MUTATION FREQUENCIES DETECTED FOLLOWING IRRADIATION
OF VIRGIN AND INSEMINATED DROSOPHILA
MELANOGASTER FEMALES

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In a series of recent publications (LEFEVRE and JONSSON 1964a, b; LEFEVRE 1965a, b), emphasis has been placed on the need for adopting proper experimental procedures in order to obtain data that accurately reflect the X-ray-induced mutability of mature sperm irradiated in the male and in the female. Utilizing such procedures, we have demonstrated that the intrinsic mutability of mature sperm irradiated in the adult male is appreciably higher than had been previously believed (LEFEVRE and JONSSON 1964b; LEFEVRE 1965a). By contrast, sperm irradiated in the female exhibit a lower mutability than that obtained following the irradiation of mature sperm in the male (LEFEVRE 1965a). This result stands in contradiction to reports of ABRAHAMSON and TELFER (1954), BONNIER and LÜMING (1953), and OSTER (1958, 1959, 1961). When sperm are irradiated in the female, it is impossible to avoid the simultaneous exposure of the maternal genome; whereas, irradiation of the male or female parent and subsequent mating to a nonirradiated parent results in the exposure of only a single genome. We have suggested that interaction between irradiated male and female genomes, as when inseminated females are irradiated, results in the loss of potential X-ray-induced mutations through failure of some mutant-bearing F₁ progeny to develop. When only one parent is irradiated, of course, this type of loss does not occur.

The original experiments involved comparisons of sex-linked recessive lethal mutation frequencies induced in mature sperm irradiated in the male and in the inseminated female. If the concept of loss of potential mutants through interaction between irradiated genomes is true, then there should be an analogous reduction in the frequency of mutations detected in maternal X chromosomes following the irradiation of inseminated females as compared with the mutation frequency detected following the exposure of virgin females that are later mated to non-irradiated males.

A more recent series of experiments has been undertaken in order to compare the frequency of sex-linked recessive lethal mutations detected following the exposure to 4,000 r of virgin and inseminated females. The results show that the presence of sperm in the female at the time of irradiation significantly reduces

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the frequency of mutations subsequently detected in maternal X chromosomes contained in eggs laid within the first four days after irradiation. A similar reduction occurs when virgin females are irradiated and then mated to separately irradiated males.

**MATERIALS AND METHODS**

Two stocks provided all of the flies used in the present experiments. A mass-bred wild-type stock, M56i, originally obtained from Amherst College, was the source of the males. The second stock, which provided females for irradiation, was \( y^2 sc^f dl-49 v ur^{a} \) (yellow-2, inversion scute-8, forked, inversion delta-49, vermilion, white-apricot). All of these are sex-linked and are fully described in Bridges and Brehme (1944).

Techniques for manipulating males and females, their irradiation, and their subsequent mating have been described in detail by Lefevre (1965a). In all experiments, females were collected as virgins and aged for three days prior to their utilization in mutation studies. Three separate experiments were conducted. In Experiment I, only the maternal genome was irradiated. Three-day-old virgin females were exposed to 4,000r and, after exposure, were confined in culture vials in groups of three with an excess of nonirradiated males. They were then subcultured at two- or three-day intervals over the course of four weeks. Recessive sex-linked lethal mutation frequencies were determined separately for each subculture by individually testing, insofar as possible, all resulting F\(_1\) daughters.

In Experiment IIA, both maternal and paternal genomes were irradiated. Virgin females, after irradiation, rather than being mated to nonirradiated males, were mated once with 7-day-old virgin males that had been separately exposed to 4,000r. Following mating, the males were removed, and the mated females, in groups of five, were subcultured five times during the course of the next two weeks.

In Experiment IIB, again both maternal and paternal genomes were irradiated, but by a different procedure. Three-day-old females that had been inseminated by mating once with 7-day-old males, were irradiated, promptly after mating, with 4,000r. These females, in groups of five, were subcultured five times during the course of the next two weeks.

