DIFFERENTIAL RADIOSENSITIVITY AS AN EXPLANATION FOR
SO-CALLED RECOVERY IN DROSOPHILA SPERM

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Baker and Von Halle (1952, 1953) demonstrated that a higher frequency of dominant lethals was obtained on the first day after X-irradiation of Drosophila melanogaster males than on the second day. This decrease in mutation frequency in second-day sperm samples has been shown to be a general phenomenon in Drosophila. (For reviews see Nordback and Auerbach 1957, Oster 1961, Luning 1961, and Mossige 1963.) An explanation which has been offered by Baker and Von Halle (1953) for this “recovery phenomenon” is that a repair of potential X-ray damage takes place, such as restitution of chromosome breaks, resulting in a reduced mutation frequency in sperm not used until the second day. It is implied in the recovery hypothesis that mature sperm used on the first and second day were of the same radiosensitivity during irradiation, and that sperm were held and used the second day if the males were not allowed to mate for 24 hours. Nordback and Auerbach (1957) found evidence that recessive lethals exhibit the decline in frequency, an observation which could be interpreted as indicating that point mutations undergo recovery, that is, revert to the original nonmutant state, during the 24 hours after irradiation.

However, Oster (1961) has suggested an alternative explanation in which actual recovery is not required. He felt that sperm used the first and second days after irradiation may have had different radiosensitivities at the time of irradiation. For example, the sperm used the first day, being nearer the genital orifice of the male and perhaps better oxygenated, might have a higher mutation frequency through an oxygen effect. Traut (1964) has found that the second-day frequency of sex-linked lethals and autosomal translocations for males not allowed to mate the first day resembled, in a series of experiments, either the first or the second day mutation frequency from males allowed to mate both days. He felt that differential sperm radiosensitivity was the best explanation for his results, and that the sperm used in the delayed matings were primarily of either the sensitive or insensitive type.

In the results of experiments reported in the present paper, the frequency of sex-linked recessive lethals in second-day sperm from males not allowed to mate the first day was always found to be intermediate between the frequencies obtained from the first and second post-irradiation days of males whose matings

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were not delayed. The results are consistent with a hypothesis of differential radiosensitivity of sperm at the time of irradiation, and mixing of sperm when matings are delayed, and do not require recovery as an explanation.

MATERIALS AND METHODS

Three experiments were conducted: in one, a rod X was used as the test chromosome; in the other two, ring-X chromosomes were used. The purpose of the rod-X experiment was to retest the "recovery phenomenon" in detail by allowing the treated males to mate with single virgin females every half-day rather than using conventional daily mass-matings. The first ring-X experiment was similar to the rod-X experiment. The ring-X was used to limit the lethals observed mainly to point mutations and a relatively small proportion of minute deletions. The second ring-X experiment was designed to determine if the recovery phenomenon could be demonstrated if the males were irradiated after nine days of exhaustive mating.

Stocks used were those maintained in the laboratory of H. J. Muller at Indiana University. The rod-X used was marked with \( y w \), the first ring-X experiment was conducted with an \( X^{c2} ec f \) ring-X, and the second ring-X experiment utilized an \( X^{c2} y B \) ring-X. Females in each of the experiments had X-chromosomes marked with \( y sc8 \) \( w sc8 \) ("winscy"). (For explanation of symbols see Bridges and Brehme 1944.) Crossing-over tests demonstrated that the former ring was intact; cytological observations by Oster (personal communication) proved that this was true for the \( X^{c2} y B \) chromosome also.

Newly hatched males were collected over a one-day period. In the rod-X and first ring-X experiments they were placed with twice their number of sisters until irradiated at 3\% to 4\% days after eclosion, by which age the recovery phenomenon is observed more consistently than in younger males (Nordback and Auerbach 1956). In the second ring-X experiment the newly hatched individual males were mated with five virgin females daily until irradiated on the ninth day after eclosion.

The irradiation given was 4000r of X rays from a General Electric Maximar X-ray machine at 240r/min, 100 kvp, 5 ma, and with 1 mm Aluminum and 7 mm lucite shielding.

