THE NATURE OF INDUCED EXCHANGES BETWEEN THE ATTACHED-X AND Y CHROMOSOMES IN *DROSOPHILA MELANOGASTER* FEMALES¹

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X-RAY induced chromosomal exchanges are generally thought to consist of the following three types: (1) "half-translocations", so-called because only two of the four pieces obtained by breaking two nonhomologous chromosomes are recovered in a fertilized egg (MULLER and HERSKOWITZ 1954; ABRAHAMSON, HERSKOWITZ and MULLER 1954); (2) "pseudocrossovers" representing exchanges occurring between nearby, but not identical, loci of two homologous chromosomes; and (3) "true crossovers" representing exchanges giving rise to chromatids equivalent to those produced by spontaneous crossing over; however, no implication of absolute identity between the molecular mechanisms involved in the induced and spontaneous processes is intended by the use of this terminology. All three types of exchanges are presumed to occur in euchromatin as well as in heterochromatin (HERSKOWITZ and ABRAHAMSON 1957).

When attached-X females not carrying a Y chromosome are irradiated, a number of viable half-translocations are produced in the form of detached X chromosomes which most frequently involve interchanges with chromosome 4 (PARKER 1954; ABRAHAMSON, HERSKOWITZ and MULLER 1956). When a Y chromosome is present (in conjunction with the attached-X at the time of irradiation) the majority of detachments involve interchanges with the Y (PARKER 1954) and are regarded as half-translocations (cf. HERSKOWITZ and ABRAHAMSON 1956) or simply as translocations (PARKER and HAMMOND 1958). In view of the probable fact that most attached-X chromosomes in existence contain a centromere derived from the Y chromosome as well as some unspecified amount of centric Y-heterochromatin (NOVITSKI 1954), and in consideration of the known homologies shared by X and Y heterochromatin (see BROSSAU 1960 for review) the hypothesis suggests itself that a substantial proportion of exchanges between the Y and attached-X chromosomes may consist of crossovers and pseudocrossovers rather than translocations.

The paucity of conventional genetic material (more specifically, of loci which by mutation or loss would give rise to lethals) in the regions under consideration precludes any attempt to demonstrate the occurrence of induced crossovers *vs.* pseudocrossovers by determining the frequency with which lethals are associated

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with the points of exchange (PATTERSON and SUCHE 1934; FRIESEN 1937; HERSKOWITZ and ABRAHAMSON 1957). In a study of nonrandom recovery of detachments, involving a Y chromosome marked distally on both arms with small X-chromosome duplications, BROSSÉAU (1964) observed that the distribution of exchanges along the length of the Y was not random; the breaks were preferentially located in those regions which are homologous to the proximal portion of the X chromosome. On the basis of this evidence, BROSSÉAU favored the hypothesis that 70 percent of the detachments analyzed were induced crossovers between homologous regions. The linear relationship of the fertility factors and of various translocated markers is known for the Y chromosome, but nothing is known of the physical distances separating these. BROSSÉAU's conclusions may, therefore, be open to the criticism that random breaks and reunions are responsible for the distribution obtained, provided that one assumes a longer distance (or simply a higher susceptibility to X rays) for those regions of greater recovered exchange. To invalidate this objection, an independent test of the distribution of breaks along the Y is necessary. This is furnished by an analysis of true translocations and gross deletions. The former represent exchanges between the Y chromosome and an autosome or the euchromatic portion of the X; the latter are losses of an interstitial segment, large enough to require two breaks. A comparison of the distribution of breaks along the Y chromosome when it is involved in detachments and when it is involved in true translocations and gross deletions would reveal the role played by homology in exchanges between the attached-X and Y chromosomes.

Experimental evidence will be presented suggesting that the relative frequency of breaks along the long arm of the Y chromosome is influenced by pairing with the heterochromatic portion of the attached-X. Further evidence will be presented showing that Y^s is involved in a significantly higher proportion of detachments than of translocations. It is proposed that this evidence argues against the belief that all heterochromatic exchanges between these two chromosomes are translocations but rather favors the view that they represent exchanges between homologous chromosomes, of a crossover-like nature.

