SOME RADIATION EFFECTS ON SEGREGATION IN DROSOPHILA

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ABSTRACT

Translocation induced in the immature oocyte, in meiotic prophase, affects division I orientation and segregation, the usual result being that the two halves of translocations are directed to opposite poles. Since interchange is usually (if not exclusively) between chromatids, this is to be expected from the creation of illegitimate conjunctions. Good agreement is obtained between patterns of segregations deduced from recovered half-translocation bearing exceptions and the kinds of disomic gametes expected as alternative recoveries from the same division I configurations. Inferences drawn from the study of compound-X females have been found to apply as well in the case of females of normal karyotype. Numerical errors occur predominantly, possibly exclusively, in division I. The rate of induced nondisjunction of specific chromosome pairs varies in relation to the structure of the entire complement, as required if radiation-induced nondisjunction is interchange dependent, but which would be unexpected if the mechanism involved effects on individual spindle fibers, chromosomes, or chromosomal bivalents.

THE question of how radiations alter chromosomal segregation is one of long standing, dating from the experiments of MAVOR (1921, 1922, 1924) in which an increase was found in both nullisomic and disomic eggs following the irradiation of female Drosophila. PATTERSON and MULLER (1930) rejected rearrangement as a possible cause of numerical aberration, based on their inference that the non-recovery of reciprocal translocations from irradiated oocytes meant that rearrangement was not inducible in female germ cells. They tacitly assumed that rearrangement occurring in meiotic prophase would have no effect on subsequent segregation. We now know this assumption was erroneous; rearrangements can be induced in the oocyte and they can affect the way segregation will occur. The inference that rearrangement plays no role in numerical aberration, being based on an erroneous conclusion, is accordingly suspect.

Our work of the past several years has shown that rearrangement that is induced by irradiating immature oocytes can play a major role in determining orientations on the division I spindle. We can now see that it requires only the uniform operation of ordinary meiotic mechanics to lead to exactly the kinds of recoveries that we make when immature oocytes are irradiated; we see this from the analysis of “half-translocations.” We can recover one of the two elements

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of a simple reciprocal translocation, showing that rearrangement is inducible in the oocyte. The analysis of exceptions carrying these half-translocations lets us see how segregation has occurred and shows us what the alternative recoveries from the same meiotic configurations must be. In many cases these will exhibit numerical rather than structural aberration. Let us ask, then, why and how segregation is affected.

Chromosomal disjunction implies an antecedent conjunction or “pairing.” While conjunction that is maintained by chiasmata minimizes segregational error, homologs may be held in conjunction without them, as is the case with some five percent of X-chromosome bivalents and with virtually all fourth chromosome bivalents. Heteromorphs, such as the Y and compound-X chromosomes, likewise maintain an achiasmate conjunction leading to rather precise disjunction. Nonhomologous chromosomes may also engage in such distributive pairing (Grell 1968) and assort apart rather regularly. Thus, it appears to be bivalent conjunction rather than bivalent homology that is recognized by the meiotic apparatus, leading to division I orientations that guarantee the assorting apart of elements comprising homologous, heteromorphic, or heterologous bivalents.

An event that leads to the conjunction of two chromosomes prior to the time when orientation on the spindle is fixed should usually result in an orientation having the centromeres of the conjoined components lying across the equator from each other. The occurrence of chromatid interchange in meiotic prophase should lead to just such heterologous conjunctions. If this be true, then the simple application of Mendel’s first law to heterologous bivalents would result, at least in the simplest cases, in the separation of the two halves of newly-induced translocations at division I. We should be able, as we are, to recover half-translocations much more readily than reciprocal translocations. The analysis of exceptions carrying these half-translocations will tell us how the homologous or heteromorphic pairing partners of the chromosomes that have undergone interchange will have oriented and segregated, and will allow us to deduce what other segregation products can be recovered from cells in which such interchanges occur.

Let us review the supporting evidence, which is derived largely from the study of half-translocations recovered as detachments of attached-X chromosomes, or as Y-chromosome fragments. We have chosen to analyze those which result from interchange with chromosome 4. These chromosomes, the compound-X, the Y and the fourth, comprise the distributive pairing pool of females that carry them; these chromosomes are useful from two standpoints. First, the meiotic problems are simpler, since the involvement of two of these in rearrangement has the effect of creating simple conjunctions—simple in that it is unlikely that multivalent configurations will arise.

