THE LACK OF EFFECT OF ETHERIZATION ON THE X-RAY MUTATION RATE IN DROSOPHILA SIMULANS

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Received January 10, 1935

The present investigation arose out of a more general study which the author undertook concerning the production of mutations in Drosophila simulans as compared with that in Drosophila melanogaster (Kossikov 1934 in press). Since for this study it was necessary to conduct X-ray mutation experiments, it seemed advantageous to increase the significance of the work by varying, in certain series, the conditions under which the treatments were given. Anesthesia by ether was decided upon as a convenient influence to use in this connection, and also one of some practical importance in the Drosophila work, since the degree of anesthetization in ordinary X-ray mutation experiments in flies may be subject to considerable variation when no particular attention is paid to this factor, and hence it is important to know whether this may constitute a source of variation in the results. According to earlier work of Hanson and Heys on Drosophila melanogaster, there did seem to be some such effect, but since we were here able to obtain further evidence on this subject, derived from a different species, it was thought of greater interest to strengthen and extend our knowledge of the phenomenon in question than to reach out in a new direction before the earlier work had had a chance to become well grounded.

MATERIAL AND METHODS

Wild-type males of Drosophila simulans (from the so-called “St. Augustine” strain) were subjected to X-rays. A part of these males were left in full narcosis during the X-ray treatment, in a special glass apparatus constructed for this purpose, while the rest, designated as “controls”, were not etherized but were X-rayed simultaneously, with the same dosage of X-rays, all other conditions being identical. A heavier and a lighter dose of X-rays was used, in different experiments, the lighter being about 1,100 and the heavier about 2,700 r, judging by the lethal rate in parallel ClB tests on Drosophila melanogaster. Imperfections in the dosimeter measurements resulted in readings about three times the actual value, but other work done at our laboratory, with another dosimeter, has agreed with previous work in showing a constant relation between the lethal rate given by the ClB method, and the actual r units, so that the former may be taken as providing a reliable method of calibration. The X-rayed males just after treatment were crossed to virgin females homozygous for the genes yellow, dusky, forked. In the second generation, recessive sex-linked
lethal mutations were registered, which could be found by the absence of non-crossover males of the wild-type in the individual cultures.

The precise registration of recessive lethal mutations in the X chromosome of *Drosophila simulans* offers some difficulties, since we have not yet got a method like the ClB method in this species of Drosophila. In order to count all the lethal mutations more exactly, however, an inversion in the X chromosome was used, which had been obtained during the preliminary part of this work, undertaken for this purpose. This inversion considerably reduces the frequency of crossing over in the region of the dusky gene.

All the cases in which there was doubt concerning the presence of a lethal were analyzed in the third generation. The flies were reared at a temperature of 25°-26°C on a yeast-containing medium, the formula of which had been worked out by C. A. Offermann (See Drosophila Information Service No. 3).

**RESULTS OBTAINED AND DISCUSSION**

Among 1,480 F₂ cultures obtained from males which were X-rayed under narcosis with an estimated dose of 1,100 r, 50 lethals were registered; this constitutes 3.4 ± 0.5 percent (the error here given being the standard error). On the other hand, out of 1,554 F₂ cultures of the control series, 47 lethals were obtained, that is, 3.0 ± 0.4 percent.

In the second series of experiments, in which the X-ray treatment was increased up to an estimated dosage of 2,700 r, 41 lethals were registered out of 384 cultures, or 10.7 ± 1.6 percent, in the narcotic series, and 56 lethals out of 479 cultures, or 11.7 ± 1.5 percent, in the control series (see table 1).

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These figures clearly show that there is no difference in the genetic effectiveness of X-ray treatment, when it is applied to non-narcotized flies and to flies that are kept under narcosis throughout the treatment.

It will be seen that the present results failed to confirm the earlier conclusions concerning the effect of etherization on X-ray mutation frequency.
in *Drosophila melanogaster*, referred to in the introduction. They agree, however, with the recent results of Hanson, investigating the same question in *Drosophila melanogaster* in a series of parallel experiments which are being published simultaneously (in press, 1934). This shows that there is no essential difference between *Drosophila simulans* and *D. melanogaster* in this respect, and the mutually confirmatory nature of our parallel sets of observations leaves no doubt about the essential correctness of the present conclusion, that is, if there is an effect of etherization, it must be a very small one, and need not ordinarily be taken into account since in the present experiments the flies were under deeper narcosis during treatment than they ever are in ordinary X-ray experiments.

In this connection it is of interest also to call to mind certain unpublished experiments of Gershenson, in which ether alone, without X-raying, was used in the attempt to produce mutations (the CIB method of detection being employed) and no significant rise in mutation frequency was found. Still earlier, Morgan (1914) had reported on the failure of ether to produce mutations, but at that time the methods of detection were not delicate enough to have given a positive result, even if the mutation frequency had been raised many times. It should be noted, moreover, as a matter of principle, that it is quite possible for an agent in itself to affect the mutation rate and still to have little or no influence in changing the mutation rate caused by another simultaneous agent, such as X-rays; in fact, the results found in the case of temperature with and without X-rays by Muller (1928, 1930) and by Timofeeff-Ressovsky (1934) are a case in point.

**LITERATURE CITED**

Hanson, F. B., 1934 Further data on the influence of physiological differences on the induced mutation rate: Anesthesia, starvation and sex. (in press)

Hanson, F. B. and Heys, F., 1933 The relation of the induced mutation rate to different physiological states in *Drosophila melanogaster*. II. Irradiation during complete anesthesia. Amer. Nat. 67: 419-428.