MATERIALS AND METHODS

The Y chromosome used was the $B^sY\gamma^+$ of BROSSÉAU (1959). This Y has attached to its long arm an X chromosome segment bearing the dominant mutant B^s (Bar eyes of Stone) and to its short arm a similar segment bearing γ^+ (wild-type allele of the mutant yellow); both duplications are distal to the fertility factors. The ordinary attached-X chromosome (reversed metacentric) used was homozygous for the marker γ . Virgin females of the constitution $\gamma/B^sY\gamma^+$ were collected for a period of three days and, on the fourth day, were exposed for six minutes to a dose of about 3000r of X-irradiation. They were then mated to γ/Y or XY , $\gamma^2 su-w^a w^a Y^s.Y^L/Y$ males (w^a = apricot eyes; su = suppressor), allowed to lay eggs for six days and were finally discarded. Exceptional offspring consisted of males and females exhibiting one but not the other of the Y markers (B^s or γ^+), and nondisjunctional types. These were individually tested and classified as bearing (1) a *detachment*: if the Y marker appeared X-linked, or (2) a *Y fragment*: if the marker segregated from the X chromosome. These fragments are of three kinds: (a) Y chromosomes which exchanged some distal portion of their short or long arms for an X or autosome telomere and are true translocations, (b) gross deletions with one break proximal and the

other distal to one of the markers, and (c) single hit deletions of the markers; these are small induced point mutations or deficiencies encompassing, at most, three adjacent genes, and which are found to occur with a frequency linearly proportional to the dose (FRYE 1957, 1959). Cases of autosomal linkage of the Y-chromosome markers and cases of nondisjunction were not further analyzed; stocks were established for all detachments and fragments. In order to determine the amount of Y material carried by the detachment, i.e., in order to localize the region of the break point along the Y chromosome, detachment males marked with γ^+ were crossed to attached-X females of the constitution $\gamma \nu f/FR2$ (FR2 is a Y fragment carrying the fertility factors of Y^L and γ^+ as a small duplication (NOVITSKI 1952); ν = vermilion eyes; f = forked bristles). If the F_1 males were fertile the detachment was tested with BROUSSEAU's Y^L fertility tester stocks; and if the F_1 males were sterile, the detachment was tested with the Y^S fertility tester stocks (BROSSEAU 1960). This was accomplished by replacing the fertile Y carried by stock detachment males with the various sterile Y's. The fertility of such males would indicate the presence, on the detachment, of those fertility factors which are absent on the tester Y chromosomes. Detachment males marked with B^S were crossed to attached-X γ/Y'' females (Y'' is an attached- Y^S chromosome (STERN 1929)). The remainder of the test was the reciprocal of the one for the γ^+ detachments. Fragments bearing B^S and those bearing γ^+ were tested for the presence of the Y^S and Y^L fertility factors, respectively, by means of the BROUSSEAU tester stocks.

Fertility tests were performed by mass mating 15 to 20 pairs of flies. The available tester stocks for factors *kl-3*, *kl-3,4* and *kl-4,5* are "leaky" (BROSSEAU, personal communication). Therefore, any culture was scored as sterile if it produced less than 20 offspring. This arbitrary criterion gave consistent and repeatable results.

In order to determine whether any fertility factors were present in the heterochromatin of the attached-X used, females with this chromosome and no Y were irradiated. The first 75 detachments recovered were tested for six of the seven fertility factors with negative results (there are no tester stocks available to demonstrate the presence of *kl-4* unless it is accompanied by *kl-3* or *kl-5*).

RESULTS AND DISCUSSION

A total of 361 detachments involving the Y chromosome and 83 Y fragments were analysed and are presented in Table 1.

Detachments were classified as captured or capped X chromosomes depending on whether they possess a Y or an X centromere, respectively (ABRAHAMSON, HERSKOWITZ, and MULLER 1956). Detachments involving a Y chromosome break in the centric region extending from *kl-1* to *ks-1* could not be assigned to either of the above two categories. The obvious discrepancy in the frequency of reciprocal products (83:11 for exchanges involving Y^S and 73:17 for exchanges involving Y^L) can readily be ascribed to nonrandom disjunction (NOVITSKI 1951; PARKER and McCURNE 1958; BROUSSEAU 1964).

