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### Authors

Hughes, Ann M.  
Hildreth, Philip E.  
Becker, Gweneth Carson.

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**University of California**  
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**Radiation Laboratory**

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Ann M. Hughes, Philip E. Hildreth,  
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GENETIC EFFECTS OF DEUTERIUM OXIDE IN DROSOPHILA MELANOGASTER<sup>1</sup>Ann M. Hughes, Philip E. Hildreth<sup>2</sup>, and Gweneth Carson Becker<sup>3</sup>

Lawrence Radiation Laboratory, University of California, Berkeley, California

Zamenhof and Demerec (1943) reported that heavy water ( $D_2O$ ) did not increase the recessive lethal mutation rate in Drosophila melanogaster when incorporated into the culture medium or injected in larvae. However, studies on the sterility-inducing ability of  $D_2O$  in mice (Hughes and Calvin, 1958; and Hughes, Bennett, and Calvin, 1959) suggested that the damage might be genetic in character. Therefore we have carried out tests on D. melanogaster to determine whether or not in addition to inducing sterility,  $D_2O$ , when incorporated into the culture medium, produces genetic effects. The genetic effects studied were sterility, the recessive lethal mutation rate in the X chromosome, meiotic and mitotic crossing over between X chromosomes, crossing over between the X and Y chromosomes in the male, and nondisjunction of the sex chromosomes in both the male and female.

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<sup>2</sup>Also associated with the Department of Zoology, University of California, Berkeley, California.

<sup>3</sup>Present address: Zoologisches Institut der Universität, Ketzertbach 63, Marburg/Lahn, Germany.

## MATERIALS AND METHODS

### Culture medium:

All flies were grown on cornmeal-molasses-agar-yeast medium. When D<sub>2</sub>O was used, it was added in place of the equivalent volume of H<sub>2</sub>O in the cooking of the medium. "Treated" flies were obtained from cultures in which eggs had been laid on medium containing the indicated concentrations of D<sub>2</sub>O. The progeny developed through eclosion on this medium. All cultures were grown at 25°C. The concentration of D<sub>2</sub>O throughout the experiments is expressed as volume percent of added heavy water and does not take into account the moisture content of the other medium constituents. This additional moisture would not decrease the D<sub>2</sub>O concentration by more than 5%--i.e., in a 20% D<sub>2</sub>O medium, the D<sub>2</sub>O concentration would not be less than 19%. The concentrations of D<sub>2</sub>O used were selected after preliminary toxicity studies.

### Toxicity studies:

Two culture bottles were prepared for each of the following concentrations of D<sub>2</sub>O: 0, 10, 20, 30, 40, 50, 70, 90, and 100%. Three pairs of Canton wildtype flies per culture were placed in the bottles for three days and then removed. Deaths of these flies during this period as well as the numbers of offspring arising in the cultures were recorded.

### Sterility studies:

Canton wildtype flies were raised on standard or on 20% D<sub>2</sub>O medium, as described above. Treated females were individually mated with either treated or untreated males; untreated females were likewise mated with treated or untreated males.

X chromosome recessive lethals:

Treated males (raised on 10 or 20% D<sub>2</sub>O medium) and control males, carrying the markers carnation (car) and vermilion (v) on the X chromosome, were individually mated with virgin females having X chromosomes homozygous for yellow<sup>2</sup> (y<sup>2</sup>), Bar (B), vermilion (v), and apricot (wa), and the inversions sc<sup>51</sup>, In<sup>49</sup>, sc<sup>8</sup> to suppress crossing over. Stocks of these flies were kindly supplied to us by Dr. H. J. Muller of Indiana University. F<sub>1</sub> females were mated individually with y<sup>2</sup>B v wa males that had been grown on standard medium. Among the F<sub>2</sub>, a culture was scored as lethal if it contained at least 20 flies but no car v males; a culture was scored as not lethal if it contained one or more car v males regardless of the total number of flies.

Crossing over between X chromosomes:

For the studies on meiotic crossing over, females heterozygous for yellow (y), crossveinless (cv), vermilion (v), forked (f), and their wildtype alleles (y cv v f/y<sup>+</sup> cv<sup>+</sup> v<sup>+</sup> f<sup>+</sup>) were raised on medium containing 20% D<sub>2</sub>O. They were mated individually to y cv v f/Y males grown on standard medium. A control series was run simultaneously. The F<sub>1</sub> generation was scored for crossovers involving these four loci.

