X-RAY EFFECTS ON DROSOPHILA PSEUDO-OBSCURA

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Received December 13, 1932

In order to procure new material for the study of problems connected with the non-disjunction of the sex chromosomes in *Drosophila pseudo-obscura*, a series of X-ray experiments was carried out. Among the species of Drosophila, *pseudo-obscura* is distinguished by the length of its X chromosome, whose genetic map is the longest recorded in any species (LANCEFIELD 1922). The work of LANCEFIELD has made a number of mutants available for experiments with this chromosome. Accordingly, the experiments were arranged to permit the detection of (1) non-disjunction of the sex chromosomes in the treated females, (2) crossover modifications in the treated X chromosomes, and (3) non-disjunction of the X chromosomes in the progeny of treated individuals. An eosin (w^e; locus 68) garnet-2 (g^2; locus 88) stock was irradiated, and the treated females were mated to wild type males. In the progeny, exceptional individuals, resulting from non-disjunction of the sex chromosomes in the mother, could be detected. The regular F_1 females were mated to males carrying in their X chromosome the dominant gene Pointed; among their offspring, the sons gave the measure of crossing over in the eosin garnet-2 interval, and the presence of the dominant sex-linked gene in the father permitted the detection of non-disjunction of the X chromosomes in the mother.

The reciprocal cross was also carried out: eosin garnet-2 males were treated and mated to wild type females. The daughters in this cross were of the same constitution as those obtained from the reciprocal mating, and were tested in the same manner. In these tests of F_1 daughters, the untreated chromosome may be considered to serve as a control. There is no control series available for the mating of irradiated female to normal male; for the reciprocal cross, however, a control was raised.

The X-ray dosage was the usual one of MULLER (1927); the Coolidge tube used at a peak voltage of 50 K.V., with a 5 m. am. current, the flies at a distance of 16 cm. from the target, with a 1 mm. aluminium filter. The exposure was sixty minutes for the females and for one set of males, but another set of males was treated for seventy-five minutes.

These experiments were carried out in the winter of 1929 and the spring of 1930, and have been reported briefly in the Carnegie Year Book for 1930 (MORGAN, BRIDGES and SCHULTZ 1930).

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THE DATA

The most striking effects were observed in the progeny of the irradiated males that were mated to normal females. There was a marked deficiency of females in the progeny from the experimental cultures, although the sex ratio in the controls was 1.16 ♀ to 1.00 ♂. This deficiency of females was most marked, as might be expected, in the series given the heaviest treatment (table 1). In that case 66 percent of the expected number of females were lacking.

TABLE 1

Progenies of treated males + ♀ ♀ × w^e g^2 ♂♂, ♂♂ *exposed to X-rays; 50 K.V., 5 m. am., 1 mm. A1 filter distance 16 cm.*

EXPOSURE MINUTES	NUMBER OF CULTURES	♀ ♀	♂♂	SEX RATIO	SEX RATIO (EXP) / SEX RATIO (CONTROL)	PERCENT ♀ ♀ DEFICIENT
60	26	349	634	0.55	0.47	0.53
75	30	185	435	0.40	0.34	0.66
0	8	554	475	1.16	..	..

There was also a high percentage of abnormalities in the progeny; 3.5 percent of the total in the sixty minute treatment, and 2.7 percent in the seventy-five minute treatment. The two largest groups consisted of mutants of the Minute type, with the usual small bristles and other characteristic peculiarities of this class (BRIDGES and MORGAN 1923, SCHULTZ 1929), and of a number of males which had no gonads but were otherwise normal (table 2). These were very similar to the "castrated" males described by GEIGY (1927, 1931) as resulting from treatment of eggs with ultra violet light. In *Drosophila pseudo-obscura*, thea bsence of testes is particularly striking, since the envelope of the gonad is colored a bright orange, and is easily visible through the abdominal wall. In these abnormal males the abdomen appeared white. On dissection it became apparent that the testes were absent, but the rest of the reproductive system was normal.

TABLE 2

Abnormalities occurring as a result of treatment.

EXPERIMENT	TOTAL ABNORMAL IN F_1	♂♂ WITHOUT TESTES	STERILE MUTANTS	F_1 MUTANTS RETAINED	F_1 ♀ ♀ TESTED FERTILE CULTURES	STERILE CULTURES	VISIBLE SEX LINKED RECESSIVES	LETHALS
60 min ♂♂	35	13	10	7	46	11	6	8
75 min ♂♂	17	1	6	2	5	7	1	..
60 min ♀ ♀	12	..	4	..	45	23	3	4
Control	..	..	..	..	..	..	..	..

In addition to these two groups there appeared such characters as "Star" eyes, "Plexate" venation of the wings, "Spread" wings, "Confluent" wing veins, and "Delta" venation. Many of these proved sterile, but some were fertile and stocks of these were established.

