# THE FREQUENCY PATTERN OF APPARENT XO MALES INDUCED BY X-RAYS IN THE SUCCESSIVE STAGES OF OOCYTES OF *DROSOPHILA* *

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In *Drosophila,* it has been generally recognized that oocyte stages differ greatly in their sensitivity to radiation-induction of several kinds of genetic effects (for a recent review, see Sankaranarayanan and Sobels 1976).

Recently, Miyamoto and Nakao (1978) have investigated the frequency pattern of *dumpy* mutations induced by X-rays in the successive stages of oocytes, by using the brood pattern technique (12 successive 1-day egg-laying periods are employed). They have revealed that the yield of the *dumpy* mutations detected in the oocytes presumably corresponding to the synaptic stage is strikingly low as compared to that recorded in the subsequent cell stages of oocyte development. They have suggested that in these oocytes some kind of repair system is operating to cause reduction in the yield of *dumpy* mutations.

More recently, evidence supporting the above suggestion has been obtained for the induction of the *yellow, white, miniature* and *forked* mutations by X-rays in the successive stages of oocytes (Miyamoto 1979).

The present report concerns apparent XO males, the majority of which were detected simultaneously in the course of the experiments on the frequency pattern for the *dumpy* mutations induced by X-rays in *Drosophila* oocytes (Miyamoto and Nakao 1978). The results obtained provide evidence supporting the above suggestion further.

## MATERIALS AND METHODS

The experimental procedures are essentially the same as those used by Miyamoto and Nakao (1978) except for the X-ray doses employed. Females of *D. melanogaster* with the genotype $sc^{S1}$ $B\ InS\ w^{a}$ $sc^{8}$ were irradiated with

* This work was done at Zoological Laboratory, Faculty of Science, Hiroshima University, Hiroshima 730.

three different doses of X-rays (1500, 3000 and 4500 R), when they were 5-day-old. The X-ray machine was operated at 200 kV, 15 mA, with 1.0 mm Al plus 1.5 mm Cu filtration to deliver X-rays at a rate of 130 R/min. Immediately after treatment, the females were mated with *y w m f*; *dp* males en masse (30 females to 150 males per bottle). For sampling the successive stages of oocytes the brood pattern technique, although rather limited in its potentialities, had to be used. The parents were transferred en masse to fresh vials daily for 12 days and allowed to lay eggs for a restricted egg-laying period of 24 h. By this procedure, the first egg-laying period represents oocytes irradiated at stage 14, and the subsequent ones represent progressively earlier stages at the time of irradiation. However, this procedure does not insure that each egg-laying period will represent specific stages of oocytes. Furthermore, the possibility of an oogonial contribution, particularly on the later egg-laying periods, cannot be excluded.

From the above mating, *y w m f* sons (apparent XO males) were detected, although the stocks in the present study were employed primarily for the detection of *dumpy* mutations (Miyamoto and Nakao 1978). Generally, these *y w m f* males result from the fertilization of a normal sperm with X-chromosome by a egg carrying no X-chromosome. Such males, lacking a Y chromosome, are sterile so that their identification cannot be verified by further tests. This may lead to somewhat of an overestimate for the yield of these exceptions, since males phenotypically identical to *y w m f* males but originating from other kinds of mutational changes may also be classified as XO males.

The statistical tests of the data obtained in the present study were made by using Kastenbaum and Bowman′s tables (Kastenbaum and Bowman 1970). Confidence intervals of 95% of the frequencies were calculated by using Fisher and Yate′s table.

## RESULTS AND DISCUSSION

The data on the induction of *y w m f* males (apparent XO males) by X-rays in the successive stages of oocytes are summarized in Table 1 and illustrated in Fig. 1. The frequencies in the table are calculated by the formula : $\frac{\text{XO males}}{\text{XX females} + \text{XO males}}$ (Definition 2 of Traut 1967, 1968).

Clearly from Fig. 1, at the exposure level of 4500 R, the frequency of the appaqent XO males varies greatly in relation to the oocyte stages irradiated. Namely, the yield of these exceptions is at the highest level in the first brood, then decreases gradually in broods 2-8, and reaches a low level in broods 9-12. The frequency patterns observed at the exposure levels of