For the investigation of mitotic crossing over, virgin Canton wildtype females and white-eyed (w) males were aged separately for three days on medium containing 20% D<sub>2</sub>O. One female was then placed with several of the males in each culture bottle, which also contained 20% D<sub>2</sub>O. A control series in which flies were raised on standard medium throughout was set up at the same time. The F<sub>1</sub> females were examined for the presence of white spots in their normally red eyes;

the mosaic patches would result from crossing over between X chromosomes, loss of the  $w^+$  chromosome, or mutation of  $w^+$  to  $w$ . In a second experiment both P and  $F_1$  individuals were raised on 20%  $D_2O$  medium.

Nondisjunction:

To determine the effect of  $D_2O$  on nondisjunction in the female,  $y\ f/y^2\ sc^{S1}\ B\ In49\ v\ w^a\ sc^8$  females grown on 20%  $D_2O$  medium were mated individually with  $y\ cv\ v\ f/sc^8.Y$  males (the  $sc^8$  attachment carries  $y^+$ ). The progeny were then examined for evidence of nondisjunction (see Figure 3). Control series were set up at the same time.

For a similar study in males,  $y/sc^8.Y$  males grown on standard or 20%  $D_2O$  medium were individually mated with  $y/y$  females. Nondisjunction of the sex chromosomes in either parent would theoretically produce equal numbers of  $+$  females and  $y$  males among the progeny. Loss of the  $y^+$  marker or of the entire Y chromosome would produce an increased number of exceptional males only. The results reported in detail below indicated that nondisjunction alone would not account for the exceptional types of offspring and that there was a loss of part or all of the Y chromosome. To determine whether the effect was caused by the loss of the Y chromosome or only part of it by breakage or crossing over, the following two experiments were carried out.

Crossing over between the X and Y chromosomes:

1. Yellow females ( $y/y$ ) were mated with males that carried yellow on the X chromosome and had a Y chromosome with Stone's Bar ( $BS$ ) attached to the long arm and the  $f$  allele of yellow ( $sc^8$  tip) attached to the short arm. For a further description of this  $sc^8.Y.BS$  chromosome, see Brosseau (1958). Expected offspring from this cross would be yellow



females and nonyellow males having Bar eyes. It would also be possible to distinguish those types of offspring that had resulted from nondisjunction from those that had resulted from crossing over between X and Y chromosomes or fragmentation of the Y. Fragmentation as used here means a deletion of the  $y^+$  or  $B^S$  segments of the Y chromosome. Another possibility for the loss of these markers would be by translocation to an autosome; in the absence of evidence for translocations having occurred, breakage or fragmentation will then refer to loss by deletion.

2. In order to recover the centromere-bearing fragments of the Y chromosome and to be sure that males carrying these fragments would be fertile, use was made of attached XY chromosomes. Females homozygous for the attached XY chromosome Ins(1)EN,dl-49, Y<sup>S</sup> car v f y<sup>+</sup> Y<sup>L</sup> were mated with y/sc<sup>2</sup>.Y.B<sup>S</sup> males. Thus a centromere-bearing Y fragment, if recovered in a male, could be tested further, because that male would also have a complete Y attached to the X chromosome and would be fertile. These fragments could then be tested for fertility by combining them in males that had either the long arm or the short arm of the Y attached to an X chromosome.

#### RESULTS AND DISCUSSION

##### Toxicity:

In the cultures that contained D<sub>2</sub>O in a concentration of 70% or more, the adults all died within a three-day period. Although the adults lived on the 40 and 50% media, no eggs were laid. At a concentration of 30% only one offspring eclosed. The results obtained from cultures having 30% or less D<sub>2</sub>O are shown in Figure 1. Our results are in general agreement with those of Zamenhof and Demerec (1943).

Variations may be attributed to the strains of flies used and the differences in methods of computing D<sub>2</sub>O concentration. D<sub>2</sub>O in concentrations of 10 or 20% were used in all the following experiments.

Sterility:

In Table 1 and Figure 2 are shown the influence of D<sub>2</sub>O on females as opposed to males. In general, D<sub>2</sub>O reduces the number of fertile matings and the numbers of offspring more sharply when fed to females than when fed to males. In contrast, in mice D<sub>2</sub>O reduced the number of fertile matings more sharply when fed to males than to females, but did not reduce the number of offspring per fertile mating in either case. (Hughes & Calvin, 1958; Hughes, Bennett, & Calvin, 1960).

