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Tobari, I. and M. Murata. National Institute of Radiological Sciences, Chiba-shi, Japan. Fertility load and frequency of lethal second chromosome in Drosophila populations with radiation histories.

It has in general been considered that most of the radiation-induced mutations are sooner or later eliminated from a population by the acts of natural selection of the irradiation is suspended. The purpose of this study is to see a recovery of genetic damages caused by the radiation-induced mutations by estimating the amounts of fertility load and the frequency of lethal second chromosomes in the populations with radiation histories. Experimental populations of D. melanogaster used in this study were identical with those reported by Murata and Tobari (1973). Three experimental populations, B, C and D, were derived from the irradiated population which had been successively exposed to 5,000r of X-rays in every generation (Tobari and Murata 1970). The populations B, C and D have been subjected to the cumulative radiation exposures of 25,000r, 50,000r and 75,000r, respectively. These populations were maintained for 75-77 generations without X-irradiation before the present experiment was carried out. The frequency of lethal second chromosomes was estimated by the Cy/Pm technique, using about 200 males taken from each of the experimental populations. To estimate the fertility load the homozygous and heterozygous flies for wild-type second chromosomes were reconstituted. For each of approximately 100 chromosomes, in homozygous as well as heterozygous condition, 10 males and 10 females were tested. Each wild-type male (or female) was mated individually to three cn bw virgin females (or males). After one week all cultures were examined for evidence of fertility. A vial was classified as sterile (S) if there were no larvae or pupae present and the parents were alive. Cultures bearing progeny were classified as fertile (F). In some of the cultures which contained no progeny, the parent of interest was dead; this type was recorded as D.

The frequency of lethal second chromosomes in a non-irradiated (control) population was estimated to be 17.8 ± 1.9%, while it was 30.7 ± 3.7%, 32.7 ± 3.8%, and 32.5 ± 3.4%, respectively, in populations B, C and D. The difference in frequency between the control populations, A, and the experimental one is statistically significant.

The proportion of fertile cultures among the total fertile and sterile cultures was computed and these fertility ratios, F/(F+S), for males and females are given in Table 1. In all

Table 1. The mean fertility ratios, F/(F+S), in the irradiated and control populations.

Population		A: Control	B: 25KR-77G	C: 50KR-75G	D: 75KR-75G
Males:					
Heterozygotes	n	99	72	100	117
	F/(F+S)	0.934±0.016	0.957±0.012	0.868±0.021	0.881±0.023
Homozygotes	n	101	99	102	105
	F/(F+S)	0.866±0.020	0.892±0.025	0.789±0.032	0.748±0.031
Homozygotes excluding complete steriles	n	99	97	94	95
	F/(F+S)	0.884±0.016	0.916±0.019	0.856±0.023	0.856±0.024
Females:					
Heterozygotes	n	105	97	101	120
	F/(F+S)	0.969±0.008	0.969±0.008	0.941±0.011	0.961±0.007
Homozygotes	n	104	99	100	100
	F/(F+S)	0.909±0.019	0.871±0.028	0.842±0.026	0.817±0.030
Homozygotes excluding complete steriles	n	102	95	94	93
	F/(F+S)	0.926±0.014	0.907±0.022	0.896±0.015	0.896±0.021

F = fertile, S = sterile, n = number of chromosomes tested

Table 2. Total sterility load (T), partial sterility load (P) and complete sterility load (C) in the irradiated and control populations.

Population	T	P	C	P:C
Males:				
A: Control	0.0755±0.0288	0.0550±0.0250	0.0205±0.0295	2.7
B: 25KR-77G	0.0703±0.0320	0.0438±0.0253	0.0266±0.0352	1.6
C: 50KR-75G	0.0954±0.0465	0.0139±0.0361	0.0815±0.0487	0.2
D: 75KR-75G	0.1636±0.0493	0.0288±0.0282	0.1348±0.0520	0.2
Females:				
A: Control	0.0640±0.0108	0.0454±0.0177	0.0186±0.0168	2.4
B: 25KR-77G	0.1066±0.0332	0.0661±0.0256	0.0504±0.0405	1.6
C: 50KR-75G	0.1112±0.0326	0.0489±0.0205	0.0623±0.0349	0.8
D: 75KR-75G	0.1623±0.0355	0.0700±0.0234	0.0923±0.0437	0.8

the populations the fertility rates are higher for heterozygotes than for homozygotes. Furthermore, in populations C and D, which had been exposed to 50,000r and 75,000r of X-rays respectively, the fertility of both the homozygotes and the heterozygotes is lower than that in the control population. The loads have been computed from the mean fertility ratios by the same method as Temin's (1966). As seen in Table 2, the total load for males and females markedly increases as an accumulated dose of X-rays increases. In populations A and B most of the total load for males and females is due to mutant genes leading to partial sterility. On the other hand, in populations C and D the total load for males is mainly due to mutant genes causing complete sterility and for females the ratio of P:C is approximately 1:1.

The results described above indicate that some of the radiation-induced mutant genes with detrimental effects on viability or fertility are maintained for a number of generations in the populations with radiation histories, although these detrimental genes may partly be eliminated by natural selection in early generations after the irradiation is suspended.

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Traut, H. Institut für Strahlenbiologie, Universität Münster, Münster, Germany.

An approximate χ^2 test as applied to mutation experiments with D. melanogaster.

The rapidly increasing interest in the development of methods for the detection of environmental mutagens has been accompanied by an interest in the statistical procedures to be employed by mutation researchers (see e.g., Armitage 1971; Berchtold 1975; Kastenbaum and Bowman 1970; Katz 1978, 1979; Traut, in press; Würgler et al. 1975). One of those procedures is the chi-square (χ^2) test. The following approximation to that test facilitates the computation of χ^2 considerably and yields nevertheless P values almost identical with those calculated in the usual way. (Note that by the help of Patau's (1942) graphs the P values belonging to the calculated χ^2 values can be obtained.) Although this approximation has already been described (Armitage 1971), it is, as far as I know, not employed to test the significance of the difference between mutation frequencies. The approximate procedure should be applied to low relative frequencies only, amounting to at most a few percent. This requirement, however, is generally complied with in mutation experiments carried out with D. melanogaster (main types of mutations studied: recessive sex-linked lethals, autosomal translocations and X-chromosomal aneuploidy). It is true that there are cases allowing the applicaiton of the following approximate formula also to high mutation frequencies (Traut, unpublished). However, it would be difficult to consider this possibility in practice and it seems, therefore, wise to use the approximate formula only when the mutation frequencies are small. As well formulae (1) to (4) presented below as the two examples illustrating the performance of the approximate test consider Yates' correction for continuity.

The following χ^2 formula is usually applied to test the significance between two mutation frequencies x_1/n_1 and x_2/n_2 (x = number of mutated units, e.g., cells, chromosomes, loci; and n = number of units analyzed), where $x_1(x_2)$ = number of mutated units and $y_1(y_2)$ = number of non-mutated units of experiment 1 (experiment 2), and $N = x_1 + y_1 + x_2 + y_2$. For low mutation frequencies (see above), formula (1)

$$(1)\quad \chi^2 = \frac{([x_1y_2 - x_2y_1] - 0.5\ N)^2\ N}{(x_1 + x_2)(y_1 + y_2)(x_1 + y_1)(x_2 + y_2)}$$