X-Y NONDISJUNCTION AND EXCHANGE INDUCED BY X RAYS IN PRIMARY SPERMATOCYTES OF THE ADULT DROSOPHILA¹

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SÄVHAGEN (1961) and STRANGIO (1961, 1962) have reported that X-irradiation of primary spermatocytes of adult *Drosophila melanogaster* results in a higher frequency of X-Y nondisjunction than X-Y exchange. Quite the reverse has been found by ZIMMERING and WU (1963) following irradiation of primary spermatocytes of the immature testis of *Drosophila*. In view of this discrepancy, the question of the relative frequency of these events in spermatocytes of the adult *Drosophila* testis has been retested. The results from this experiment indicate that X-Y exchange exceeds that of X-Y nondisjunction in agreement with the previous report of ZIMMERING and WU but not with those of SÄVHAGEN and STRANGIO. An account of this experiment, and attempts to reconcile the discrepancy, are presented below.

MATERIALS AND METHODS

P₁ males and females of the same composition employed previously by ZIMMERING and WU were used in the present experiment. The X chromosome of the male was marked with the mutant γ (yellow body) and v (vermilion eyes), and the Y chromosome, symbolized $B^S Y \gamma^+$ (BROSSEAU, NICOLETTI, GRELL, and LINDSLEY 1961) carries the mutant B^S (Bar of Stone, an extreme allele of Bar) at the tip of Y^L , and the γ^+ marker at the tip of Y^S . These males were irradiated with 1200r of X rays 12 to 24 hours after eclosion and individually mated daily with three females of the composition $\gamma w f$ (w , white eyes; f , forked bristles). Unirradiated males, 12 to 24 hours old and mated in the same way, served as controls. The experiment was carried out at a temperature of approximately 25°C.

Regular offspring from these crosses are phenotypically γ females ($\gamma v/\gamma w f$) and $w f B^S$ males ($\gamma w f/B^S Y \gamma^+$). Following an exchange between X and Y^L , probably facilitated by the presence of X-derived heterochromatin situated proximal to the B^S marker (ZIMMERING 1959, 1962), an X chromosome which carries the γ^+ marker and a centric fragment bearing the B^S marker are generated. The former product is recovered as a phenotypically + female ($\gamma v \cdot \gamma^+/\gamma w f$). The latter is recovered as a phenotypically $\gamma w f B^S$ male ($\gamma w f/FR-B^S$), although such males may also arise (1) from the deletion of the γ^+ marker and (2) following certain exchanges, at a four-strand stage, between the arms of the Y chromosome when these arms are oriented with respect to each other as are X chromosomes in the ordinary attached-X (reverse metacentric) female. An exchange between X and Y^S proximal to the γ^+ marker yields an X chromosome which carries the B^S marker and a centric fragment bearing the γ^+ marker. The former product is recovered as a phenotypically γB^S female ($\gamma v \cdot B^S/\gamma w f$). The latter is recovered as a phenotypically $w f$ ($\gamma w f/FR-\gamma^+$) male, although such males may also arise (1) from the deletion of the B^S marker

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and (2) as the reciprocal product from the kinds of exchanges between Y arms mentioned above. From the recovery of both X and Y chromosomes following nondisjunction, phenotypically B^S females ($\gamma v/\gamma w f/B^S Y \gamma^+$) are obtained. The reciprocal product is recovered as a phenotypically $\gamma w f$ male ($\gamma w f/0$). Such males may also arise (1) from loss of X or Y and (2) following certain exchanges between the arms of the Y chromosome when they are disposed in a manner similar to that of X chromosomes in the tandem metacentric female, resulting in the loss of both markers. As indicated previously by ZIMMERING and WU (1963) pairing between Y arms may be appreciably facilitated as a result of the presence of X-derived heterochromatin associated with and proximal to both the B^S and γ^+ markers. It is clear, therefore, that while it is possible to distinguish unambiguous cases of X-Y nondisjunction and exchange by simple examination of phenotypes of exceptional female offspring, each class of exceptional males may be of mixed origin, that is, may represent more than one kind of event in the treated male. Briefly, then, the classes of female progeny are: (1) regular—yellow; (2) recovery of X and Y following nondisjunction—Bar; (3) exchange between X and Y^L —wild type; and (4) exchange between X and Y^S —yellow Bar.

