EFFECTS OF X RAYS ON GENETIC LOADS IN A CAGE POPULATION OF *DROSOPHILA MELANOGASTER*¹

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IN assessing the hereditary effects of radiation on human populations, it is very important to know the population dynamics of mutant genes induced by radiation and their effect on population fitness. It is still controversial whether radiation-induced mutations are deleterious in the heterozygous condition, though they appear to be mostly deleterious in the homozygous condition. A pioneering experiment on this problem was conducted by WALLACE (1956; see also WALLACE and KING 1951) with *Drosophila melanogaster*. He examined the changes in frequency of lethal second chromosomes in populations, either acutely or chronically irradiated with gamma rays, and found that the frequency of lethal chromosomes increases in successive generations when the populations are chronically irradiated. Examining the viability of lethal heterozygotes, he later (1963) concluded that shortly after irradiation started, lethal genes showed a deleterious effect on the heterozygote viability but later developed an enhancing effect on viability, i.e., overdominance. CARSON (1963) also noted a transitory increase in population size and egg-to-adult viability in *Drosophila* populations which had received 1,000r of X rays per week over a period of two years. On the other hand, PROUT (1954), TORROJA (1964) and SANKARANARAYANAN (1964) reported that radiation-induced mutations are more or less deleterious in the heterozygous condition. Mouse data obtained by LÜNING and SHERIDAN (1964) indicate that radiation does not necessarily decrease the population fitness.

In view of this circumstance, a new type of experiment was initiated with *Drosophila melanogaster* in order to obtain more penetrating information on this problem. This experiment is featured in examining how the so-called *random* and *inbreeding* loads change under continuous irradiation. From the rate of increase in these genetic loads, we can estimate the heterozygous effect of mutant genes. As a special type of inbreeding load, the frequency of lethal second chromosomes has also been determined. As will be seen in the following, the results obtained show that mutations are on the average slightly deleterious in the heterozygous condition.

MATERIALS AND METHODS

The base population employed in this investigation was the same as that used in the previous studies (TOBARI 1966a,b). This base population was originally made up by intermixing 20 males and 20 females taken from each of 24 different laboratory stocks of *Drosophila melanogaster*.

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Before the present experiment was started this intermixing population was kept at constant temperature (25°C) for about 3 years (or approximately 72 generations) in a plastic population cage (40 × 30 × 13cm) provided with 15 cups containing medium. The population therefore has been considered to have reached a genetic equilibrium. The equilibrium frequency of lethal plus semilethal second chromosomes has been estimated to be 24–25%.

Two thousand virgin females and 2,000 males were randomly sampled from several culture cage-cups withdrawn from the base population. These flies were divided into two groups: one of which was used for an irradiated population and the other for a nonirradiated control population.

In the irradiated population, 1,000 males were exposed to a dose of 5,000r of X rays every generation. Immediately after irradiation they were crossed at random with 1,000 virgin females in a plastic population cage. Several culture cage-cups with eggs were withdrawn from the population on the 6th to 9th day after mating. From these cage cups 1,000 virgin females and 1,000 males were collected and used as a group of parents for the next generation. For the control population the same procedure was adopted to keep the population. Thus, both irradiated and nonirradiated populations were maintained with a total 2,000 flies in every generation.

The X irradiation was administered at a rate of 100r per min in 200 KVP, 5mmA with 0.5mm Al + 0.5mm Cu filtration.

All the populations were kept at constant temperature (25°C) throughout the experiment.

To estimate the genetic loads, in terms of random (A) and inbreeding (B) loads as proposed by MORTON, CROW and MULLER (1956), and the frequency of lethal second chromosomes, about 200 virgin females and 350 males were sampled from several culture cage-cups randomly withdrawn from each of the irradiated and the control populations on the 2nd, 5th, 10th and 15th generation, which corresponded to accumulated doses of 10, 25, 50 and 75kr, respectively.

The mating scheme for estimating the genetic loads was as follows (Figure 1): about 200 single-pair matings per population were made. In the next generation a single male was mated with two virgin females from a different single-pair mating, i.e., a male (δ_B) from a culture B was put into a vial with a female (φ_A) from A, and two days later the same male was transferred into a new vial with a new female (φ_D) from D. In the third generation after irradiation, virgin females and males were taken from each vial and four types of matings were arranged with them, that is, random, single first-cousin, half-sib and full-sib matings.

