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Test for sex-linked lethals in irradiated mice

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Sex-linked lethals have proved such a useful measure of overall mutation frequencies in *Drosophila*, that it seemed worth while to devise a method for detecting such lethals also in mice. The following scheme was used.

Tabby males were irradiated and mated to females heterozygous for Bent-tail and Brindled in coupling, thus:

$$\frac{Bn\ +\ Mo^{br}}{+\ +\ +}\ ♀ \times \frac{+\ Ta\ +}{\qquad}\ ♂ \text{ (Irradiated)}$$

All the daughters (F_1 ♀♀) must be heterozygous for Tabby, and any induced sex-linked mutation would be carried on the Tabby chromosome. Bent Brindled daughters were kept for testing by wild-type males, thus:

$$\frac{Bn\ +\ Mo^{br}}{+\ Ta\ +}\ (F_1\ ♀) \times \frac{+\ +\ +}{\qquad}\ ♂$$

Because Bent-tail is incompletely penetrant in females, F_1 females that were Brindled but not phenotypically Bent were tested. Twelve per cent of all tested F_1 females were proved by the test to have lost Bent-tail by crossing-over.

Any induced lethal located between the loci of *Bn* and Mo^{br} would be indicated by the absence of $+Ta+$ males among the progeny of the F_1 ♀♀. One son of this genotype was taken as diagnostic of the absence of a lethal. Because Bent-tail males have reduced viability it was not practicable to use the segregation ratios to look for evidence of lethals outside the marked segment.

Altogether 79 Tabby males were irradiated. One of these died before mating and 17 failed to produce any daughter suitable for testing. Of the remaining 61 males, the first six were given a dose of 600 r. and one was given (inadvertently) 820 r. The litters produced after 600 r. were too small and the remaining 64 males were given 500 r. The radiation was given under nembutal anaesthesia with body shielding.

The males were mated immediately after irradiation. The first 5 males were given between 2 and 5 females simultaneously and these were examined for vaginal plugs and replaced after insemination. The remaining 57 of the males that produced daughters for testing were given 4 females after irradiation. Vaginal plugs were not looked for and the females were replaced by another four after one week. These were removed after the second week. Thus the majority of males were kept for only 2 weeks after irradiation.

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The time of conception, judged as being 20 days before birth, of the tested daughters was as follows:

Days after irradiation	0–1	2–3	4–5	6–7	8–9	10–11	12–13	14–21	Total 0–21
No. of F_1 ♀♀	27	37	20	28	23	8	4	8	155

Out of the 61 males that provided daughters suitable for testing, 18 males provided only one daughter, 16 provided two, 17 provided three and 10 provided between four and seven.

In total 155 F_1 ♀♀, proved to be $Bn+Mo^{br}/+Ta+$, were tested. Of these, 154 produced at least one $+Ta+$ son and were thus proved to be free of any lethal in the Bn–Mo^{br} segment. One produced only a $+Ta\ Mo^{br}$ son and so was proved free of a lethal in the Bn–Ta segment. There were, in addition, 21 F_1 ♀♀ which did not carry Bn. All these produced at least one Ta son and so were proved free of a lethal in the Ta–Mo^{br} segment. If 0/154 are taken as the observed numbers, the upper 95% confidence limit for the mutation rate of lethals is 2·4%.

Table 1. *Segregation of* Bn, Ta *and* Mo^{br} *in the* F_2 ♂♂

Non cross-overs:	+ *Ta* +	299	411
	Bn + *Mo*^{br}	112	
Cross-overs:			
between *Bn* and *Ta*	+ + *Mo*^{br}	35	51
	Bn Ta +	16	
between *Ta* and *Mo*^{br}	+ *Ta Mo*^{br}	10	14
	Bn + +	4	
		Total	476

Table 2. *Recombination frequencies*

Segment	Recombination (%)
Bn–Ta	10·7 ± 1·44
Ta–Mo^{br}	2·9 (95% confidence limits: 1·6–4·9)
Bn–Mo^{br}	13·6 ± 1·57

One hundred and fourteen F_1 ♀♀ were proved negative by one litter. Thirty-three ♀♀ required 2 litters, six required 3 litters, one 4, and one 6 litters. In no case, however, was there a deficiency of Ta sons such as to suggest the presence of a lethal outside the Bn–Mo^{br} segment. In the few cases where more than two litters were required, this was because the females produced small litters and not because larger numbers were required before a Ta son appeared.

