, for example, Novitski and Peacock 1970). Finally, it may be noted that the possibility that the bridges produced by X-rays involve the longer chromatid more frequently, thus eliminating about half the SCO-2 (L'), is an unlikely explanation to account for the observed results, since, for example, a deficiency of similar magnitude would be expected in (L') NCO's as compared with (S) NCO's. Examination of the relevant data in Table 1 shows clearly this is not the case.

**LITERATURE CITED**