FIGURE 1.—Mating scheme for estimating the genetic loads, A and B statistics.

The egg-to-adult survival was studied in the progenies of the above four types of matings. These progenies should have inbreeding coefficients, F , equal to 0, $1/16$, $1/8$ and $1/4$, respectively. Three virgin females and 4 males were placed together in a separate vial with boiled yeast medium, allowing them to lay eggs for 12 hours, and then discarded. Thereafter, the numbers of eggs laid on food were counted under a binocular microscope. All progeny emerging from these eggs were scored.

To see the frequency of lethal second chromosomes, the remaining 150 out of 350 males were crossed individually to Cy/Pm virgin females. A single F_1 $Cy/+$ male taken from each cross was backcrossed to Cy/Pm females. In the F_2 generation $Cy/+$ females and $Cy/+$ males were intercrossed. In the next generation two types of flies, Curly and non-Curly, emerging from each cross were counted separately before the progeny could appear. When the frequency of non-Curly flies (which is expected to be 33.3% if the chromosome tested is normal) was zero, namely, when non-Curly flies did not appear, the chromosome tested was defined as one carrying a lethal gene or genes.

RESULTS

Survival from egg to adult stage in outbred and inbred progenies: The average percentages of survival from egg to adult stage with different degrees of inbreeding in the nonirradiated population are given in Table 1, together with the number of culture vials and standard error in each datum. The mean number of eggs per vial counted ranges from 74 to 110. In all generations tested except for one, generation V, the egg-to-adult survival is markedly depressed with increasing degree of inbreeding. Analysis of variance of the angular transformation of percent survival has shown that there are significant differences between inbreeding levels but the differences between generations are not significant (Table 2). Thus, all of the data were pooled for each level of inbreeding and the grand means were computed. They are 85.1%, 84.8%, 79.7% and 72.6% for $F = 0, 1/16, 1/8$ and $1/4$, respectively.

For the irradiated population the percentages of egg-to-adult survival in different degrees of inbreeding are presented in Table 3. This table also includes the number of culture vials and standard error for each datum. The grand means of the control population are also given in the table. The number of eggs per vial counted ranges from 80 to 110 on the average, with one exception of 150 at $F = 1/16$ in the 10kr dose level.

TABLE 1
The percentages of egg-to-adult survival with different degrees of inbreeding in the control population

Generation tested	$F = 0$		$F = 1/16$		$F = 1/8$		$F = 1/4$	
	n	Percent	n	Percent	n	Percent	n	Percent
II	93	85.8 ± 1.1	87	86.5 ± 1.2	98	84.0 ± 1.5	101	67.1 ± 2.4
V	106	82.5 ± 1.4	103	88.4 ± 0.8	105	80.4 ± 1.3	107	76.2 ± 1.2
X	103	87.9 ± 1.1	103	86.0 ± 1.1	105	80.6 ± 1.4	105	72.1 ± 1.6
XII	105	83.4 ± 1.4	103	82.3 ± 1.3	106	79.4 ± 1.3	109	73.0 ± 1.7
XIV	110	84.6 ± 1.3	105	83.5 ± 1.4	108	76.9 ± 1.6	105	76.5 ± 1.4
XV	104	86.8 ± 0.9	95	82.2 ± 1.2	105	77.6 ± 1.3	106	70.2 ± 1.6

n: Number of culture vials tested.

TABLE 2

Analysis of variance of egg-to-adult survivals in the control population

Source of variation	SS	df	MS	F
Between inbreedings	311.13	3	103.71	22.69**
Between generations	17.05	5	3.41	
Error	68.60	15	4.57	
Total	396.78	23		

** Significant at the 1% level.

TABLE 3

The percentages of egg-to-adult survival with different degrees of inbreeding in the irradiated population

Accumulated dose (r)	F = 0		F = 1/16		F = 1/8		F = 1/4	
	n	Percent	n	Percent	n	Percent	n	Percent
Control (0)	621	85.1 ± 0.5	596	84.8 ± 0.5	627	79.7 ± 0.6	633	72.6 ± 0.7
10,000	81	70.9 ± 2.5	63	59.6 ± 3.2	66	62.5 ± 2.8	73	51.9 ± 2.7
25,000	102	66.7 ± 1.7	100	62.8 ± 2.1	101	59.1 ± 2.0	106	45.1 ± 1.8
50,000	103	62.6 ± 2.5	93	55.5 ± 2.6	89	52.1 ± 2.1	97	37.3 ± 2.1
75,000	99	59.5 ± 2.1	90	53.4 ± 2.2	92	49.1 ± 2.1	103	29.6 ± 1.7

n: Number of culture vials tested.