The second useful attribute of these chromosomes is that aneuploidy involving them will have minimal effects on the recovery and testing of exceptions. The adult will be male or female according to whether the zygote has one or two X chromosomes. Trisomy for chromosome 4 has little phenotypic consequence, and simple schemes can be used to circumvent the deleterious effects of fourth chromosome monosomy. Finally, Y-chromosome hyperploidy usually has little effect
on the viability or fertility of either sex, so the simple precaution of handling \( Y \) chromosomes and their derived fragments as supernumerary chromosomes eliminates the possibility of major problems of viability or fertility of the exceptions. Hence, we work with these chromosomes, compound-\( X \), \( Y \), and \( 4 \), because they make it possible for us to ask our questions with experiments of relatively simple design.

Breakage and rejoining of chromosomes, resulting in the recovery of half-translocations, can occur in any stage of oocyte development yet studied (Par-ker 1963; Parker and Williams 1970), and the resulting interchanges occur between chromatids rather than between whole chromosomes (Parker 1969). The best evidence for this is the duplicated recovery of markers present only singly in the treated female (e.g., Williamson 1969, 1970) and the nonrandom recoveries of reciprocal interchange products, due to the properties of heteromorphic dyads (Parker 1969). Hence, interchange should lead to novel conjunc-

Recovery of one half of a translocation raises the question of when the two halves separated: was it at the first or at the second meiotic division? Chromatid interchange makes it simple to get the answer in experiments involving the detachment of compound-\( X \) chromosomes (Figure 1). If the two halves of the

**Figure 1.**—Hypothetical segregation following interchange between the compound-\( X \) and a fourth chromosome, assuming (1) no effect of interchange on segregation and (2) regular assort-

ment apart of chromosome \( 4 \) homologs at division I. All capped-detachment-bearing exceptions will be hyperploid, having a free maternal chromosome \( 4 \) that is the homolog of the cap if the translocation halves separated at division I (Gamete C-1) or the sister of the cap if separation was at division II. This model does not properly describe the half-translocation recoveries made.
translocation move to the same pole at division I, but to opposite poles at division II, as shown in the top half of the figure, then the capped detachment will be recovered along with the intact sister of the chromatid that furnished its distal, capping end (Gamete A-2). Well over ninety percent of the recoveries are not of this type, and the high incidence of the 3- and 4-break rearrangements leads us to believe that the few cases of sister recovery that are made may have resulted from the formation of trivalents rather than by the segregation shown. At the most, only a few of the separations of translocation halves can have occurred at division II (PARKER 1969). However, neither does the alternative given in the lower half of the figure properly describe segregation. If homologous chromosomes assort apart even when one is involved in interchange with a heterolog, then the exceptions carrying capped detachments will always be hyperploid for the cap. Yet a very large number, on the order of forty percent, of the capped detachments are recovered in hypoploids having no free maternal chromosome 4.

If the heterologous conjunction of two chromosomes, resulting from interchange, prevents the conjunction of either of these with its normal pairing partner, then the latter should either behave as a univalent or else should display novel distributive pairing relationships, depending on the initial composition of the distributive pairing pool and the nature of the removals from it. Homologous centromeres might then move to the same pole at division I or move apart, seemingly independently, as illustrated in Figure 2. Evidence that this is the case (PARKER 1969) is given by the nearly equal recovery of half-translocations with or without the intact homolog of the chromatid furnishing the cap (Gametes E-1 and G-1).

Another novelty of the pattern of assortment revealed by the analysis of half-translocation exceptions is that it will also lead to the formation of nullisomic and of disomic gametes (Gametes E-2 and F-2). Please note in the diagram that the nullo-4 gamete has a compound-X, while the diplo-4 gamete is nullo-X. Our expectations have been realized in this case, as well as in others where tests have been made. Eggs disomic for chromosome 4 are virtually always nullo-X when attached-X females without a Y are irradiated (PARKER and WILLIAMSON 1970), and are usually nullo-X and virtually always nullo-Y when the treated attached-X females carry a Y (PARKER 1970 and Table 1). The former finding agrees with expectations based on the analysis of detachments carried out by PARKER (1969), while the latter shows agreement with WILLIAMSON’s (1974) findings where Y fragments are capped by chromosome 4, that some 80-90 percent of segregations are of the types illustrated in Figure 3.