Table 1. The incidence of *y w m f* males (apparent XO males) from the cross *y w m f; dp* males by X-irradiated $sc^{S1}$ *B InS* $w^a$ $sc^8$ females in *Drosophila*

| Brood | After irradiation of 1500 R | | After irradiation of 3000 R | | After irradiation of 4500 R | | Control | |
|---|---|---|---|---|---|---|---|---|
| | No. XO males/Total | Percent XO males | No. XO males/Total | Percent XO males | No. XO males/Total | Percent XO males | No. XO males/Total | Percent XO males |
| 1 | 11/562 | 1.96** | 9/247 | 3.64** | 10/109 | 9.17** | 8/18361 | 0.04 |
| 2 | 48/4157 | 1.15** | 97/1678 | 5.78** | 73/1114 | 6.55** | | |
| 3 | 56/5079 | 1.10** | 68/1647 | 4.13** | 25/400 | 6.25** | | |
| 4 | 25/5417 | 0.46** | 85/2790 | 3.05** | 67/1121 | 5.98** | | |
| 5 | 32/4971 | 0.64** | 68/3061 | 2.22** | 84/2114 | 3.97** | | |
| 6 | 21/5510 | 0.38** | 61/3881 | 1.57** | 101/2947 | 3.43** | | |
| 7 | 18/5213 | 0.35** | 34/3759 | 0.90** | 52/2686 | 1.94** | | |
| 8 | 10/4808 | 0.21** | 17/3761 | 0.45** | 30/3684 | 0.81** | | |
| 9 | 5/4649 | 0.11 | 5/3736 | 0.13 | 13/3740 | 0.35** | | |
| 10 | 3/3804 | 0.08 | 5/3616 | 0.14 | 4/3688 | 0.11 | | |
| 11 | 0/2068 | — | 8/3477 | 0.23** | 8/3281 | 0.24** | | |
| 12 | 0/1574 | — | 1/1784 | 0.06 | 0/2764 | — | | |
| 1-12 | 229/47812 | 0.48** | 458/33437 | 1.37** | 467/27648 | 1.69** | | |

** Significant at the 1% level from the control.

3000 and 1500 R are essentially similar to that 4500 R level. These frequency patterns are well in line with those for the *dumpy* mutations (Miyamoto and Nakao 1978) and the *yellow, white, miniature* and *forked* mutations (Miyamoto 1979). Such stage dependency for the X-ray induction of apparent XO males agrees well with that elucidated in the loss of X-chromosome induced by 3000 R of X-rays in the successive stages of oocytes (Grell *et al.* 1966).

In addition to the above stage dependency, Fig. 1 indicates the existence of some kind of dose dependency for the X-ray induction of these exceptions. In order to clarify this point further, 12 daily broods are classified roughly into two groups according to their striking difference in the radiosensitivity to the induction of such exceptions: broods 1-7 and broods 8-12. The first 7 broods are characterized by their highly radiosensitive nature, while the later 5 broods show a good deal of radioresistance. Table 2 and Fig. 2 represent these data. As shown in Fig. 2, the yield of the apparent XO males increases with exposure faster than linearly in broods 1-7, while in broods

8-12 it increases in an approximately linear manner throughout the three exposure levels.

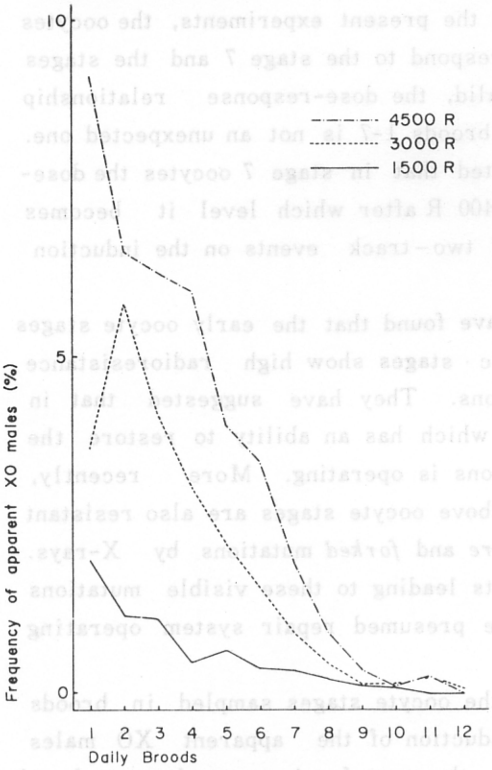


Fig. 1. Frequency pattern of apparent XO males induced by X-rays in the oocytes of *Drosophila* at different stages (Data from Table 1).

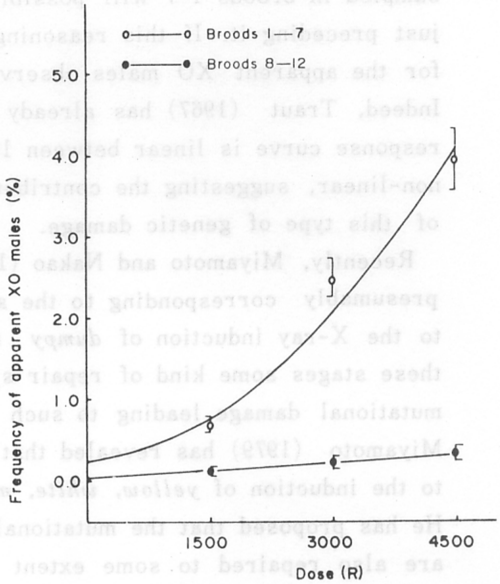


Fig. 2. Dose-response relationships for apparent XO males induced by X-rays in oocytes of *Drosophila*. The curves have been drawn according to the estimated regression coefficient.

