COMPARATIVE FREQUENCY OF X-RAY INDUCED CROSSOVER-SUPPRESSING ABERRATIONS RECOVERED FROM OOCYTES AND SPERM OF *DROSOPHILA MELANOGASTER*

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THE incidence of dominant lethality is of the same order of magnitude in oocytes as in sperm of *Drosophila melanogaster* given the same radiation exposure (SONNENBLICK 1940; BATEMAN and CHANDLEY 1963). However, when oocytes of females bearing free X chromosomes are irradiated, the frequency of X-ray induced translocations is only 1/100 of that recovered from sperm given the same X-ray exposure (KANELLIS and RADU 1943). This is puzzling since dominant lethality is usually attributed to gross chromosomal rearrangements. PATTERSON and MULLER (1930) suggested that spatial relations between chromosomes (tightly packed together in the sperm head, loosely dispersed in other cells) might alter the relative proportion of exchanges and restitution. CATCHESIDE (1938) provided some evidence that spatial relations among chromosome arms affect aberration yield: among aberrations detected in F_1 larvae of irradiated *D. melanogaster* males, he found a higher proportion of rearrangements involving only one chromosome arm (paracentric inversions) than would be expected from random breakage and reunion.

In a study designed to investigate the contribution of spatial relations of chromosomes to the difference in frequency of translocations recovered from sperm and oocytes, GLASS (1955a) attempted to determine whether the frequency of one type of X-ray induced intrachromosomal aberration, inversions, recovered from oocytes approaches the frequency recovered from sperm. He reported the recovery of a considerably higher frequency of inversions (detected by means of crossover suppression) from oocytes than translocations, a finding which he interpreted as support for the suggestion of PATTERSON and MULLER (1930). However, it has recently been shown that in a large proportion of translocations with distal breakpoints, the frequency of recombination is low, comparable to that of inversion heterozygotes (ROBERTS 1965). Since there had been no cytological studies of suspected inversions in GLASS's experiments, we felt that a reexamination of the relative frequency of inter- and intrachromosomal aberrations recovered from irradiated oocytes and sperm was needed.

In the present study we examined oocytes for X-ray induced crossover-suppressing aberrations, using a screening technique and radiation exposure which

had been previously used in sperm (ROBERTS 1965), and we examined salivary chromosomes to determine the nature of the recovered aberrations. Surprisingly, neither of the two crossover-suppressing aberrations recovered from oocytes was an inversion, and the frequency of intrachromosomal as well as interchromosomal rearrangements recovered from oocytes was much lower than the frequency recovered from sperm.

MATERIALS AND METHODS

A technique by which all five major chromosome arms can be simultaneously screened for crossover-suppressing aberrations employs a stock with markers spaced approximately 50 crossover units apart (ROBERTS 1965). The markers are: scute (sc, 1–0.0, scutellar bristles missing), forked (f, 1–56.7, bristles short and gnarled), aristaless (al, 2–0.0, aristae reduced), black (b, 2–48.5, body, legs, veins blackened), speck (sp, 2–107.0, black speck in wing axil), veinlet (ve, 3–0.2, longitudinal wing veins interrupted), scarlet (st, 3–44.0, eye color scarlet), and claret (ca, 2–100.7, eye color clear ruby). Since there is little recombination in the short fourth chromosome, it was not marked.

Wild-type females of an aberration-free strain, Canton-S, which had eclosed less than 6 hours previously, were irradiated with 4000r of X rays (250 kvp, 30 ma) delivered over a period of 5 to 6 minutes. The females were mass-mated to marker males, and the 1 to 4 day brood fraction was used. The oocytes were therefore irradiated before the first meiotic division (i.e. irradiated in stage 7 or earlier [KING, RUBINSON and SMITH 1956]). Individual F_1 females were backcrossed to marker males. Twenty to 25 F_2 progeny were scored for crossover suppression in the five marked arms. Control crossover values had been previously established by means of a cross between unirradiated Canton-S males and marker females, and binomial confidence limits (95% level) (MAINLAND, HERRERA and SUTCLIFFE 1956) had been established for each chromosome arm. Recombination in each chromosome arm of an irradiated sperm recovered over the marker chromosome in an F_1 female was compared with the control value, and in order to provide an objective criterion of crossover reduction, we used 95% level confidence limits to determine significant crossover reduction. Although this is a screening procedure for crossover-suppressing aberrations, marker assortment can indicate when a crossover suppressor is a translocation.

Stocks were made of all chromosomes which had statistically significant crossover reduction, and the salivary chromosomes were examined in order to determine the type of rearrangement and the location of breakpoints.

