TRANSACTIONS, ILL. STATE ACAD. SCI.
Vol. 68, No. 1 (1975)

AN ANALYSIS OF X-RAY INDUCED SEMI-LETHALS IN *DROSOPHILA MELANOGASTER*

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ABSTRACT - Oregon R males exposed to 800, 1600 or 3200R were mated to M-5 and the F_2 offspring that had ratios of 1 wild to 6-9 Bar males were cultured to F_3 and F_4. Fourteen out of 1572 F_2 cultures were selected. These semi-lethals were all mosaics for lethals. There was no predominate type of mosaic, but all combinations of wild, complete lethal, lethal, and semi-lethals were observed. Over half of these chromosomes when retested in F_4 yield a different viability than recorded in F_3. Of the ninety apparent lethals, four upon retesting were viability mutants. By conventional methods of lethal detection about 0.5% lethals would be overlooked.

INTRODUCTION

In a search for X-ray induced lethals in a specific region of the X-chromosome several of the apparent lethals turned out to be semi-lethals when placed in a balanced system for maintenance. In an attempt to understand how this could have occurred, X-ray induced recessive sex-linked semi-lethals with viability close to lethals and with no visible change from the wild type were selected. Then individual F_2 and F_3 females heterozygous for these semi-lethals were isolated and offspring examined for variability as to lethality.

Semi-lethals along with other viability mutants were first produced and recognized by Muller (1928). Spencer and Stern (1948) reported that 65 out of 989 lethals when cultured for another generation acted as semi-lethals. Although this group of mutations with lowered viability is a relatively large one, it has not been used often, for these mutations are not readily detected and because of variability are difficult to use in quantitative studies. Auerbach (1947) pointed out that gonadic mosaicism for a lethal acted as a basis for spurious semi-lethality and that chemically induced mutations had a higher frequency than those produced by X-rays.

Table 1. The induction of lethals and semi-lethals on the X chromosome by 800R, 1600R and 3200R of X-rays.

Non Lethals	Complete Lethals[1]	Lethals[2]	Semi-Lethals[3]	Ratio of Complete Lethals to Semi-Lethals
		800R		
548	14(2.49%)	2(0.36%)	2(0.36%)	7 to 1
		1600R		
720	31(4.42%)	3(0.41%)	5(0.69)	6.2 to 1
		3200R		
304	41(11.8%)	4(1.3%)	7(2.25%)	5.9 to 1

[1]No wild type males appeared in F_2, F_3. [2]An occasional wild type male appeared, but the ratio of Bar eye to wild greater than 10:1 in F_2. [3]Ratio of Bar eye to wild type, 9.9 to 6.5:1 wild type.

MATERIALS AND METHODS

Oregon R males 3 days old were exposed to 800, 1600, or 3200R of X-rays generated by a General Electric Maximar 250-III unit at 225 KV, 15 ma at 32 cm, 305 R/min in air. The males were immediately presented to an excess of Muller-5 ($sc^{8\,7}$ *BInS* w^a sc^8) females and given one hour to mate once. Thus only mature irradiated spermatozoa were used. All cultures were kept at 24°C and 60% humidity. The F_1 females that had mated with their brothers were isolated in a vial containing agar, cornmeal, molasses, corn syrup, oatmeal and brewer's yeast. The term "complete lethals" were given to those cultures in which no wild type males appeared in an F_2 in which at least 10 Bar eye males had been classified. The majority of these "complete lethals" had a minimum number of 20 Bar eye males in the F_2. If in these cultures an occassional wild type male appeared so that the ratio of wild to Bar eye males was one wild to ten or higher Bar eye males, these were classified as "lethals." Of course this is an arbitrary decision as to what constitutes a lethal and for those cultures that had a ratio of 1 wild to 9.9 Bar eye males or less (but statistically significant not to be a 1:1 ratio) were termed to be "semi-lethal." Those cultures that had less than a total of 10 males were classified as "no decision."

Normally in our mutagenesis studies, a vial containing the F_2 of unetherized flies was placed under the binocular wide field microscope and if only one male was observed that possessed the phenotypically marked X chromosome to be tested, the culture was classified as nonlethal. However, with practice some semi-lethals could be detected, for in these vials, it took somewhat longer to find the wild type males. The flies were etherized and the ratio of wild type males to Bar eye males were determined. Those semi-lethals that had ratios of one wild to 7-10 Bar eye males were retained and retested for lethality and mosaicism, for it was thought that it was these lethals that were mistaken for complete lethals and that the semi-lethality was revealed when placed in a balanced chromosome system. As many of the virgin heterozygous females as could be obtained from these cultures were isolated and mated to M-5 males to produce a F_3. The males were all classified in the F_3 to obtain the ratio of wild to Bar males and this procedure was repeated in the F_4.

Every complete lethal obtained in the F_2 was cultured to a minimum of ten vials in the F_3 by isolation of heterozygous females and the offspring examined for wild type males.

RESULTS

All the complete lethals, lethals, and semi-lethals that were induced by 800, 1600, and 3200R of X-rays are listed in Table 1. Of the 90 apparent complete lethals retested for lethality four of these produced wild type males. One apparent complete lethal

Table 2. An analysis of 14 apparent semi-lethals for gonadic mosaicism by isolating F_2 heterozygous females.

Mosaic for wild, semi-lethal, lethal, complete lethal	3
Mosaic for wild, semi-lethal, lethal	3
Mosaic for wild, semi-lethal, complete lethal	2
Mosaic for wild, complete lethal	3
Mosaic for semi-lethal, complete lethal	2
Mosaic for semi-lethal, lethal	1
Non mosaics	0

Table 3. F_3 females heterozygous for apparent semi-lethal isolated mated with M-5 and lethality of X chromosome in the F_3 compared to F_4.