Furthermore, there are nonrandomly high coincidences of segregational error of X and of fourth chromosomes when karyotypically normal females are irradiated. Between one-fourth and one-fifth of the nullo-X gametes are diplo-4 (TRAUT and SCHEID 1971; PARKER and BUSBY 1973), and about seventy-five percent of the diplo-4 gametes are nullo-X, with many of the “nondisjoining” fourth chromosomes appearing to have exchanged proximal segments with the only other kind of acrocentric present, the X (PARKER and BUSBY 1973). We also find that diplo-X eggs frequently are also nullo-4, about forty percent being of this con-
Figure 2.—Hypothetical segregation following interchange between the compound-X and a fourth chromosome, assuming (1) interchange-conjunction of the two chromosomes directs them to opposite poles at division I and (2) the homolog of the interchange-involved fourth chromosome behaves as a univalent. This model accounts for the usual recoveries, capped detachments accompanied by the homolog of the chromatid furnishing the cap (Gamete G-1), capped detachments without an accompanying free maternal chromosome 4 (Gamete E-1), diplo-4 nullo-X (Gamete F-2), and compound-X nullo-4 (Gamete E-2).

Table 1

<table>
<thead>
<tr>
<th>Genotype treated</th>
<th>Triplo-4 incidence percent</th>
<th>Number</th>
<th>Sex of triplo-4 exceptions:</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sex of triplo-4 exceptions:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C[1]RM/0^*$</td>
<td>0.8</td>
<td>311</td>
<td>96.6</td>
<td>11</td>
<td>3.4</td>
</tr>
<tr>
<td>$C[1]RM/Y^+$</td>
<td>1.7</td>
<td>216</td>
<td>81.2</td>
<td>50</td>
<td>18.8</td>
</tr>
</tbody>
</table>

* Data from Parker and Williamson (1970).
† Data from Parker (1970).
FIGURE 3.—Two of four hypothetical segregations following Y-4 interchange in compound-X females, assuming the interchange-conjoined chromosomes go to opposite poles at division I. Y fragment recoveries show these two to account for some 80–90% of all segregations; fourth-chromosome nondisjunction (Gametes I-2 and J-1) conforms to the predictions of this model.

3%; PARKER and BUSBY 1973). Hence, it is not surprising that fourth chromosomes that have lost the marker placed on the short, heterochromatic left arm commonly show a marked change in behavior when distributive pairing properties are measured (PARKER and BUSBY 1973). This is to be expected from a change in size and content, with a number of these chromosomes carrying some loci from the proximal segment of the X. Furthermore, X chromosomes have been recovered with either 4L or 4R linked. These occur with a nonrandomly high frequency in the XXY female exceptions. In fact, more are recovered from this source than from all other genotypes combined in the progeny of irradiated females (PARKER, WILLIAMSON and GAVIN, 1974; PARKER, 1974).

If numerical aberration induced by oocyte irradiation results from interchromosomal events, then the incidence of specific kinds of gametic disomy should to some extent depend upon the structure of the entire chromosomal complement. It has been found (Table 1) that the presence or absence of a Y chromosome in attached-X females affects the incidence of disomy for chromosome in attached-X females affects the incidence of disomy for chromosome 4, this occurring about twice as frequently when the attached-X females have a Y chromosome (PARKER
1970) as when they do not (PARKER and WILLIAMSON 1970), consistent with the reported competition of X and Y chromosomes for fourth chromosome interchange (PARKER 1954, 1965).

In females with free X’s, the elimination of acrocentrics, accomplished by the fusion of the euchromatic arms of X and of 4, is accompanied by a pronounced lowering of the incidence of XXY females, to be expected if many of these results from the effects of X-4 interchange on segregation (Table 2; PARKER, unpublished). With females having such attached X-4 chromosomes, X-4 interchange would either occur between homologs or else would be internal to one of the chromosomes, giving rise to pericentric inversions that would be undetectable in many cases, and to compound-X or compound-fourth chromosomes, and should operate either to assure further the assorting apart of these homologs, or else would have no effect, as reported by WILLIAMSON (1969) in the case of Y fragments arising from interchange between the two Y arms. Rearrangements do occur, as shown by the recoveries of the above-mentioned compound chromosomes, but the incidence of XXY trisomy is lower than when X and 4 are separate acrocentrics, in accord with expectations, with about one-half of the “nondisjunctions” resulting from the formation of new compound X’s.