In all experiments, the males were divided after irradiation into two equal groups. In the rod-X and first ring-X experiments males of the first group were allowed to mate within an hour after irradiation with single virgin females. A new virgin female was provided for each of these males each half-day for three days. The second group of males was kept isolated from females for one day and each of these males was mated on the second and third post-irradiation days with a different female each half day. In the second ring-X experiment, half the males were allowed to mate the first and second days after irradiation with five new females per male each day; the other males were not allowed to mate the first day and were each given five females on the second day. The males were numbered and record was kept of successful matings based on the appearance of offspring. Matings took place at 26 ± 1°C, and the transfer of males to new females was done without etherization to minimize disturbance of the males. Female offspring from these mating broods were tested for sex-linked recessive lethals by standard techniques. \( F_2 \) cultures without males bearing the treated X-chromosome and with at least 12 males bearing the untreated X, this number being obtained by retests if necessary, were classified as lethals.

RESULTS

From Figure 1a (solid line) and Table 1 it can be seen that the mutation frequency of the second day sperm was lower than that of the first day sperm when the males carrying a rod-X chromosome were mated immediately after irradiation. However, delaying the mating for one day (broken line) resulted in a mutation frequency in the first sperm from these males that was intermediate between the first and second non-delayed sperm samples.
Figure 1.—Percent X-ray induced sex-linked recessive lethals in sperm samples from *Drosophila melanogaster* males mated immediately (solid line) or delayed one day after irradiation (broken line). The dotted line represents the lethal frequencies obtained from those males which, although given females the first day, did not mate effectively, but did on the second day. Each point is presented with its standard error. Figure 1a, with rod-X chromosome; Figure 1b, with ring-X chromosome; Figure 1c, with ring-X chromosome irradiated after eight days of exhaustive mating.

A similar response is seen in Figure 1b, in which the ring-X chromosome was used. The second-day mutation frequency is again lower than that of the first day. The second-day frequency for males not allowed to mate the first day is again intermediate between those of the non-delayed sperm samples. The use of the ring chromosome here shows that these relations apply to point mutations.

The lethal frequency data presented in Figure 1 and Table 1 are, except for the special case noted, confined to results from those males which had mated
TABLE 1

Percent sex-linked recessive lethals induced in males carrying a rod- or a ring-X chromosome

<table>
<thead>
<tr>
<th>Experiment</th>
<th>0.5</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>2.5</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rod-X</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>not delayed*</td>
<td>14.0(48/343)</td>
<td>13.7(43/315)</td>
<td>11.7(21/179)</td>
<td>5.9(6/102)</td>
<td>9.0(5/54)</td>
<td>0.0(0/24)</td>
</tr>
<tr>
<td>delayed</td>
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<tr>
<td>Ring-X</td>
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<tr>
<td>not delayed</td>
<td>12.0(50/415)</td>
<td>12.9(33/256)</td>
<td>10.9(27/247)</td>
<td>7.0(15/213)</td>
<td>9.0(10/112)</td>
<td>11.0(4/36)</td>
</tr>
<tr>
<td>delayed</td>
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<td></td>
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<tr>
<td>voluntarily delayed†</td>
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<td></td>
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<tr>
<td>Ring-X with exhaustive</td>
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<tr>
<td>pre-irradiation mating</td>
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<td>(daily mating broods)</td>
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</tr>
<tr>
<td>not delayed</td>
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<tr>
<td>delayed</td>
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</tbody>
</table>

* The males were allowed to mate either immediately after X-irradiation (not delayed) or after 24 hours (delayed).
† The data marked “voluntarily delayed” are from males which, although given females the first 24 hours, did not produce offspring with them, but did on matings the second day.
‡ Absolute numbers are given in parentheses, lethals as numerators, totals as denominators.
successfully in every mating through the mating scored. It was found that the decline in mutation frequency from the first to the second day was accentuated by using this procedure, presumably by allowing more homogeneous sperm samples to be selected. The one exception to this treatment, shown by the dotted line, Figure 1b, should be noted in particular: here a low lethal frequency was found on the second day from males which had been given females immediately after irradiation, but had not successfully inseminated them until the first mating on Day 2. This low mutation frequency for males which were given the opportunity to, but did not, mate the first day may reflect the existence of differences in the maturation and use of sperm in different males, such that fewer mature sperm were available in the unsuccessful males than in those which successfully inseminated females the first day.

An unusually low lethal frequency was found the first day after irradiation when males had been exhaustively mated for eight days prior to irradiation (Figure 1c). Unlike the previous experiments, however, the second day mutation frequency was higher (although not significantly so) than that found on the first day after irradiation. Nevertheless, when similarly treated males were delayed in their mating for one day, the mutation frequency was intermediate between the other two values, as had also been observed in the other experiments.