All irradiations were carried out with a Keleket constant-potential X-ray machine operated at 250 kv and 15 ma and provided with a 1 mm aluminum filter. At a target distance of 45 cm, the dose-rate was approximately 300 r/min. All exposures were timed to deliver 4,000r, and the exposures were monitored before and after irradiation with a calibrated Victoreen dosimeter. The females were placed in perforated size 000 gelatin capsules for exposure, which was carried out at room temperature.

All flies were raised in an insectary maintained at 25°C and 50% relative humidity.

**RESULTS**

In Table 1 are recorded the number of sex-linked recessive lethal mutations induced in maternal X chromosomes following exposure to 4,000r of 3-day-old virgin and inseminated females. The data are organized to show separately the mutation frequencies derived from subcultures made during the first four days after irradiation, from the fourth to the seventh day, and from the 7th to the 14th day. In all three experiments, the mutation frequency progressively declined after irradiation, the highest mutation frequency being observed in subcultures made during the first four days after irradiation. After the first week, however, the mutation frequencies remained relatively constant; for this reason, the data from all subcultures after the first week have been combined. Although not shown by Table 1, the fertility of irradiated females is lowest immediately following irradia-
### TABLE 1

**Frequency of sex-linked recessive lethal mutations detected at successive intervals in maternal X chromosomes following exposure of 3-day-old virgin and inseminated females to 4,000r**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Genomes irradiated</th>
<th>Subcultures</th>
<th>0-4 days</th>
<th>4-7 days</th>
<th>7-14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Maternal only (virgin ♀ ♀)</td>
<td>285</td>
<td>3,832</td>
<td>117</td>
<td>299</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.44 ± 0.42%</td>
<td>5.29 ± 0.46%</td>
<td>8,566</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.116</td>
<td>2,211</td>
<td>= 5.29 ± 0.46%</td>
<td>820</td>
</tr>
<tr>
<td>IIA</td>
<td>Maternal + paternal (virgin ♀ ♀ + ♀ ♀ separately)</td>
<td>62</td>
<td>1,116</td>
<td>38</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.56 ± 0.69%</td>
<td>4.6 ± 0.7%</td>
<td>826</td>
<td></td>
</tr>
<tr>
<td>IIB</td>
<td>Maternal + paternal (inseminated ♀ ♀)</td>
<td>155</td>
<td>3,303</td>
<td>149</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.69 ± 0.37%</td>
<td>4.21 ± 0.34%</td>
<td>5,259</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Maternal + paternal (IIA + IIB)</td>
<td>217</td>
<td>4,419</td>
<td>187</td>
<td>195</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.91 ± 0.33%</td>
<td>4.29 ± 0.31%</td>
<td>6,085</td>
<td></td>
</tr>
</tbody>
</table>

Tests of significance

- **IIA vs. IIB:** $x^2 = 1.3, P = 0.25$
- **IIA vs. IIB:** $x^2 = 0.3, P = 0.6$
- **II vs. II:** $x^2 = 21.5, P < 0.0001$
- **II vs. II:** $x^2 = 3.2, P = 0.07$

* 7-28 day subcultures.

...tion, but it shows progressive improvement during the first week. After seven days, the fertility of irradiated females approximates that of nonirradiated females of comparable age, and at the same time, the mutation frequency levels off. Mutants detected after the seventh day often occurred in clusters, but in the present experiments no effort was made to identify, by tests for allelism, the individual clusters of lethal mutations that occurred in the late subcultures. For this reason, standard errors have not been calculated for the mutation frequencies detected a week or more following irradiation.