RESULTS AND DISCUSSION

Since the first indications of an increase above control values of nondisjunctional and exchange females were observed in Brood 7 (in agreement with the reports of SÄVHAGEN and of STRANGIO) only the data beginning with those from Brood 7 through Brood 10 are included in Table 1. From those broods, then, which contained the largest relative proportion of primary spermatocytes at the time of irradiation, that is, Broods 7 and 8, it may be seen that of all female offspring, three cases of induced nondisjunction (0.18 percent) as compared with ten cases of exchange (0.60 percent) were observed in Brood 7, and no cases of nondisjunction (0.00 percent) and nine cases of exchange (1.33 percent) were found in Brood 8. Taken in conjunction with the previous findings of ZIMMERING and WU (1963) the evidence suggests that X-irradiation of primary spermatocytes

TABLE 1

Progeny from Broods 7 to 10 from matings of a single 12 to 24 hour old $\gamma v/B^S Y \gamma^+$ irradiated with 1200r (I) or not irradiated (C) with three $\gamma w f$ females each day for a period of ten days

Brood		Females				Males			
		Regular γ	Nondisjunction B^S	Exchange +	Exchange γB	Regular $w f B^S$	Loss of $B^S w f$	Loss of $\gamma^+ \gamma w f B^S$	Loss of X, Y, or γ and $B^S \gamma w f$
7	(C)	5432	0(0.00)	0(0.00)	0(0.00)	4223	1(0.02)	1(0.02)	1(0.02)
	(I)	1636	3(0.18)	4(0.24)	6(0.36)	1325	20(1.41)	28(1.97)	49(3.45)
8	(C)	3149	1(0.03)	0(0.00)	0(0.00)	2560	0(0.00)	1(0.04)	2(0.08)
	(I)	668	0(0.00)	5(0.74)	4(0.59)	523	12(2.12)	11(1.94)	20(3.53)
9	(C)	3837	0(0.00)	0(0.00)	0(0.00)	3158	1(0.03)	0(0.00)	0(0.00)
	(I)	1850	1(0.05)	0(0.00)	1(0.05)	1430	13(0.89)	10(0.68)	11(1.37)
10	(C)	3301	0(0.00)	0(0.00)	0(0.00)	2624	0(0.00)	1(0.04)	1(0.04)
	(I)	6315	1(0.02)	1(0.02)	0(0.00)	4880	20(0.41)	21(0.43)	16(0.32)

Frequencies of exceptional females are based on female offspring only, and those of exceptional males on male offspring only. These frequencies are shown in italics. See text for further details.

of the immature or adult testis of *Drosophila* yields a greater frequency of X-Y exchange than X-Y nondisjunction. These results are not in agreement with those reported by SÄVHAGEN and by STRANGIO. On the other hand, the present data on the frequency of exceptional males in Broods 7 and 8, indicating a relatively high breakage frequency (or failures of restitution), are consistent with their results.

One problem that remains is that of reconciling the present data (and previous data of ZIMMERING and WU 1963) with the reports of SÄVHAGEN and of STRANGIO. Some comments on the experimental designs employed by each of these latter authors may help throw some light on this problem.