Though no lethal was found, one sex-linked visible was found. This was a recessive anaemia, and was subsequently proved to be located between Bn and Ta, at a distance of 3 cross-over units from Ta (Falconer & Isaacson, 1962). The F_1 ♀ which produced this visible was conceived 11 days after the irradiation of her father with 500 r.

As an incidental result, our experiment yielded new recombination data for the three sex-linked genes used (Tables 1 and 2).

DISCUSSION

At the outset of the experiment, we made a rough estimate of the frequency of lethals to be expected. The estimate was based on two sets of published data, disregarding the possibility that, as a result of natural selection, loci able to mutate to lethals might be rarer on the X-chromosome than on the autosomes (Berg, 1937; see, however, Auerbach & Moser, 1953). (1) Russell *et al.* (1951, 1958, 1959) found an average mutation frequency of 25×10^{-8}/r/locus in their study of seven specific loci in irradiated spermatogonia. In spermatozoa and spermatids, mutation frequency was about twice as high. About 75% of the mutations were recessive lethals. The published cross-over distance (Falconer, 1954; Phillips, 1954) from *Bn* to Mo^{br} was 17%. We assumed that this length of chromosome would carry at least 100 genes able to mutate to a lethal; since the X-chromosome of *Drosophila melanogaster*, with a genetical length of 70, carries at least 1000 genes and probably more, this seemed a conservative estimate. Thus, for a dose of 500 r given to spermatozoa and spermatids we expected a lethal frequency of

$$25 \times 10^{-8} \times 100 \times 2 \times 500 \times 75 \times 10^{-2} = 18 \times 10^{-3}$$

or approximately 1·8%. (2) Carter (1957*a*) based his calculations on the reduction in litter size when the sons of irradiated ♂♂ were mated to their daughters. He arrived at an estimate of 1 recessive lethal per 300 r. As in our experiments, post-meiotic stages alone were tested. Since a segment of 17 crossover units forms 1% of the whole genome of 1620 units (Carter, 1955), a dose of 500 r would be expected to result in a lethal frequency of $5/3 \times 1\% = 1{\cdot}7\%$. Thus, both calculations led to the same estimate. Our failure to find lethals in a sample of 154 is not significantly different from this value.

Meanwhile, Carter (1959) has reported on a pilot experiment in which Haldane's method for the detection of autosomal recessive lethals (Haldane, 1956; Carter, 1957*b*) was put to the test. Carter concluded from his data that the minimal X-ray dose required to induce one lethal in spermatogonia is about 800 r. Haldane (1960), on recalculating Carter's data, arrived at the lower value of 600 r. This corresponds to about 300 r for the more sensitive post-meiotic stages and is therefore compatible with Carter's previous results that have been mentioned before.

Although, therefore, neither Carter's nor our data are in disagreement with previous work, they both show that the frequency of recessive lethals is not considerably higher than the expected minimum. Our experiments were undertaken in the hope that this might be so, and Haldane, too, considered this possibility when outlining his method for the detection of autosomal lethals (1956). If, as seems to be the case, lethals are not more frequent than visibles at specific loci then, as Carter has already pointed out, the detection of lethals is not superior to that of specific visibles in efficiency as expressed by the number of animals to be raised and scored. According to Carter & Lyon (1961), however, extrapolations from specific loci experiments to the effect on the genome as a whole are not justified; if this is so, tests for recessive lethals may retain their value because of the more generalized conclusions they permit.

The basic reason why the recessive-lethal methods are so efficient in *Drosophila* and so inefficient in the mouse is, of course, the difference in chromosome number. In *Drosophila*, tests for sex-linked lethals scan one-fifth of the whole genome, tests for autosomal lethals two-fifths or four-fifths depending on whether the tests are carried out on one autosome or on both. In the mouse, the proportion of the whole genome scanned will remain small even if it becomes possible to hold the genes on one or two of the autosomes together by inversions.

SUMMARY

Tabby males were irradiated with 500 r and mated to *Bn* Mo^{br} ♀♀. Daughters conceived during the first 3 weeks after irradiation were tested for the presence of sex-linked recessive mutations in their progeny, each daughter representing one irradiated gamete. One visible, but no lethal, was found in the *Bn–*Mo^{br} segment among 154 tested gametes; one gamete was proved free of lethals or visibles in the *Bn–Ta* segment, and 21 gametes in the *Ta–*Mo^{br} segment. Among the whole 176 tested gametes there was no indication of a lethal in the adjoining segments.

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