RESULTS

Two hundred irradiated genomes (numbered 500 to 700), or a total of 1,000 chromosome arms, were scored genetically for crossover suppression. Of the three cases in which crossover suppression was significant, one (No. 687), in which the crossover reduction was of borderline significance, proved not to be an aberration upon cytological examination. Descriptions of the two recovered aberrations are found in Table 1. The percentages of intra- and interchromosomal rearrangements recovered from oocytes in the present experiment were the same—less than 1% of total genomes studied. The experiment (ROBERTS 1965) using the same stocks, screening techniques, and X-ray exposure provides, for comparison, the crossover-suppressing aberrations recovered from sperm. In the two sets of data (Table 2), the difference between the frequency of crossover-suppressing aberrations recovered from sperm and the frequency recovered from oocytes is evident.

TABLE 1

X-ray-induced aberrations recovered from oocytes

Aberration Number	Recombination			Genetic data	Cytological data
	Arm involved	Number of recombinants	Percent recombinant		
591	2L	0/100	0	T(2;3)	T(2;3)28D;69D
619	2L	11/188	5.9	no T	Dp(2;2)26A-28E (tandem duplication)

TABLE 2

Aberrations induced by 4000r X rays in spermatozoa and oocytes

	Number of gametes tested	Number of chromosome arms tested	Intrachromosomal* rearrangements recovered		Interchromosomal† rearrangements recovered		Total rearrangements recovered	
			Number	Percent	Number	Percent	Number	Percent
Oocytes	200	1000	1	0.5	1	0.5	2	1.0
Spermatozoa	360	1800	32	8.9	70	19.5	102	28.4

* Inversions, transpositions, duplications.
† Translocations.

DISCUSSION

As stated in the introduction, KANELLIS and RADU (1943) recovered T(2;3)'s with much lower frequency from oocytes (0.1%) than from spermatozoa (11.6%), although both types of germ cells received the same radiation exposure (4500r of X rays). However, GLASS (1955a) reported that the frequency of recovery of inversions from oocytes (0.6%) was much closer to the frequency of recovery of inversions from sperm (2.2%) than the frequency of recovery of translocations from the two germ cells. He interpreted this finding as evidence that lack of proximity of breaks in nonhomologous chromosomes was responsible for the low frequency of translocations recovered from oocytes.

In the experiments of GLASS, sperm and oocytes were irradiated simultaneously with different X-ray exposures (600 to 2000r) for each series. The genomes of each series were marked for detection of inversions involving either the X, 2, or 3; in most series only part of an arm was marked. In contrast, the markers used in the present study were placed so that the entire length of each of the five major chromosome arms could be simultaneously scored for each irradiated genome examined, and a higher X-ray dose (4000r) was used. One thousand chromosome arms were screened in the present experiment, compared to 978 screened by GLASS. A higher frequency of crossover-suppressing aberrations should have been recovered from oocytes tested in the present experiment than from the experiment of GLASS, in which a comparable marker arrangement on chromosome 2 and 2000r of X rays was used (1.8% "inversions" recovered). However, the opposite was true (Tables 1 and 2).

The explanation for these differences in experimental results probably lies in the following differences in the design of the present experiment and those of GLASS: (1) The criteria used for determining the presence of inversions in GLASS's experiments were presumably tests for crossover suppression. Data obtained in the present experiment indicate that without rigid and objective criteria for determining when the amount of crossover suppression in a given chromosome arm is significant, many false positives are obtained. Sample size, for example, is important, false positives appearing relatively frequently with samples of 15 flies or less. Use of statistical tables of binomial confidence limits (95% level) in our experiments reduced the number of false positives to a minimum, so that only an occasional arm with crossover suppression of borderline significance had no detectable aberration present on cytological examination (No. 687 in the present series). (2) Another possible explanation of the different results obtained in the two experiments is the possibility of undetected aberrations in the marker or wild-type stocks. The Canton-S strain used in the present experiments was reputedly aberration-free; control studies and the failure to recover the same aberration twice indicate that the strain was, in fact, aberration-free. (3) Finally, but most important, cytological study of suspected aberrations is essential in order to insure that any aberrations that might be present in the wild-type or marker stocks are not recovered repeatedly, to confirm or eliminate cases of borderline significance, and to establish the nature of recovered aberrations; no cytological studies were reported in GLASS's paper.