Comments on SÄVHAGEN's experimental scheme: SÄVHAGEN irradiated males of the constitution γ^{16}/γ^+Y , an X chromosome marked with γ and a Y chromosome carrying the γ^+ marker (MULLER 1948), and mated daily with $\gamma w sn$ females (*sn*, singed bristles). Phenotypically wild-type female offspring were selected for further testing since these could have been the result of X-Y nondisjunction ($\gamma^{16}/\gamma w sn/\gamma^+Y$) or X-Y exchange ($\gamma^{16} \cdot \gamma^+/\gamma w sn$). SÄVHAGEN states: "In order to distinguish between these possibilities, each wild-type female has to be tested individually by crossing them to $\gamma w sn/\gamma^+Y$ males. If the wild-type females are due to induced crossing over, there will appear two classes of non-recombinant females ($\gamma w sn$ and wild-type) while there will be four nonrecombinant classes in the offspring from nondisjunctional females ($\gamma w sn$, wild type, γ , and $w sn$).” It may be seen, however, that a test of this kind may not be adequate to distinguish between these two possibilities for the following simple reason. If the genotype of an exchange female is indicated as

then crossing over between *sn* and the centromere would result in a "shift" of the γ^+ marker from the γ^{16} to the $\gamma w sn$ chromosome. This would have the effect of creating two additional "noncrossover" female classes (that is, noncrossovers for the γ -*sn* region) which would appear γ ($\gamma^{16}/\gamma w sn$) and $w sn$ ($\gamma w sn \cdot \gamma^+/\gamma w sn$). Since the frequency of crossing over between *sn* (locus: 21.0, BRIDGES and BREHME 1944) and the centromere would run about 40 percent, these additional classes would be expected with some appreciable frequency. Consequently, four "noncrossover" classes would be expected from the wild-type F_1 female whether it originated from nondisjunction or exchange in the treated male. (Most certainly, if the female progeny from the tested wild-type female were simply scanned to determine if two or four "noncrossover" classes appeared, this kind of genetic test would be totally inadequate). The view of the present authors is that SÄVHAGEN's genetic test to distinguish between cases of nondisjunction and exchange may not have been adequate to permit a decision concerning the relative frequencies of these two events.

Comments on STRANGIO's experimental scheme: STRANGIO irradiated males

carrying an unmarked X and the $B^sY\gamma^+$ and mated with $\gamma apr ec$ (yellow body, apricot eyes, echinus (rough) eyes; STRANGIO 1961) or with $sc^{s1} In-S apr$ females (a multiply inverted X bearing the mutant apricot; STRANGIO 1962). Phenotypically B^s females were selected for further testing to determine if the B^s marker had been shifted to the unmarked X, i.e., following an exchange, or had remained associated with the Y chromosome, i.e., from the recovery of both X and Y following nondisjunction in the treated male. STRANGIO (1962) reported that "exceptional Bar females (represented) primary nondisjunction of X and Y in the male parent on most occasions." Now, it may be seen that neither of STRANGIO's experiments permitted an identification of X-Y exchange which places the γ^+ marker on the X, i.e., from exchanges between X and Y^L , since the recovery of an $X^+ \cdot \gamma^+$ chromosome in F_1 female progeny yields a phenotypically wild-type female, indistinguishable from regular females. Since (1) no mention is made of the possibility of this kind of exchange and (2) to arrive at the frequency of such exchanges would have necessitated testing virtually all regular females, it seems likely that all exchanges of this type were simply unidentified as such. Since approximately half of all exchanges between X and $B^sY\gamma^+$ are of this type (Table 1, and ZIMMERING and WU 1963), then on the basis of this fact alone, STRANGIO would have found 50 percent fewer exchanges than the present authors. On the other hand, there is no doubt that a simple genetic test of the B^s females would have provided the kind of information required to distinguish between nondisjunction and exchange. This may be illustrated as follows.