X chromosome recessive lethals:

As shown in Table 2, D<sub>2</sub>O in concentrations of 10 and 20% did not significantly increase ( $P = 0.8$  to  $0.9$ ) the frequency of recessive lethal production above that for the controls; these results are in agreement with those of Zamenhof and Demerec. However, among the F<sub>1</sub> females there was an increase in sterility, which was significant in the 20% D<sub>2</sub>O experiment ( $\chi^2 = 12.1949$ ,  $P < 0.001$ ) but not significant in the 10% D<sub>2</sub>O experiment ( $P = 0.1$  to  $0.2$ ). Among the F<sub>1</sub> females there was also an increase, though not significant ( $P = 0.2$  to  $0.3$ ), in the crossover frequencies between the X chromosomes, as evidenced by the recovery of recombinant types among the F<sub>2</sub> individuals.

Crossing over between X chromosomes:

Among 3174 control offspring from  $y \underline{cv} \underline{v} \underline{f}/y^+ \underline{cv}^+ \underline{v}^+ \underline{f}^+$  mothers and 3499 offspring from mothers of the same genotype raised on 20% D<sub>2</sub>O medium, there was no appreciable difference in the meiotic crossover rate between

the two groups. In the control group there was 14.5% crossing over between y and cv, 24.1% between cv and v, and 21.8% between y and f; in the treated group the comparable percentages were 14.0%, 22.4%, and 22.0%. Crossing over in the treated series was greater than in the control only between y and f, and this was not a significant increase ( $P > 0.60$ ).

In the studies on mitotic crossing over, there was no significant increase ( $P = 0.8$  to  $0.9$ ) over the control series when the red-eyed females and white-eyed males were aged and mated and their progeny grown on 20%  $D_2O$  medium (control = 0.71%, treated = 0.76%). When the parents of the red-eyed females and white-eyed males were also grown on 20%  $D_2O$  medium, there was an increase in crossover rate from 0.20% (2096 eyes examined) in the control to 0.34% (2672 eyes examined) in the treated series. The difference in rate is not statistically significant ( $P = 0.3$  to  $0.5$ ). The variation between the control series of the two experiments was greater than that between the control and treated series in either experiment.

Nondisjunction:

The possible progeny that would result from the cross of y f/y<sup>2</sup> B v w<sup>a</sup> females with y cv v f/sc<sup>8</sup>.Y males are shown in Figure 3; the exceptional progeny that would result from nondisjunction in the female are indicated by asterisks. The XXX females usually are not viable, and none were found. In an examination of 11,178 progeny from control females, 8 XXY females and 28 XO males were found. Among 12,743 progeny from females raised on 20%  $D_2O$  medium there were 27 XXY females and 43 XO males. The combined frequency of these exceptional females and males was increased from 0.3%

in the control series to 0.6% in the treated series; this increase is statistically significant at the 1% level ( $\chi^2 = 7.5767$ ,  $P < 0.01$ ).

(Nondisjunction in the male would result in  $\underline{f}$  females,  $\underline{B}/\underline{+}$   $\underline{v}$  females,  $\underline{y}$   $\underline{f}$  males, and  $\underline{y}^2$   $\underline{B}$   $\underline{v}$   $\underline{w}^a$  males. In the  $D_2O$  treated series, three  $\underline{B}/\underline{+}$   $\underline{v}$  females were found. In the control series, three  $\underline{f}$  females, two  $\underline{B}/\underline{+}$   $\underline{v}$  females, 13  $\underline{y}$   $\underline{f}$  males, and eight  $\underline{y}^2$   $\underline{B}$   $\underline{v}$   $\underline{w}^a$  males were found.)