In the present experiments, two crossover-suppressing rearrangements were recovered, neither an inversion (Table 1). The low frequency (less than 1%) of both intra- and interchromosomal rearrangements recovered from oocytes contrasts sharply with that obtained from sperm: 8.9% intrachromosomal rearrangements (mostly inversions) and 19.5% interchromosomal (translocations). It should be pointed out that the crossover-suppressing translocations recovered may represent a fraction of the total yield of translocations, because some translocations with proximal breaks do not suppress crossing over (BROWN 1940; ROBERTS 1965). Possible explanation for the present results will now be considered.

Aberrations induced in oocytes must pass through meiosis, whereas those induced in sperm can be passed to the zygote directly. Therefore, aneuploid segregation must be considered as a possible explanation of differential recovery of newly formed aberrations from sperm and oocytes. If there were 100% directed segregation of the elements of a newly formed translocation, all newly arisen translocations would be lost. For example, if translocation occurred only when nonhomologously paired chromosomes were broken or if translocation were to always ensure segregation of the involved elements, no translocations should be recovered from oocytes; after disjunction, each functional egg nucleus receiving one element of the translocation would be duplicated or deficient. However, translocations are recovered from oocytes, although with a very low frequency, thereby ruling out nonhomologous pairing with subsequent disjunction of the elements as the only configuration in which translocations can be produced, and also ruling

out 100% directed segregation of the elements of a newly formed translocation. Since chromosomes at the time of irradiation are in the four-strand stage, with independent assortment of the elements of a translocation, one fourth of the nuclei formed from an oocyte containing a translocation between two chromatids should contain both elements. Only one of the four egg nuclei formed is functional, but since there are four times as many chromatids present in oocytes as in sperm, with all other factors being equal, one would expect to recover one fourth as many translocations from oocytes as from sperm, owing to aneuploid segregation.

When asymmetrical dyads are present at the second meiotic division of Drosophila females, the shorter element is preferentially recovered (NOVITSKI 1951). Meiotic drive, therefore, might make the recovery of a translocation with a distal break in one chromatid and a proximal break in another highly improbable, although there should be little effect on the recovery of exchanges of equal lengths of arms (if both breaks are heterochromatic, for example). Aberration 591, the translocation recovered from irradiated oocytes, does not have grossly unequal arms.

Four kinds of intrachromosomal aberration may be recovered—inversions, transpositions, duplications and deficiencies. The presence of four times as many chromatids in oocytes as in sperm provides opportunity for the induction of duplications in oocytes. (Duplications are not expected to be induced in sperm and none were recovered in the present experiment, but BAUER (1939) has reported the recovery of duplications from X-irradiated sperm.) The only intrachromosomal aberration recovered from oocytes was a tandem duplication (No. 619, Table 1), which can be considered an asymmetrical crossover resulting from breaks in two chromatids. The reciprocal product, a large deficiency, would probably not be recovered owing to lethality. The induction of duplications and deficiencies should be more frequent than inversion in oocytes if breaks in different chromatids of a tetrad are as likely to recombine as breaks in the same chromatid. In that case, the eucentric aberrations that result from random rejoining of two breaks should be duplications and deficiencies the three fourths of the time that two chromatids are involved. Prior to the recovery of No. 619, it was not anticipated that a tandem duplication could be detected as a crossover suppressor. A detailed account of the behavior of this rearrangement will be reported in a future communication.

Recombination in oocytes might lead to loss of inversions if an inverted segment were to include a chiasma within its limits. The contribution of such a mechanism to the differential yield of inversions from the two types of irradiated germ cell cannot be estimated owing to uncertainty as to the significance of chiasmata as indicators of chromosome arms which have exchanged (COOPER 1949) and the extent of terminalization of chiasmata if they do in fact form in early oocytes, when recombination is presumed to occur (PLOUGH 1917), and persist into the irradiated oocyte stages. If such a mechanism were operating in oocytes, loss should be greater for long inversions and slight to negligible for short inversions. There should be no segregation loss of inversions from females, since any of the

four chromatids which might be rearranged could contribute to a viable egg nucleus, but no inversions were recovered from irradiated oocytes in the present experiment.

Although, in contrast with sperm, only one fourth of the translocations induced in oocytes should be recovered if the elements segregate independently, the actual fraction of translocations recovered from irradiated oocytes is usually considerably less than one fourth that recovered from sperm. In the experiment of KANELLIS and RADU, for example, only one hundredth as many translocations were recovered from oocytes as from sperm. It appears that even in the case of newly induced translocations, where aneuploid segregation can be an important factor in reducing the yield from meiotic stages (HERSKOWITZ and SCHALET 1957), aneuploid segregation is inadequate to account fully for the difference in translocation yield between premeiotic (gonial), meiotic (oocyte), and postmeiotic (sperm) stages unless one postulates an *ad hoc* hypothesis of segregation of translocation elements as discussed above. The low frequency of intrachromosomal rearrangements recovered from oocytes (0.5%) as compared with sperm (8.9%) is further evidence that aneuploid segregation is not responsible for the much lower yield of X-ray induced aberrations recovered from oocytes than from sperm, and that a given radiation dose induces fewer gross aberrations in oocytes than in sperm.