From the experiment involving the cross of $X^+/B^sY\gamma^+$ males with $\gamma apr ec$ females, nondisjunctive F_1 B^s females would have the composition $+/\gamma apr ec/B^sY\gamma^+$ and F_1 B^s exchange females the composition $+ \cdot B^s/\gamma apr ec$. By simply crossing these females by their $\gamma apr ec/B^sY\gamma^+$ brothers and scoring F_1 females, then, disregarding crossovers between γ and ec (only about 5 percent), four classes of regular female progeny in equal numbers are expected from XXY (nondisjunctive) females, i.e., wild type ($+/\gamma apr ec$), Bar ($+/\gamma apr ec/B^sY\gamma^+$), yellow-apricot-echinus ($\gamma apr ec/\gamma apr ec$), and apricot-echinus-Bar ($\gamma apr ec/\gamma apr ec/B^sY\gamma^+$). Secondary nondisjunctive females, $+/\gamma apr ec/B^sY\gamma^+$ are phenotypically Bar and hence indistinguishable from the regular Bar females. From exchange females, the two classes of female progeny expected in the absence of a crossover between ec and the centromere are yellow-apricot-echinus ($\gamma apr ec/\gamma apr ec$) and Bar ($+ \cdot B^s/\gamma apr ec$), while two additional classes are recovered following a crossover in the ec -centromere region, namely, wild type ($+/\gamma apr ec$) and yellow-apricot-echinus-Bar ($\gamma apr ec \cdot B/\gamma apr ec$). Since the frequency of crossing over in the ec -centromere region is about 50 percent, then the yellow-apricot-echinus-Bar females should constitute roughly 25 percent of all female progeny. Since Bar females which are also phenotypically yellow are expected normally only from exchange females but not from nondisjunctives, there would have been no difficulty in distinguishing between these two possibilities. Furthermore, from the experiment involving the cross of $X^+/B^sY\gamma^+$ males with $sc^{s1} In-S apr$ females, nondisjunctive Bar female progeny would have the composition $X^+/sc^{s1} In-S apr/B^sY\gamma^+$ and would yield four classes

of regular female progeny when crossed with their $sc^{s1} In-S apr/B^s Y \gamma^+$ brothers, namely, wild type ($+/sc^{s1} In-S apr$), apricot-Bar ($sc^{s1} In-S apr/sc^{s1} In-S apr/B^s Y \gamma^+$), Bar ($+/sc^{s1} In-S apr/B^s Y \gamma^+$), and apricot ($sc^{s1} In-S apr/sc^{s1} In-S apr$). Secondary nondisjunctional females, $+/sc^{s1} In-S apr/B^s Y \gamma^+$ are phenotypically Bar and indistinguishable from regular Bar females. On the other hand, exchange females, $+ \cdot B^s/sc^{s1} In-S apr$, would yield only two classes of female progeny, namely, Bar ($+ \cdot B^s/sc^{s1} In-S apr$) and apricot ($sc^{s1} In-S apr/sc^{s1} In-S apr$) owing to the suppression of effective exchange between the X chromosomes. Clearly, then, it would have been simple to make a distinction between nondisjunction and exchange in these cases. Unfortunately, STRANGIO (1961) provides no data on the number of Bar females tested (or how the test was carried out) and the later report (STRANGIO 1962) appears to be based on the analysis of eight Bar females.

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SUMMARY

X-irradiation of primary spermatocytes of adult $\gamma v/B^s Y \gamma^+$ *Drosophila melanogaster* males was demonstrated to produce a greater proportion of X-Y exchanges than X-Y nondisjunction. This result is consistent with the previous findings of ZIMMERING and WU (1963) following irradiation of the prepupal testis, but is in disagreement with those reported by SÄVHAGEN (1961) and by STRANGIO (1961, 1962) from experiments on adult males. While the reasons for this discrepancy are not fully established, a closer examination of the experimental schemes employed by SÄVHAGEN and by STRANGIO suggests that (1) the genetic test used by SÄVHAGEN to distinguish between nondisjunction and exchange may have been faulty thus providing no basis for a decision as to the relative frequency of occurrence of these events, and (2) STRANGIO's experimental design failed to permit the identification of exchanges between X and Y^L , a class constituting approximately 50 percent of all X-Y exchanges. However, the present authors cannot explain why tests which STRANGIO presumably carried out to distinguish between nondisjunction and X- Y^s exchanges gave evidence that most of these cases were nondisjunctional, since, on the basis of the present evidence and that of ZIMMERING and WU (1963) the vast majority of such cases should have proven to be X- Y^s exchanges.

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