The effects observed in the progeny of the treated females were much less extreme. There was in F_1 a slight deficiency of males, somewhat more than might be expected as a result of the poorer viability of the eosin garnet-2 males as compared with their wild type sisters. In the absence of a control series, the rather wide fluctuations of the sex ratio in *Drosophila pseudo-obscura* in different experiments make it difficult to place much weight on this result. The occurrence of sex linked lethals in the mother would lead to such a deficiency of males and would thus be expected in progenies from X-rayed mothers.

TABLE 3

Progenies of treated females w^e g^2 ♀♀ × + ♂♂, ♀♀ treated as the ♂♂ in table 1.

EXPOSURE (MINUTES)	NUMBER OF CULTURES	♀♀	♂♂	SEX RATIO
60	37	887	547	1.60

The number of abnormal specimens[2] was much lower in this group than in the progeny of treated males, a fact which gains significance when it is remembered that new sex-linked recessives in the treated chromosomes may be detected in this cross, which is not the case when males are treated. In spite of this, only 0.8 percent of the total progeny were variants. Of these, one was an occurrence of Notch—probably an oogonial mutation, since three such females, all of which were sterile, appeared in one culture. There occurred also a singed male (sterile), and several mosaics, which may have been due to fractional mutation[3]; a male with one wing curled, another with one miniature wing, and a third male with the venation plexus on one wing.

It is clear that the effects in F_1 are much more extreme when the males are treated. This is further evidenced in the tests of F_1 daughters. Here again (the last four columns of table 2) the males treated for sixty minutes are much harder hit than the corresponding females.

Those of the mutants that could be carried on were studied. It developed that a number of them carried translocations, indicating as might be ex-

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[2] Only one male resulting from primary nondisjunction in the treated females was found.

[3] These were not tested. Their occurrence is of interest in connection with the work of PATTERSON (this journal, 1933, pp. 32–52) on *D. melanogaster*, where mosaics of this kind do not occur in the female. Among the progenies of X-rayed males, five "fractional" mutations were found among the 52 aberrants of the present experiment. These also were not tested; further data are necessary for the study of this problem.

pected, that these occur frequently following the radiation of sperm in this species. Six chromosome aberrations were found: three translocations, one involving the second and third autosomes, the others the second chromosome and the Y; a high non-disjunction stock, probably a translocation, since in such cultures sterile males, probably carrying duplications for a part of the X, appeared; and finally, two crossover reducers, one inhibiting crossing over between eosin and garnet-2, the other between yellow and eosin. The sex-linked recessives which appeared included yellow, a prune-like eye color located some twenty units to the left of yellow, an extreme short vein character associated with a total loss of the testes, an echinus-like eye, a rudimentary-like wing, and a "bubble" wing which proved allelomorphic to the similar mutant found by LANCEFIELD (1930) in race B.

DISCUSSION

The deficiency of females in progenies from X-rayed males is a phenomenon discovered by MULLER in his experiments of 1927 on *Drosophila melanogaster*. He offered the explanation that "dominant lethals" occurred in the X chromosomes as a result of irradiation, and that females heterozygous for an X chromosome carrying such a lethal died. Since the Y chromosome is genetically almost empty, the males in the progeny have an advantage over the females who receive from their fathers a possibly injurious X instead of the innocuous Y. This manifests itself in the changed sex ratio.

In MULLER'S data on *D. melanogaster*, the maximum effect found was an 18 percent deficiency of females, and this of doubtful statistical significance. The present data on *D. pseudo-obscura* show a much greater depression of the sex ratio, the deficiency of females reaching sixty-six percent of the number to be expected on the basis of the control values. It will be remembered that the X chromosome of *D. pseudo-obscura* is very long; in fact, it contains about forty-three percent of the haploid chromatin, as contrasted with twenty-eight percent in the *melanogaster* X chromsome. The more extreme results with *D. pseudo-obscura* are then to be expected on MULLER'S hypothesis, since the longer the X chromosome, the greater the chance that a "dominant lethal" will arise, hence the greater the depression of the sex ratio. The problem is complicated, however, by such factors as the comparative extent of the "inert region" of the X in the two species, and the relative importance of different regions and different chromosomes in the production of dominant lethals. It is entirely likely that these represent long deficiencies, or translocations resulting in chromosomes with two spindle fibres, which would be lethal.