Semi-lethal	F_3 and F_4 similar	F_3 and F_4 different
SL 3200-1	6	21
3200-2	24	16
3200-4	5	1
3200-5	7	9
3200-3	6	2
3200-6	5	12
3200-7	4	9
SL 1600-1	11	19
1600-2	8	4
1600 3	9	4
1600-4	4	11
1600-5	3	1
SL 800-1	7	3
800-2	4	8
	103	120

Table 4. SL3200-1 an apparent semi-lethal that had a ratio 9.4B:1 + ♂ in the F_2 and the mosaic mature of F_3 and the variability of the F_4.

F_2 Female	F_3 offspring	F_3 Female	F_4 offspring
A	semi-lethal 3.29B:1+ (30)*	A[6]#	5 non lethal, 1 semi-lethal
B	non lethal 1.25B:1+ (30)	B[2]	2 semi-lethals
C	complete lethal 14B:0+ (14)	C[5]	2 semi-lethals, 2 lethals, 1 complete
D	complete lethal 11B:0+ (11)		
E	no decision 8B:1+ (9)	E[1]	1 non lethal
F	semi-lethal 9B:1+ (10)	F[3]	1 non lethal, 2 semi-lethal
G	semi-lethal 3.75B:1+ (19)	G[2]	1 semi-lethal, 1 complete lethal
H	semi-lethal 6.5B:1+ (15)		
I	non lethal 1.23B:1+ (49)	I[3]	3 semi-lethals
J	semi-lethal 3.27B:1+ (64)	J[3]	3 non lethals
K	complete lethal 32B1:0+ (32)	K[4]	1 semi-lethal, 1 lethal, 2 complete

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* Number in parenthesis represented the total number of males counted.

\# Number in brackets represented the total number of F_3 isolated to obtain F_4.

was in the 800R series, two in the 1600R data and one as a result of exposure to 3200R. Upon further analysis two of these were lethals in that an occasional wild type male appeared, and two were classified as semi-lethals in that the ratio of wild type to Bar males was in the range of one wild to 5-9 Bar eye. The majority of lethals produced by X-rays were complete lethals. Semi-lethals as defined above were produced 1/6th as frequently as complete lethals. Lethals in which an occasional wild type male appeared were still less frequent.

All fourteen of the X-ray induced semi-lethals when cultured to F_3 turned out to be mosaics (Table 2). There was no predominate type of mosaic, but all combinations of wild, complete lethal, lethal and semi-lethal were obtained. The further analysis of these lethals to F_4 generation revealed that over half the chromosomes that were retested yielded a different viability than recorded in F_3 (Table 3).

Since Tables 2 and 3 represented the summary of F_3 and F_4 generations of the fourteen semi-lethals that were studied, the analysis of one of the semi-lethals SL3200-1 is presented in Table 4. It was the first one detected in 3200R series test. In this particular semi-lethal, there was a mixture of non-lethal, semi-lethal and complete lethal X chromosomes as revealed by the F_3 and F_4 generations. The only reasonable way that this data in Table 4 can be explained is on the basis that this apparent semi-lethal was a mosaic for a lethal.

DISCUSSION

Eleven of the fourteen apparent semi-lethals were gonadal mosaics for wild type and viability mutations, the other three were gonadal mosaics for semi-lethal and lethal mutants (Table 2). However, since the distinction between semi-lethal and lethal mutations is an arbitrary one, in that the ratio of one wild to ten Bar eye or larger was considered a lethal, it was possible that some semi-lethals, if the culture conditions were poor, would appear as lethals or even a complete lethal. Also some lethals if raised under favorable conditions would be classified as semi-lethals and ideal food environment could also result in some semi-lethals tabulated as non-mutants.

The fourteen gonadal mosaics all contained lethal or complete lethal mutations, however, these would have been overlooked, for in the conventional method of detecting lethal mutants, an appearance of one wild type male, the culture would have been classified negative. Yet 14 lethal containing cultures out of 1572 is 0.89% which would have been missed. This incidence of X-ray induced of X-ray gonadal mosaicism is still less than results of chemical treatments as reported by Auerbach (1947), Carlson and Southern (1962) and Browning and Altenberg (1961).

Of the ninety apparent lethals, four were misclassified, which is 4.44%. Of the total of 1572 cultures that were closely examined, the four false lethals would be 0.25%. Thus if in the analysis of X-ray induced lethals, by the conventional method in which a vial is examined under low power the presence of 1 male containing the X chromosome that was irradiated is found and the vial is classified as a non-mutant, there would be about 0.5% of the total lethals that would be missed. To reduce the error the induced lethals should all be cultured to the F_3 to detect the semi-lethals.

Gonadal mosaicism may be due to the damage of one strand of DNA; or if the injury shown as a change after several replications. The orientation of the nucleus at the first division may also play a role in mosaicism. There was no relative increase in semi-lethals at the lower dose 800R. The variability of the semi-lethals was of such an extent that 54% of the chromosomes bearing the semi-lethal in F_3 were classified differently in the F_4.

This variability of the viability of some of the mutants could simulate mosaicism or be classified as unstable genes. Since true gonadal mosaicism is most likely to occur in the F_1 female and appears in F_2 offspring, the apparent mosaicism in F_2 and F_3 females is most likely due to the variability of the semi-lethals.

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