Table 2. Frequencies of apparent XO males (*y w m f* males) induced by X-rays in *Drosophila* oocytes

| Dose | Broods 1-7 | | | Broods 8-12 | | |
|---|---|---|---|---|---|---|
| | No. XO males/Total | Percent XO males | | No. XO males/Total | Percent XO males | |
| 1500 | 211/30909 | 0.68** | (0.59-0.78) | 18/16903 | 0.11 | (0.06-0.17) |
| 3000 | 422/17063 | 2.47** | (2.25-2.72) | 36/16374 | 0.22** | (0.15-0.30) |
| 4500 | 412/10491 | 3.93** | (3.56-4.32) | 55/17157 | 0.32** | (0.24-0.42) |
| Control | 8/18361 | 0.04 | (0.02-0.09) | | | |

** Significant at the 1% level from the control.

The numbers in parentheses indicate 95% confidence intervals for the mutation frequency.

Since almost none of the stage 14 oocytes were sampled because of their relatively high radiosensitivity to the induction of dominant lethal mutations by X-rays under the doses employed in the present experiments, the oocytes sampled in broods 1-7 will possibly correspond to the stage 7 and the stages just preceding it. If this reasoning is valid, the dose-response relationship for the apparent XO males observed in broods 1-7 is not an unexpected one. Indeed, Traut (1967) has already reported that in stage 7 oocytes the dose-response curve is linear between 100 to 400 R after which level it becomes non-linear, suggesting the contribution of two-track events on the induction of this type of genetic damage.

Recently, Miyamoto and Nakao (1978) have found that the early oocyte stages presumably corresponding to the synaptic stages show high radioresistance to the X-ray induction of *dumpy* mutations. They have suggested that in these stages some kind of repair system which has an ability to restore the mutational damage leading to such mutations is operating. More recently, Miyamoto (1979) has revealed that the above oocyte stages are also resistant to the induction of *yellow, white, miniature* and *forked* mutations by X-rays. He has proposed that the mutational events leading to these visible mutations are also repaired to some extent by the presumed repair system operating in these oocytes.

Present results have made clear that the oocyte stages sampled in broods 8-12 are more resistant to the X-ray induction of the apparent XO males than those sampled in broods 1-7. Under the transferring procedure employed in the present study, oocytes sampled in broods 8-12 presumably correspond to the ones at the synaptic stages (see Miyamoto and Nakao 1978). According to Koch *et al.* (1970), the synaptic stage oocytes are characterized by their highly radioresistant nature. They are even more efficient in repairing radiation induced lesions (e. g., chromatid deletions and chromatid breaks) than the stage 7 oocytes with a system for the repair. In the meantime, it has been reported that the majority of the apparent XO males induced by X-irradiation of *Drosophila* oocytes seem to originate from chromosome breakage, although to a lesser extent result from primary non-disjunction (Traut 1967, 1968; Traut and Scheid 1970). Taking all these findings into consideration, the present observations can be interpreted to signify that chromosome breakage events may be repaired efficiently in the oocyte stages sampled in broods 8-12 and presumably corresponding to synaptic stages.

The interesting point in this connection is that in the dose-effect relationship elucidated in the present study for the apparent XO males a striking difference is noted between the oocytes sampled in broods 1-7 and those in broods 8-12: the exponential dose-effect curve versus the linear one. This possibly implies that there exists a marked difference in the mechanisms of the induction of these exceptions between these two oocyte groups. It may be utilized as supporting evidence for the high efficiency of the oocytes presumably corresponding to the synaptic stages in repairing X-ray-induced chromosome breaks. However, explanations of this kind are purely speculative, of course, and it does not seem profitable to pursue the nature underlying the difference in the dose-response relationship further at this time, because available information is still limited.

## SUMMARY

The frequency pattern of the apparent XO males induced by X-rays (1500, 3000 and 4500 R) in the successive stages of oocytes of *Drosophila* was investigated by using the brood pattern technique. The inseminated females were transferred daily to fresh vials for 12 days. Under this procedure, the first egg-laying period represents oocytes irradiated when they are at stage 14, and the subsequent ones represent progressively earlier stages of oocyte development at the time of irradiation.

The results obtained indicate that throughout the three exposure levels the yield of the apparent XO males detected in the first 7 day egg-laying periods (1st-7th day) is relatively higher than that in the subsequent 5 day periods (8th-12th day).

This finding seems to indicate that the oocytes sampled in the subsequent 5 day egg-laying periods (8th-12th day) are relatively radioresistant to X-ray induction of the apparent XO males as compared with those sampled in the first 7 day egg-laying periods (1st-7th day). This feature corresponds well to that elucidated previously in the *dumpy* mutations and the *yellow*, *white*, *miniature* and *forked* mutations.

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