Spatial relations of chromosomes may contribute to differential aberration yields in that the tightly packed chromosomes observed in sperm heads (WOLF 1939) may become involved in rearrangements more readily than chromosomes in oocytes, owing to the closer proximity of breakpoints in sperm. (The volume of the male pronucleus, where rejoining apparently occurs [MULLER 1940; OSTER 1955], is unknown, so the chromosomes may not be so tightly packed at time of rejoining as in sperm). If tight packing is, in fact, responsible for the higher aberration yield from sperm than oocytes, the present data indicate that it must increase the probability of intrachromosomal rearrangements as well as interchromosomal rearrangements.

The low frequency of gross chromosomal aberrations recovered from oocytes might conceivably result from the induction of fewer breaks in the chromosomes of oocytes than in sperm given the same radiation exposure. However, GLASS (1955b) obtained identical dose curves for small X-ray induced deficiencies with sperm and oocytes, suggesting that chromosome breakage in both germ cells is equal. The low frequency of inversions as well as translocations recovered from oocytes suggests that the high frequency of dominant lethals obtained from 1 to 4 days oocyte brood fractions (PARKER 1959, BATEMAN and CHANDLEY 1963) may not be due to gross chromosomal rearrangements. The low frequency of inversions induced in oocytes suggests that the contribution of large deficiencies (which, although not recoverable owing to lethality, might be expected to originate from comparably placed breaks) to the class of dominant lethal oocytes is equally low. A similar argument can also be made against another suggested source of dominant lethals (LEA 1946), aneucentric rearrangements between nonhomologous chromosomes, since the translocation data considered in the light of the predicted

loss from aneuploid segregation and the low inversion yield suggest that the corresponding eucentric rearrangements, translocations, are produced infrequently in oocytes compared with sperm. PARKER (1959), from similar considerations, has suggested that anaphase II bridges resulting from sister-strand union in stage 7 oocytes may be an important source of dominant lethals, and HERSKOWITZ and SCHALET (1957) have estimated that chromosomal nondisjunction and aneuploidy resulting from recovery of "half-translocations" in egg nuclei can account for approximately one ninth of the dominant lethality induced by irradiating oocytes. The heterogeneous nature of the class of dominant lethals is also suggested by differences in the time of expression of dominant lethality in embryos of insects (VON BORSTEL and REKEMEYER 1959), but the event or events responsible for the majority of dominant lethals are, as yet, unknown.

Pairing of homologous chromosomes in oocytes may be an important factor in reducing the aberration yield from these germ cells. Detachment of attached X chromosomes, the most frequently recovered gross chromosomal rearrangement from irradiated oocytes, appears to involve mostly the partially homologous Y and fourth chromosomes in stage 7 oocytes, and PARKER (1963) has suggested that detachments in stage 7 oocytes depend primarily on homologous pairing relations, whereas those in stage 14 oocytes are more likely to be dependent on chance associations. (The observation that regions of the Y chromosome that are homologous to the X are preferentially involved in detachments in stage 7 oocytes (BROSSEAU 1964) agrees with this interpretation.) The stage 14 oocyte, like the sperm, has no chromosome rejoining until the time of fertilization (PARKER 1963). Irradiated stage 14 oocytes, by analogy, might yield more inversions, as well as translocations than earlier oocyte stages since both types of rearrangement appear to be dependent, to a considerable extent, on random associations of chromosomes in sperm. Pairing by homology may cut the inversion yield from early oocytes by minimizing looping and folding within chromosome arms, as it might reduce the translocation yield from early oocytes by minimizing associations between arms of nonhomologous chromosomes.

SUMMARY

One thousand chromosome arms from irradiated oocytes (stage 7 and earlier) were screened for crossover-suppressing aberrations; two such aberrations were recovered, but neither was an inversion. The yield of intrachromosomal rearrangements recovered from oocytes was an order of magnitude below that recovered from sperm given the same radiation exposure. Possible explanations for the differences in aberration yield from the two germ-cell stages and their relation to the problem of dominant lethality in oocytes are considered.

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