The objection might be raised at this point that we have usually used experimental designs that would permit the recognition only of the kinds of errors that our model proposes, those occurring at division I. Division II errors, with certainty, would not arise by the mechanism we have advanced. However, the early data of ANDERSON (1931) concerning the crossover status of nondisjoining X’s had already demonstrated, by marker heterozygosity, that most X nondisjunction induced by X-irradiation occurs at division I. ANDERSON believed that a minority of his XXY females resulted from division II nondisjunction, but the X’s in his experiment lacked adequate marking in the proximal region to support this conclusion. MERRIAM and FROST (1964), studying spontaneous nondisjunction of the X’s, demonstrated clearly that one cannot use homozygosis frequencies derived from attached-X’s as a basis for one’s expectations for homozygosis in XXY exceptions. We have recently looked at the frequency of homozygosis of two proximal markers, carnation and suppressor of forked, and have found the incidences to be no higher than might be expected from spontaneous crossing over

### TABLE 2

<table>
<thead>
<tr>
<th>Genotype irradiated</th>
<th>Dose</th>
<th>Number of offspring</th>
<th>Number Female exceptions</th>
<th>Number XXY</th>
<th>Number C[1]/RM</th>
<th>X0 Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>X/X ; 4/4</td>
<td>0</td>
<td>3,558</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>X/X ; 4/4</td>
<td>3 kR</td>
<td>3,520</td>
<td>27</td>
<td>27</td>
<td>0</td>
<td>153</td>
</tr>
<tr>
<td>X-4/X-4</td>
<td>0</td>
<td>3,751</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>X-4/X-4</td>
<td>3 kR</td>
<td>7,197</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>282</td>
</tr>
</tbody>
</table>

Induction of XXY females in stocks where X and fourth chromosomes are acrocentrics or are fused (Robertsonian translocation) to form a submetacentric.
and interchange events induced proximal to these markers. Out of 137 analyzed XXY exceptions, seven were homozygous for one or the other of these markers. Of these seven, one was a new compound-X, one was a Robertsonian fusion of X and 4, and three were without a maternal chromosome 4. It is likely that some "induced crossing over" may also have been involved in this region, but the demonstration of this would have required the marking of the short right arm of the X. In any case, the segregational errors of the X occur predominantly, and perhaps exclusively, in division I when they result from exposure to ionizing radiation.

We have considered so far only what happens when immature oocytes are irradiated. According to Traut (1970), the treatment of mature oocytes does not lead to an increased incidence of trisomy. If it does not, why not? The analysis of half-translocations induced in mature, stage 14 oocytes suggests that it should not do so, and in fact imposes the firm requirement that orientation for division I be already fixed at the time the cells are irradiated, or at least at the time interchange is actually effected (Busby 1971; Williamson 1973). Thus, our experiments point the way toward defining the stage of oogenesis at which distributive pairing relationships become fixed and no longer subject to alteration by interchange.

This is not to say that once this stage is reached the normality of segregation is assured. Segregational error may come about in other ways, as shown by the meiotic mutants of Sander et al. (1968), or as shown by the induction of division I nondisjunction by low-temperature treatment of mature oocytes reported by Tokunaga (1970). Our conclusions speak only to the question of nondisjunction induced by irradiating immature oocytes, but we certainly think that any event, spontaneous or mutagen-induced, that leads to chromatid interchange in the immature oocyte would produce results similar to those obtained by irradiation.

Any model, ours or otherwise, that seeks to interpret radiation-induced effects on segregation must account for these effects' being less inducible in stages treated near the time of division, that is, in the mature oocyte, than in those treated much earlier, in immature stages. If numerical aberration results either from generalized damage to chromosomes, affecting the normality of centromere function, or from damage to the cell's ability to mobilize a spindle properly, we may ask why these effects, which we know can persist for more than a week, are expressed only at division I rather than occurring also at division II only some five minutes later. Our model accounts for this in that interchange, once it is accomplished in the immature oocyte, will persist and will affect division I orientations. It is required only that interchange precede orientation; should this sequence be reversed, orientation remains unaltered by interchange.

LITERATURE CITED


RADIATION AND SEGREGATION IN DROSOPHILA