The results of the study of nondisjunction of the X and Y chromosomes in the male ( $\underline{y}/\underline{y} \times \underline{y}/\underline{sc}^8 \cdot \underline{Y}$ ) are shown in Table 3. The frequency of the total exceptional flies recovered from the treated series was significantly higher than that from the control series ( $\chi^2 = 20.8137$ ,  $P < 0.0001$ ). Since the increase in exceptional females is significant only at the 5% level ( $P = 0.05$  to  $0.04$ ) and the increase in exceptional males is highly significant ( $\chi^2 = 17.4569$ ,  $P < 0.0001$ ), it seems likely that the genetic accident involved is not solely nondisjunction but perhaps also a loss of the  $\underline{y}^+$  marker or the entire Y chromosome. In some cases a single event early in spermatogenesis probably would have resulted in the production, by a single male, of two or more exceptional flies. Even when each cluster is counted as a single event, there were 57 single events in the control and 109 events in the treated series. The difference is still highly significant ( $\chi^2 = 19.7099$ ,  $P < 0.0001$ ).

Crossing over between the X and Y chromosomes in the male:

The exceptional types of offspring and the numbers of these recovered from the crosses between  $\underline{y}/\underline{y}$  females and  $\underline{sc}^8 \cdot \underline{Y} \cdot \underline{B}^S$  males are presented in Table 4. The exceptional  $\underline{y}^+ \underline{B}^S$  daughters and  $\underline{y}$  sons could be explained by nondisjunction in either parent. The apparent rate of nondisjunction was not significantly increased when the males were raised

on 20% D<sub>2</sub>O ( $\chi^2 = 0.0858$ ,  $P = 0.8$  to  $0.7$ ). The other four exceptional classes may be accounted for by crossing over between the X and Y chromosomes. The distribution of clusters that may have resulted from crossing over between the X and the short arm of the Y are shown in Table 5. The reciprocal classes resulting from a crossover between the X and the short arm of the Y would be: (1) an X chromosome, carrying y, attached to the Y centromere and the long arm of the Y carrying BS, and (2) the short arm of the Y, carrying y<sup>+</sup>, attached to the X centromere. The egg fertilized by the first type would produce a yellow female with Bar-shaped eyes and that fertilized by the second type a wildtype (+) male. The reciprocal classes resulting from a crossover between the X and the long arm of the Y would be yellow males with Bar-shaped eyes and wildtype (+) females. If crossing over is to account for the exceptional offspring, then the reciprocal types should occur in nearly equal frequencies, discounting viability or other differences that might reduce the recovery of one type to less than that of the other. The reciprocal classes from each type of assumed crossover are very similar in frequency; among the controls there were 10 y BS males and no + females; however, the y BS males arose from two cultures, one of which contained a single y BS male and the other contained nine. A single event could have accounted for this cluster of nine. If we assume that these exceptional types have resulted from crossing over, then in the D<sub>2</sub>O-treated cultures the rate of crossing over has been increased greatly over that in the controls. There was no significant increase in the frequency of crossing over between the long arm of the Y and the X chromosomes in the treated series ( $P = 0.1$  to  $0.2$ ), but there was an extremely significant increase in the frequency of crossing over between the short arm and the X

( $\chi^2 = 58.0516$ ,  $P < 0.0001$ ). Fragmentation of the Y chromosome would not account for the reciprocal classes, as only partial Y's would be produced and no X chromosomes with Y fragments attached would be found. These were recovered; therefore fragmentation, even though it may occur, cannot account for these exceptional female types. In the control culture that produced nine  $y \underline{B}^S$  males, fragmentation and loss of the  $y^+$  tip of the Y during an early gonial division might very well have accounted for this high number of exceptions, since no reciprocal type that would be expected from crossing over was recovered. The number of exceptional males recovered was always higher than the reciprocal female class in both the treated and control series in this and the next experiment to be described; this increase might well have been a result of Y chromosome breakage.

The types and the numbers of exceptional offspring that resulted from the crosses between Ins(1)EN, dl-49,  $Y^S$  car v f y· $Y^L$  females and  $y/sc^8$ · $Y \underline{B}^S$  males are presented in Table 6. The frequency of nondisjunction was not significantly increased by  $D_2O$  treatment ( $P = 0.2$  to  $0.1$ ). Only one fly that might have resulted from crossing over between the long arm of the Y and the X chromosome was recovered, and this from the control group. As was explained earlier, this could have resulted from fragmentation of the Y because it was recovered as a Y fragment and not attached to an X chromosome. The frequency of recovery of types that could have resulted from crossing over between the short arm of the Y and the X chromosome is higher in the treated than in the control series and is significant at the 5% level ( $\chi^2 = 4.3122$ ). There is a greater discrepancy between the reciprocal types recovered than among those recovered in the last experiment (the males being in much higher