In this connection, MULLER'S attempt to test the assumption that "dominant lethals" occur at random throughout the chromosomes may be mentioned. On this assumption he deduced a relation between the depression of the sex ratio, the percentage of eggs laid that hatched into adult females and the proportion of the total haploid chromatin contained by the X chromosome. He found this relation not to hold in his data, nor does it hold in HANSON'S (1928) data even when correction is made for the "inert" region (PAINTER 1931, MULLER and PAINTER 1932, DOBZHANSKY 1932) of the X. Many more eggs die than should according to formula. MULLER therefore considered that some type of effect on more than one chromosome might be responsible. The explanation of the discrepancy, offered by EFROIMSON (1931), that an autosomal deficiency of a given length is more likely to be a dominant lethal than one in the X chromosome, on considerations of genic balance, seems unlikely. For example, a relatively small section of the X is involved in PATTERSON'S "viability gene" (1932) which behaves as a dominant lethal under his experimental conditions, although BURKART (1931) appears to have obtained from the "Blond" translocation a deficiency for this section which survives as a Minute. Furthermore deficiencies in the autosomes, involving several units of the map, are known to be viable. It seems preferable to adopt the view that the many possible types of multiple chromosome aberrations resulting from X radiation are responsible for the excess mortality.

The depression of the sex ratio seems to be the easiest way of measuring the gross X-ray effects in those experiments where the interest is centered on the magnitude of the effect rather than on the detailed analysis of the various types of disturbance. In this respect, *D. pseudo-obscura* is an especially favorable object due to the extent of the effect. Yet by matings of the treated males to attached X females in *D. melanogaster*, a converse deficiency of males may be obtained (BARTH 1928); this is, of course, due to the dominant lethals plus the sex linked recessive lethals. These two quantities combined give a discrepancy of sufficient magnitude to be workable in *D. melanogaster*. The technique has the advantage of being less laborious than the usual ClB method of MULLER, and therefore may have a certain use in the further analysis of the effects of X-rays on chromosomes, and particularly in exploratory work with other agents.

It may be remarked in the present work, as in that with *D. melanogaster*, that the X-ray effect is noticeably less upon the female than upon the male (MULLER 1930, PATTERSON and MULLER 1931). In *D. melanogaster* this has also been shown to be the case for the immature germ-cells of the male as compared with the mature sperm (HARRIS 1929, HANSON 1929) suggesting that the difference in effect may be perhaps correlated with the difference between haploid and diploid. It has been indicated that the difference between the effect on male and female in *D. melanogaster* is due largely to a difference in the number of chromosome abnormalities (PAT-

Terson and Muller 1931). It is possible that the usual number of these abnormalities may be produced in the diploid cell, but that they are eliminated because a greater proportion of types, which cannot undergo division normally, are produced. It should also be remembered however, that the relative distances between chromosomes are greater in the immature cells than they are in the sperm, hence translocation might less frequently be successful. When these considerations are taken into account, the observed differences are perhaps to be expected.

The males without gonads found in F_1 from the treated males deserve special mention. Their condition is not due to loss of the Y chromosome, since XO males of this species are normal in testis size. The lack of testes must then be either a developmental abnormality caused by X-rays, or a rather frequent kind of dominant mutation, affecting either the initial establishment of the germ track, or the later development of the gonad. It should be recalled here that among the mutants induced by X-rays, one sex-linked recessive caused a loss of the testes, associated with a short vein character in the wings. The male sterility observed by Muller in his experiments with *melanogaster* may be due to the same phenomenon, which is less easily observed in *D. melanogaster*, since the color of the testis envelope is relatively inconspicuous.

An accurate comparison of the effects of X-rays on *D. melanogaster* and *D. pseudo-obscura* cannot be made at present since no strictly comparable data are available. The comparison might be interesting, in view of the results of Timoféeff-Ressovsky (1931) on *D. funebris*, where the data, also unfortunately not strictly comparable, indicate a lower lethal mutation rate than in *melanogaster*. In *D. pseudo-obscura* it is the impression that dominant mutations are more frequent than in *D. melanogaster*, both spontaneous mutations and those from X-rays.

SUMMARY

1. X-ray experiments were made with *Drosophila pseudo-obscura*, which were so arranged that non-disjunction could be detected in the treated females, and modifications of crossing over and disjunction of the X chromosomes could be detected in the F_1 both from treated males and females.

2. In the progeny of treated males, instead of the sex ratio of 1.16 ♀ : 1.00 ♂ observed in the controls, there occurred a marked decrease from the expected number of females. Further a high percentage of abnormalities was found. They consisted mostly of Minutes and males without gonads, and a number of other mutants in addition. Six chromosome aberrations were found as a result of treatment.

3. As has been found in the work with *D. melanogaster*, the effects noted in the progeny from treated females were less extreme than in those from treated males.

4. The more extensive depression of the sex ratio observed in *D. pseudo-obscura* than in *D. melanogaster*, in the progenies from treated males, may be correlated with the difference between the relative sizes of X and autosomes in the two species. The longer the X relative to the autosomes, the greater the chance of injury to the females carrying such chromosomes that have been irradiated.

5. The depression of the sex ratio in *D. pseudo-obscura* affords the simplest technique available for measuring gross X-ray effects on chromosomes. A similar technique in *D. melanogaster* is available from the mating of treated males to attached X females.

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