In each experiment (Figure 1), the frequency of sex-linked recessive lethals associated with the sperm samples from males delayed one day in mating was intermediate between the first and second day frequencies of males mated immediately after irradiation. The regularity of this effect makes its validity likely, although some of the standard errors of the mutation frequencies are relatively large.

DISCUSSION

The observation that the second-day mutation frequency appears dependent upon whether the males mated immediately after irradiation is consistent with the hypothesis that sperm are at different stages of readiness for ejaculation and that with these different stages are associated differences in radiosensitivities. The further hypothesis is proposed that if males with sperm in the last day of readiness for release are not allowed to mate, sperm mixing may occur, since this would explain the intermediate mutation frequency found when mating was delayed.

In the ring-X experiment with older males (Figure 1c) the mutation frequency rose from an unusually low one on the first day to a frequency on the second day which was similar to that observed in the other two experiments in “recovered” sperm. A mating ratio of five females to one male was used per day from eclosion until irradiation in this experiment. This exhaustive pre-irradiation mating may have made a relatively pure sample of unusually insensitive sperm (possibly situated farther back in the testis, away from air) available for the first day’s mating. The higher frequency in the second day sperm from the excessively mated flies may have resulted from the presence among them of cells which were more nearly spermatids at irradiation, a phenomenon which probably caused the
rise seen on Day 3 in the experiments with younger males. Even though there was a rise from the first to second day, an intermediate frequency was observed in males delayed the first day, suggesting here a mixing of sperm otherwise used separately on the first two post-irradiation days. The observation that a rise, instead of the usual decline, may occur on the day after irradiation is exactly opposite to expectations based on the recovery hypothesis but is readily explainable through that of differential radiosensitivity and differential use of sperm.

The unusually low mutation rate observed in the ring-X experiment with younger males (Figure 1b) on the second post-irradiation day for males which even though placed with females the first day, failed to successfully inseminate them, is of especial interest. It is probable that fewer of the sperm of these males were fully mature and ready for insemination at the time of irradiation than were present in the males that mated on the first day. Hence the sperm released on the second day were presumably less mature, and less radiosensitive, at the time of irradiation. Thus the especially low mutation frequency from these sperm again fits the expectation for sensitivity differences rather than recovery.

Differential sperm sensitivity has also been used to explain the recent results reported by LeFevre and Jonsson (1963). They found that the decline in mutation frequency depended upon the rate of sperm utilization rather than (as would be expected with a recovery hypothesis) on time. This technique of comparing lethal frequencies from individual post-irradiation copulations had been used earlier by Mossige (1963), who however, gave a different interpretation to her essentially similar results.

Further evidence that recovery from irradiation-induced damage does not occur during storage of mature sperm under various conditions has been reported by Trosko (1964). The concept of mixing of sperm of different radiosensitivities has been used by Muller, Herskowitz, Abrahamson, and Oster (1954), who were concerned with cell selection after irradiation of immature sperm, rather than with the use of irradiated mature sperm under different mating conditions.

The observations reported here, although not constituting final proof in themselves, are consistent with the concept that the decline in mutation frequency from the first to the second day after irradiation reflects both a differential X-ray sensitivity of sperm at different stages, and a mixing of these sperm when matings are delayed. Thus they do not require the assumption of a recovery process.

I wish to express my great appreciation to Dr. H. J. Muller, Dr. R. R. Rinehart and Dr. H. Traut for their generous help in preparing the manuscript, and to Patricia Radcliffe for technical assistance.

**SUMMARY**

A decline in the sex-linked lethal mutation frequency from the first to the second day after X-irradiation was observed with rod- and ring-X chromosomes when males irradiated three to four days after eclosion were mated singly to females at either half-day or daily intervals. A rise in frequency was observed, however, if males had been exhaustively mated for eight days after eclosion and
were then irradiated. In all cases an intermediate mutation frequency was observed if males were not allowed to mate on the first day. On the other hand, the frequency for the second day for males which on the first day were given the opportunity to mate, but failed to do so effectively, was even lower than the second day frequency for males that mated on the first day. All these results support the concept of a differential sensitivity to X rays associated with position in the genital tract, and mixing of sperm if matings are delayed, rather than a recovery process.

LITERATURE CITED