frequency), but probably most of this effect is due to the poor viability caused by the attached XY chromosomes. This same effect is to be noted in the nondisjunctional classes in which the males are recovered much more often than the females. The nondisjunctional females would have one attached XY, one free X, and an additional Y with X segments at each end; the females bearing the crossover chromosome would have an attached XY from the mother and an X chromosome with a partial Y attached from the father. The exceptional males that were thought to carry the sc<sup>8</sup> fragment attached to either an X centromere (assuming crossing over) or a Y centromere (assuming Y fragmentation) were individually mated to virgin attached XY females of the strain described above. The offspring in all cases were y car v f females and car v f males. When these car v f males were then mated to y/y females, the offspring were y females and wildtype males. It is not possible to determine to which of the centromeres, X or Y, the sc<sup>8</sup> fragment was attached. Undoubtedly some fragments were attached to X centromeres, since we obtained the reciprocal class that resulted from crossing over. The stock carrying the sc<sup>8</sup> fragment was inadvertently lost, so it could not be further tested. The assumed reciprocal of this chromosome, a yellow-bearing X chromosome with B<sup>S</sup> now attached to it, was tested by obtaining it in males that had in addition only the long arm of the Y with the y<sup>+</sup> allele attached or only the short arm of the Y. In the former case each male tested was sterile, which would be expected if the Y attachment did not contain the fertility factors from the short arm of the Y; in the latter case the y B<sup>S</sup> males were fertile, which also would be expected if the Y attachment carried all the fertility factors of the

long arm. Thus it appears that the long arm of the Y has become attached to the X chromosome by crossing over between the X and the short arm of the Y.

It has been shown (Lindsley, 1955) that  $Y^S$  rather than  $Y^L$  preferentially pairs with the X chromosome, and this is verified by our experiments. We obtained only one fly (+ female, Table 4) that is strong evidence for crossing over between the long arm of the Y and the X chromosome, yet we obtained many cases that may be interpreted as having resulted from crossing over between the short arm of the Y and the X chromosome.

#### SUMMARY

The genetic effects of deuterium oxide incorporated into the culture medium were studied in Drosophila melanogaster. After preliminary toxicity studies, concentrations of only 10 or 20%  $D_2O$  were used. Concentrations of 10 or 20%  $D_2O$  had no effect on recessive lethal production, nor were mitotic or meiotic crossing over between X chromosomes in the female influenced by 20%  $D_2O$ . The induction of sterility, nondisjunction in the female, and crossing over between the X and the short arm of the Y were strongly influenced by 20%  $D_2O$ .



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TABLE 1

Sterility produced in Canton stock by D<sub>2</sub>O

	Control	20% D <sub>2</sub> O		
Total matings	51	49	55	46
% Sterile matings	6	29	55	59

TABLE 2

Effect of D<sub>2</sub>O on recessive lethal production, induction  
of sterility, and crossing over in D. melanogaster

	Control	10% D <sub>2</sub> O	20% D <sub>2</sub> O
Total cultures	461	1543	1499
% Recessive lethal cultures	0.21	0.26	0.20
% Sterile cultures (F <sub>1</sub> )	6.9	9.5	12.9
% Crossover cultures (F <sub>1</sub> )	0.0	0.65	0.61

TABLE 3

Results of study for nondisjunction between the X and  
Y chromosomes in the male; y/y females x y/sc<sup>B</sup>-Y males

Treatment	Total flies	Exceptional flies		
		<u>+</u> females	<u>y</u> males	Total per thousand
Control	75,040	25	44	0.92
20% D <sub>2</sub> O	70,528	39	88	1.60

TABLE 4

Exceptional offspring resulting from the cross of  
y/y females x y/sc<sup>8</sup>.1.B<sup>S</sup> males

Treatment	Total flies	Male non-disjunction		Other exceptionals					
		<u>y<sup>+</sup>B<sup>S</sup></u>	<u>y</u>	Females		Males			
				<u>y</u>	<u>B<sup>S</sup></u>	<u>+</u>	<u>+</u>	<u>y</u>	<u>B<sup>S</sup></u>
Control	28,799	16	30*	7	0		12	10**	
20% D <sub>2</sub> O	22,866	10	29	38	1		46	4	

\*Includes a cluster of 9 in one culture.

\*\* Includes a cluster of 8 in one culture.