Fragments include, as previously mentioned, true translocations, gross deletions and single-hit deletions. Gross deletions differ from translocations in that the broken Y-chromosome arm is capped by its own telomere rather than an X or autosomal telomere. These two types of fragments are, therefore, quite analogous regarding their contribution to the distribution of breaks along the Y. Single-hit deletions of the Y markers would be exclusively included among the recovered fragments which appeared to have retained all of the fertility factors. An estimate of the relative frequency of translocations and deletions among the fragments can be obtained from those exceptional females which exhibited autosomal linkage of the Y markers; and those in which the marker appeared linked

TABLE 1

Distribution of breaks along the Y chromosome recovered as detachments or fragments

Type of detachment	Regions on the Y							<i>ks-1</i>	<i>ks-2</i>	γ^+	Break in:
	<i>B</i> ^s	<i>kl-5</i>	<i>kl-4</i>	<i>kl-3</i>	<i>kl-2</i>	<i>kl-1</i>	<i>c</i>				
Capped-X(<i>B</i> ^s)	52	0	12	0	9		76				Y ^L
Captured-X(<i>B</i> ^s)								3	8		Y ^s
Captured-X(γ^+)	4	3	0	0	10						Y ^L
							101				
Capped-X(γ^+)								2	81		Y ^s
Fragments											
(γ^+)	19	0	0	2	37	13					Y ^L
(<i>B</i> ^s)								1	1	10	Y ^s

to the attached-X. The complementary class would be recovered as fragment-bearing males, presumably representing true translocations. There were nine females of the first type recovered (none of the second type) and 57 fragment males (additional females and males which arose from multiple events, such as simultaneous detachment of the attached-X with an autosome, fragmentation of the Y and nondisjunction of the sex chromosomes are not considered here). Since one would expect to observe three fragment males for every two translocation females, the corrected data are 14/71, i.e., a frequency of 20 percent true translocations among recovered fragments. This estimate is of the same order of magnitude as the one derived in the case of irradiated males by ZIMMERING and WU (1964).

In order to compare the distributions of breaks along the Y when it is involved in detachments and when it is involved in translocations or gross deletions, one can only consider captured X chromosomes. The reason for this limitation is that broken Y chromatids, capped by an X or autosomal telomere, are recovered from an unequal dyad where the sister-strand is a normal Y chromatid. This is true of captured X chromatids but is not true in the case of capped X's which are recovered from dyads where the nonexchange strand is an attached-X. There appears to be no obvious justification for assuming that the coefficient of non-random disjunction (*c*) may be the same for both types of dyads. The data involved in the comparison in question have been extracted from Table 1 and are presented in Figure 1 accompanied by formal diagrams of the dyads which yielded them. Mention must be made here that the three captured X's with a Y break between *kl-4* and *kl-5* probably represent exchange points between *kl-3* and *kl-4*. This observation does not alter the validity of the argument which follows and will be further discussed below.

There appears to be no difference in the relative distribution of breaks within Y^s but the data are so few that they exclude any meaningful analysis. In the case of Y^L, there is a definite suggestion that the distribution of exchanges recovered as detachments differs from the distribution generated by breaks giving rise to fragments. Considering the following three regions: (I) from *kl-1* to *kl-2*, (II)

FIGURE 1.—Diagrammatic representation of the dyads from which captured X chromosomes and Y fragments were recovered. Heavy lines represent heterochromatin, thin lines euchromatin.