In Experiments IIA and IIB, the paternal as well as the maternal genome was irradiated. The mutation frequencies detected at successive intervals after irradiation were quite similar in these two experiments. As shown in Table 1, the mutation frequencies are insignificantly different during both the 0 to 4 day and 4 to 7 day intervals. After the first week, however, tests of significance are difficult to apply because of the lack of information as to the independence of the mutations identified. Under the extreme assumption, which is certainly not true, that the mutations were all of independent origin, the mutation frequency of 3.9% observed in Experiment IIA during days 7 to 14 is not significantly different from the frequency of 3.10% observed during the same time interval in Experiment IIB ($x^2 = 1.3; P = 0.25$). Because the mutations were not, in fact, all of independent origin, the difference in the two mutation frequencies is of even less significance than indicated by the chi-square test. Thus, the mutation frequencies detected in Experiments IIA and IIB may be fairly added together to give values that can be compared with those obtained in Experiment I, where only the maternal genome was irradiated.
As shown in Table 1, the mutation frequency detected during the first four days after irradiation is significantly higher in Experiment I than in Experiment II (A and B combined). In the later subcultures, the mutation frequencies detected in Experiment I, though higher, are not significantly higher than those observed in Experiment II. Eggs laid most promptly after irradiation were more mature at the time of irradiation than were eggs laid later. Thus, fertilization with irradiated sperm appears to modify the detected mutation frequency of only those female germ cells that were at a relatively mature stage at the time of irradiation.

DISCUSSION

All experiments quantitatively concerned with the mutability of cells that have been exposed to radiation involve the implicit assumption that the frequency of mutations detected after irradiation is an adequate measure of the frequency of mutations initially induced in the exposed cells. In the particular case of germ cells, mutations can be detected only in those that (1) survive the exposure, (2) complete their maturation, (3) are successful in fertilization, and (4) give rise to viable, fertile offspring that can be tested for the presence of newly induced mutations. As a consequence, it is never safe to assume that the detected mutation frequency and the induced mutation frequency are the same, because the functioning gametes may not comprise an unbiased, random sample of the total population of irradiated germ cells.

In an experiment previously reported (LEFEVRE 1965a), a significant reduction in the detected mutation frequency was observed when mature sperm were irradiated in the female rather than in the male. Further data have since been accumulated following exposure to 4,000r of 7-day-old virgin males which were then mated, immediately and in sequence, to three nonirradiated females. A total of 5,035 fertile F₁ daughters produced from these matings were tested for the presence of newly induced sex-linked recessive lethal mutations, and 778 such mutations were found, a frequency of 15.45%. In a precisely parallel fashion, 7-day-old virgin males were mated individually in succession to three females which, after insemination, were irradiated with 4,000r. A total of 8,019 fertile F₁ daughters were tested for the presence of newly induced sex-linked recessive lethal mutations in the paternal X chromosome, and 947 mutations were found, a frequency of 11.81%. The difference in the two mutation frequencies is highly significant (χ² = 31.1; P = <0.00001).

Unfortunately, this experiment does not provide irrefutable evidence of an interaction between irradiated genomes, because the sperm are in two different environments at the time of their irradiation. In the male, mature sperm are stored in the seminal vesicle; in the female, they are located in the ventral receptacle and in the two spermathecae. The chemical environment, as well as the oxygen concentration, may be sufficiently different in male and female sperm storage organs to modify the induced mutation frequency. If so, the lower mutation frequency detected when sperm are irradiated in the female, rather than in the male, can not be taken as proof that interaction between irradiated genomes
eliminates some mutant-bearing progeny; the induced mutation frequency may have been lower in the female environment.

To avoid this shortcoming, the present experiments were devised in which sex-linked recessive lethal mutations induced in maternal X chromosomes were detected following the irradiation of virgin and inseminated females. In both situations, the irradiated female germ cells are located in the ovary and complete their maturation before coming into contact with sperm. The presence or absence of sperm in the female receptacle and spermathecae at the time of irradiation can hardly affect the ovarian environment; in either case, egg mutability should be the same. Whatever the true situation may be, the difficulty is completely avoided in Experiment IIA where virgin females were irradiated and later mated to separately irradiated males. As shown in Table 1, the mutation frequency detected among eggs which were relatively mature at the time of irradiation, and which were laid during the first four days after irradiation, was significantly reduced when both eggs and sperm had been irradiated, as compared with Experiment I in which only the female was irradiated.