TABLE 5

Combinations in which the exceptional flies ( $y B^S$  females and  $\varphi c^8$  males) resulting from crossing over between the X and the short arm of the Y, as summarized in Table 4, were recovered

<u>Number of exceptionals in each culture</u>		<u>Number of cultures*</u>	
<u><math>y B^S</math> females</u>	<u>+ males</u>	<u>Deuterium</u>	<u>Control</u>
1	0	13	4
1	1	5	0
1	2	1	1
1	3	3	0
2	0	2	1
2	1	2	0
2	2	3	0
2	3	1	0
0	1	13	7
0	2	3	0
0	3	0	1

\*Total cultures examined: control, 407; deuterium, 323.

TABLE 6

Exceptional offspring resulting from the cross of  $Y^S$  car v f y· $Y^L$  females x  $y/sc^8$ · $Y$ · $B^S$  males

Treatment	Total flies	Male non-disjunction		Other exceptionals			
		$B^S$	<u>car v f y</u>	<u>y</u> $B^S$	<u>car v f</u>	<u>car v f</u>	<u>car v f y</u> $B^S$
Control	26,791	3	18	3*	0	10*	1
20% $D_2O$	29,084	2	32	5	0	23**	0

\*Includes a cluster of 2 y  $B^S$  females and 2 car v f males in the same culture.

\*\*Includes a cluster of 2 car v f males in each of two cultures.