from *kl-2* to *kl-5*, and (III) from *kl-5* to *B^s*, since the ratio of detachments to fragments in regions I and III are similar, one would expect an equivalent and intermediate ratio in region II, irrespective of whether *c* differs for the fragment and detachment dyads or whether it is inversely related to length. The data for regions I and III were pooled (tested separately these two sets of data do not differ significantly; $P = .7$) and compared to the data from region II, by means of FISHER's exact method for treatment of 2×2 tables. The analysis indicates the difference to be barely significant at the 10 percent level of confidence ($P = .098$). An argument which may add weight to such a low fiducial level is that if *c* does vary inversely with length, the data in region II are biased against the hypothesis that they may be different: one might expect a higher proportion of fragments with a break between *kl-2* and *kl-3* among fragments than of detachments with an exchange between *kl-4* and *kl-5* (or *kl-3* and *kl-4* as discussed below) among detachments. The contribution of single-hit deletions to the number of recovered fragments in region III cannot be estimated with any degree of accuracy. Emphasis must be placed on the fact that, were this contribution substantial, the difference in relative frequency of fragments and detachments in regions II and III would be minimized. Pooling the data for these two regions and comparing it to the data for region I would, in this case, lead to the same type of results as were previously obtained.

Turning now to an inter-arm comparison, breaks along *Y^s* constitute 16 percent of the fragments and 40 percent of the detachments under consideration (Figure

TABLE 2

Distribution of breaks along the Y chromosome recovered as detachments (grouped data)

	<i>B</i> ^s to <i>kl</i> -5	<i>kl</i> -5 to <i>kl</i> -1	<i>kl</i> -1 to <i>ks</i> -1*	<i>ks</i> -1 to <i>ks</i> -2	<i>ks</i> -2 to <i>y</i> ⁺
BROSSEAU (1964)	56	34	177	5	89
	29	17	27	7	35

* This region includes the centromere and the locus of *bb*⁺.

1). This difference is significant at the 2 percent level of confidence and can be interpreted as showing that homology between the attached-X and Y chromosomes modifies the relative frequency with which *Y*^s and *Y*^L undergo exchanges with the attached-X to a degree which is significantly different from random breakage of the Y.

A comparison of the distributions of detachments obtained by BROSSEAU (1964) with that illustrated in Table 1 lends further support to the thesis that homology governs the type of exchange which attached-X and Y chromosomes will undergo. BROSSEAU used the same *B*^s*Y**γ*⁺ chromosome, but a different reversed metacentric: *γ*² *su-u*^a *w*^a *bb* (*bb* = bobbed bristles). The difference in radiation dose administered in the two experiments should not interfere with the comparison since all types of exchanges appear to follow two-hit kinetics (HERSKOWITZ and MULLER 1953; PARKER 1953). Table 2 summarizes the results of BROSSEAU's and of the present experiments. For purposes of analysis, the Y chromosome was subdivided into five regions and the data were pooled to fit this scale. In both experiments there is a high frequency of breaks in the centric region and in the regions distal to *KS* and *KL*. Yet the two distributions are significantly deviant from one another at the 1 percent level of confidence, suggesting qualitative differences in the heterochromatin of the two attached-X's. This may, in turn, be translated into different pairing affinities of these two chromosomes for the *B*^s*Y**γ*⁺, yielding different distributions of exchanges.

Finally, a comment is in order regarding *kl*-4. In Table 1 it can be seen that 12 capped X's were recovered from exchanges occurring between *kl*-3 and *kl*-4: no reciprocal products were found. Exchanges between *kl*-4 and *kl*-5 yielded three captured X's and here, again, no reciprocal products were recovered. The same phenomenon is evident in BROSSEAU's data where six capped X's of the first type and three captured X's of the second type mentioned above are listed. This gives a total of 18 exchanges between *kl*-3 and *kl*-4 and six exchanges between *kl*-4 and *kl*-5 without reciprocal products. This discrepancy could be readily explained by making the uncomfortable assumption that both attached-X chromosomes bear *kl*-4. As mentioned in the previous section, it is not possible, to date, to test for the presence of this fertility factor alone. This fact added to the foregoing remarks casts an aura of mystery around *kl*-4, the existence of which cannot be doubted on the basis of complementation tests (BROSSEAU 1960).

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SUMMARY

Evidence has been presented that X-ray induced exchanges between an attached-X and marked Y chromosomes are not translocations but are influenced by the homologies existing between these two chromosomes and are probably the result of crossing over. This evidence was based on a comparison of the distribution of breaks on Y^L when this arm is involved in an exchange with the attached-X and when it is involved in translocations and gross deletions. More critical evidence was obtained by comparing the relative proportion of exchanges involving Y^S (40 percent of all detachments) with the relative proportion of translocations and gross deletions involving the same arm (16 percent of all Y fragments).