Thus, insofar as the more mature gametes, whether male or female, are concerned, mutants are detected in reduced frequency when both genomes have been irradiated, as compared with the frequency detected when only the male or only the female has been irradiated. In the case of oocytes, at least, the initially induced mutation frequency could not have been different under the two experimental conditions of irradiation: 1. without sperm present, and 2. with sperm present in the female at the time of irradiation. The reduced mutation frequency detected following the irradiation of both maternal and paternal genomes must result from the loss of some mutant-bearing progeny as a result of interaction between the two irradiated genomes.

In all three experiments performed, the mutation frequency detected among eggs laid four to seven days after irradiation was lower than that observed among eggs laid during the first four days. Moreover, the reduction in mutation frequency when both genomes were irradiated, though noticeable, was not statistically significant as in the case of eggs laid earlier. Eggs laid during the 4 to 7 day interval were at a less advanced stage of maturation at the time of irradiation than were the eggs laid during the first four days. A still further reduction in mutation frequency was detected among eggs laid a week or more after irradiation, at which time the irradiated females showed a return to nearly normal fertility. These facts indicate that eggs laid seven or more days after irradiation with 4,000r were in the oogonial stage at the time of irradiation. Among these eggs, the detected mutation frequency was not significantly lower when both genomes were irradiated as compared with the situation in which only the female was irradiated.

Rather than reflecting an intrinsically lower mutability of oogonia than of oocytes, the progressive reduction in mutation frequencies that is observed in Experiment I may be correlated with the fact that mutants derived from irradiated oogonia are virtually free from chromosomal aberrations (including translocations, inversions, cytologically evident deficiencies, and dominant lethals);
whereas mutants derived from irradiated oocytes may be associated with chromosomal aberrations of one sort or another (Glass 1955; Valencia and Valencia 1964). Oogonial chromosomes may be relatively unbreakable; aberrant oogonia may not survive to become functional ova; or aberrant chromosomes may be selectively eliminated in the polar bodies. For whatever reason, the process of oogenesis seems to assure that chromosomal mutants will not be present in functional eggs that develop from irradiated oogonia.

A similar reduction in mutation frequency is found in Experiments IIA and IIB when irradiated oocytes are fertilized by irradiated (rather than normal) sperm; it is not found, however, when irradiated oogonia are fertilized by irradiated sperm. We suggest that the same phenomenon may be at work here as in Experiment I; that is, fertilization with irradiated sperm leads to the preferential loss of precisely those mutants that are associated with aberrant chromosomes. Thus, the detected mutation frequency is significantly reduced when irradiated sperm are used to fertilize irradiated oocytes. Chromosomal mutants, however, will not be present in the germ line following irradiation of oogonia, so that subsequent fertilization by irradiated sperm does not effect a further reduction in the frequency of detected mutations. This interpretation can be tested by a comparative cytological examination of samples of mutants obtained (a) during the first four days (or less) after irradiation of virgin females, (b) from the same subcultures following irradiation of inseminated females, and (c) from subcultures made a week or more after irradiation of virgin females.

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**SUMMARY**

Sex-linked recessive lethal mutation frequencies were detected at successive intervals after irradiation with 4,000r of 3-day-old females, which were then mated with nonirradiated males. The mutation frequencies detected in this experiment were compared with those obtained in parallel experiments in which irradiated females were mated with males that had been separately exposed to the same dose of radiation, and with those detected following the irradiation of inseminated females. The irradiation of both paternal and maternal genomes, rather than only the maternal genome, results in a highly significant reduction in the frequency of mutations detected among eggs laid during the first four days after irradiation. Among eggs laid later, a small, but insignificant, reduction in frequency of mutations is detected when both genomes are irradiated.

The suggestion is made that interaction between the two genomes, when both have been irradiated, leads to the elimination of a proportion of mutants induced in relatively mature germ cells. Since a similar loss of mutants does not occur following the irradiation of immature female germ cells, it is further suggested that mutants associated with chromosomal aberrations are preferentially eliminated by interaction of the two irradiated genomes.