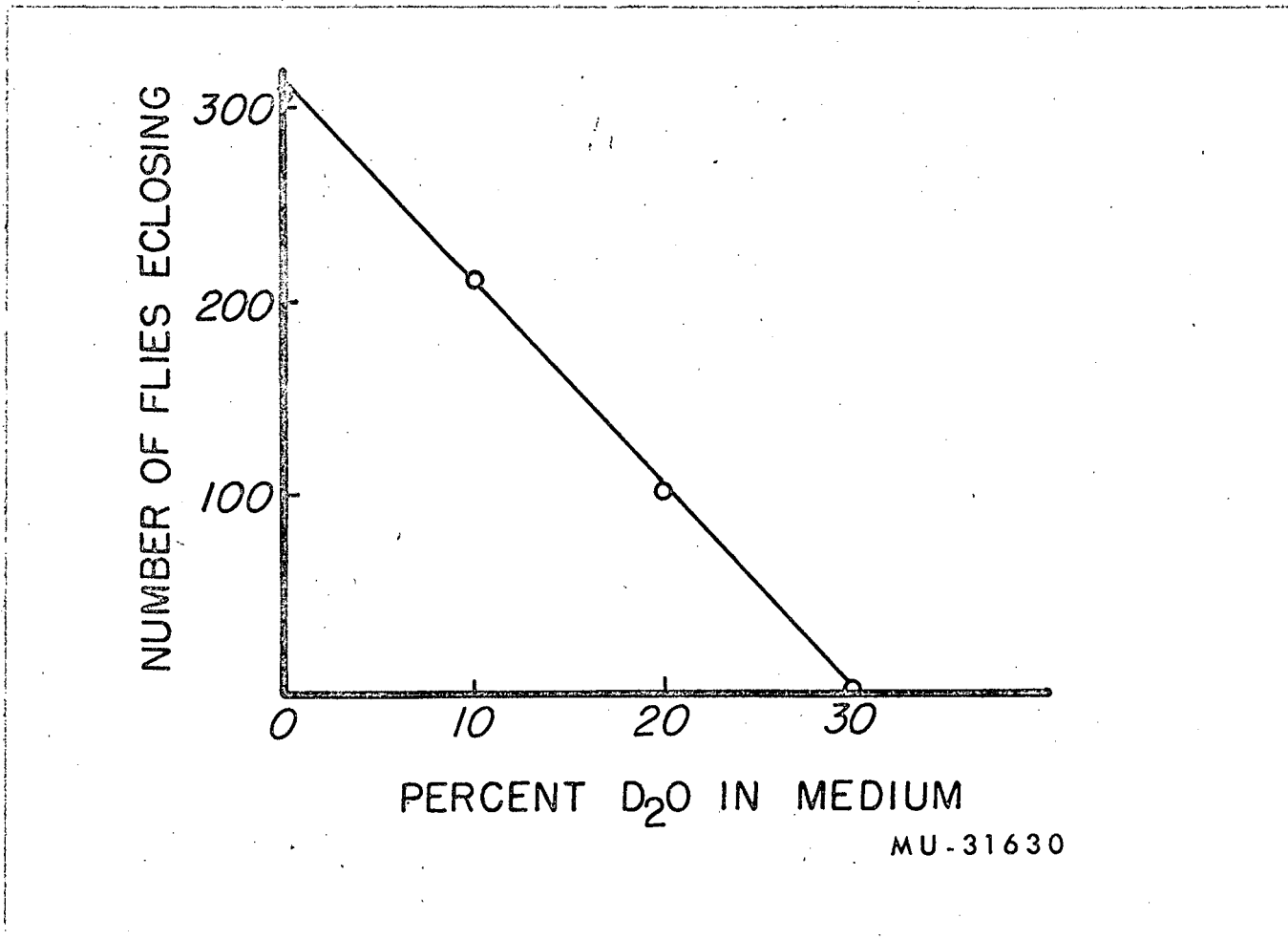
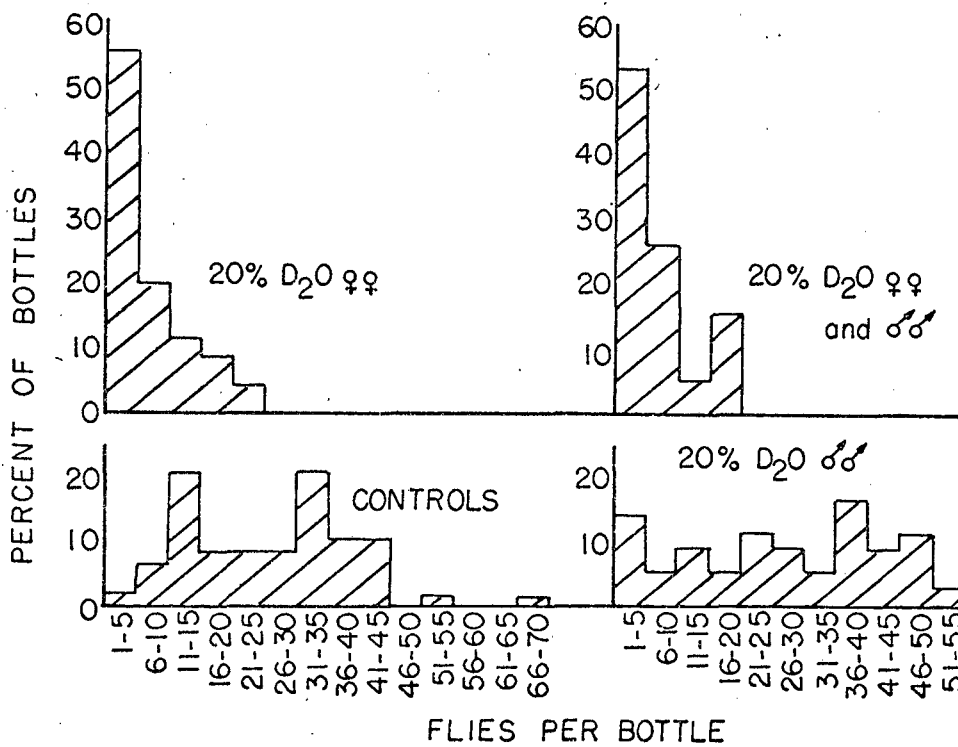
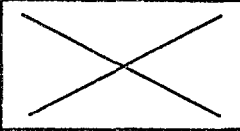


FIGURE 1.--Numbers of offspring recovered when eggs were laid and developed through eclosion on the indicated medium.



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FIGURE 2.--Numbers of offspring recovered when parents were raised on the medium indicated.

♀ \ ♂	$y\ cv\ v\ f$	$sc^B \cdot Y$
$y\ f$	$y\ f\ ♀$	$f\ ♂$
$y^2 B v w^a$	$y \frac{B}{+} v\ ♀$	$B v w^a\ ♂$
$\frac{y\ f}{y^2 B v w^a}$	$y \frac{B}{+} xxx\ ♀^*$	$\frac{B}{+} xxy\ ♀^*$
○	$y\ cv\ v\ f\ xo\ ♂^*$	

MU-31629

FIGURE 3.--Cross used to study nondisjunction in  $B_2O$ -treated

$\underline{y\ f/y^2\ B\ v\ w^a}$  females.

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