LITERATURE CITED

- ABRAHAMSON, S., I. H. HERSKOWITZ, and H. J. MULLER, 1954 Genetic proof for half-translocations derived from irradiated oocytes of *Drosophila melanogaster*. (Abstr.) *Genetics* **39**: 955. — 1956 Identification of half-translocations produced by X-rays in detaching attached-X chromosomes of *Drosophila melanogaster*. *Genetics* **41**: 410-419.
- BROSSEAU, G. E., JR., 1958 Crossing over between Y chromosomes in male *Drosophila*. *Drosophila Inform. Serv.* **32**: 115. — 1960 Genetic analysis of the male fertility factors on the Y chromosome of *Drosophila melanogaster*. *Genetics* **45**: 257-274. — 1964 Non-randomness in the recovery of detachments from the reversed metacentric compound X chromosome in *Drosophila melanogaster*. *Can. J. Genet. Cytol.* **6**: 201-206.
- FRIESEN, H., 1937 Mechanism of crossing over in males of *Drosophila melanogaster*. *J. Genet.* **35**: 141-150.
- FRYE, S. H., 1957 Frequency of minute chromosome rearrangements in relation to X-ray dose in *Drosophila melanogaster*. (Abstr.) *Genetics* **42**: 371. — 1959 Further investigation concerning the frequency and analysis of minute rearrangements of the yellow region in relation to X-ray dose in *Drosophila melanogaster*. (Abstr.) *Genetics* **44**: 511.
- HERSKOWITZ, I. H., and S. ABRAHAMSON, 1956 Induced changes in female germ cells of *Drosophila*. I. Dependence of half-translocation frequency upon X-ray delivery rate. *Genetics* **41**: 420-428. — 1957 Induced changes in female germ cells of *Drosophila*. IV. Dependence of induced crossover-like exchanges in oocytes and oogonia upon X-ray intensity. *Genetics* **42**: 444-453.
- HERSKOWITZ, I. H., and H. J. MULLER, 1953 Evidence against the healing of X-ray breakages in chromosomes of female *Drosophila melanogaster*. (Abstr.) *Genetics* **38**: 669.
- MULLER, H. J., and I. H. HERSKOWITZ, 1954 Concerning the healing of chromosome ends produced by breakage in *Drosophila melanogaster*. *Am. Naturalist* **88**: 177-208.
- NOVITSKI, E., 1951 Non-random disjunction in *Drosophila*. *Genetics* **36**: 267-280. — 1952 The genetic consequences of anaphase bridge formation in *Drosophila*. *Genetics* **37**: 270-287. 1954 The compound X chromosomes in *Drosophila*. *Genetics* **39**: 127-140.
- PARKER, D. R., 1953 Observations on X-ray induced detachments of attached-X chromosomes in *Drosophila*. (Abstr.) *J. Tenn. Acad. Sci.* **28**: 185. — 1954 Radiation-induced exchanges in *Drosophila* females. *Proc. Nat. Acad. Sci. U.S.* **40**: 795-800.
- PARKER, D. R., and A. E. HAMMOND, 1958 The production of translocations in *Drosophila* oocytes. *Genetics* **43**: 92-100.

- PARKER, D. R., and J. McCRONE, 1958 A genetic analysis of some rearrangements induced in oocytes of *Drosophila*. *Genetics* **43**: 172-186.
- PATTERSON, J. T., and M. L. SUCHE, 1934 Crossing over induced by X-rays in *Drosophila* males. *Genetics* **19**: 223-236.
- STERN, C., 1929 Untersuchungen über Aberrationen des Y-Chromosoms von *Drosophila melanogaster*. *Z. Ind. Abst. Vererb.* **51**: 253-353.
- ZIMMERING, S., and C. K. WU, 1964 Meiotic X-Y exchange and nondisjunction induced by irradiation in the *Drosophila* male. *Genetics* **50**: 633-638.