TABLE 4

Analysis of variance of egg-to-adult survivals in the irradiated population

Source of variation	SS	df	MS	F
Between inbreedings	957.79	3	319.26	105.36**
Between doses of X rays	415.27	4	103.82	34.36**
Error	36.33	12	3.03	
Total	1409.39	19		

** Significant at the 1% level.

In all the levels of inbreeding the egg-to-adult survival is consistently decreased with increasing amount of the accumulated dose of X rays. The effects of radiation and inbreeding levels on survival are both significant in a statistical sense (Table 4). Furthermore, a greater viability depression due to X irradiation can be found at a higher level of inbreeding.

The random load (A) and inbreeding load (B): The basic principle to estimate the genetic loads is that proposed by MORTON, CROW and MULLER (1956). They have suggested that $-\log_e$ (survival fraction) is a linear function of the inbreeding coefficient, F, if there is no interaction between genes at different loci, in the sense that their effects on survival are multiplicative. The random load, A, is estimated by calculating the intersect at $F=0$ and the inbreeding load, B, is

estimated by the regression coefficient of the linear regression equation of $-\log_e(S) = A + BF$, where S stands for the proportion of survivors. Both A and B value were calculated in practice by the method of weighted regression analysis proposed by MALOGOLOWKIN *et al.* (1964) with the data in Table 3.

The magnitudes of the A and B are summarized in Table 5, together with the B/A ratio and graphically presented in Figure 2. The random load, A , is significantly increased by X irradiation. It first increased rapidly and then gradually approached the equilibrium value. The approach to the equilibrium is rather rapid, and this strongly suggests that the radiation-induced mutations are deleterious in the heterozygous condition. If they were completely recessive or overdominant, the random load would increase almost linearly in early generations such as we examined. It is noted that the A -value in the control population is nearly the same as that obtained by others (DOBZHANSKY, SPASSKY and TIDWELL 1963; STONE, WILSON and GERSTENBERG 1963; TORROJA 1964; MALOGOLOWKIN *et al.* 1964; SANKARANARAYANAN 1964; METTLER, MOYER and KOJIMA 1966).

On the other hand, the inbreeding load, B , is strongly affected by radiation and increased with an increase in dose, although the relation is not linear; the rate of increase in B -value per kr tends to decrease as the dose increases. That is, it is approximately 0.054, 0.021, 0.020 and 0.014 for 0–10kr, 10–25kr, 25–50kr and 50–75kr, respectively. These rates of increase again suggest that the newly arisen mutations are deleterious in the heterozygous condition.

The B/A ratio in the control population is estimated to be 4.3, while in the irradiated population it varies from 3.2 to 4.7. No difference in B/A ratio can be found between the control and irradiated populations.

Frequency of lethal second chromosomes: The frequencies of second chromosomes carrying lethals are given in Table 6 and graphically in Figure 2. In the control population the frequency ranges from 17.3% to 19.4% with a mean of $17.8 \pm 1.85\%$. This figure is well in agreement with that obtained in the previous studies (TOBARI 1966a,b), where the frequency of lethals has been estimated to be 15.5–17.0%.

Figure 2 shows that X irradiation brings about a fairly rapid increase in the frequency of lethal chromosomes. However, the relationship between the frequency and the accumulated dose is obviously curvilinear similar to that for

TABLE 5

Estimates of A and B statistics, and B/A ratios

Accumulated dose (r)	A	B	B/A
Control (0)	0.1469 ± 0.0047	0.6269 ± 0.0420	4.27
10,000	0.3601 ± 0.0307	1.1680 ± 0.2437	3.24
25,000	0.3859 ± 0.0214	1.4838 ± 0.1820	3.85
50,000	0.4517 ± 0.0322	1.9795 ± 0.2629	4.38
75,000	0.4928 ± 0.0298	2.3305 ± 0.2584	4.73

FIGURE 2.—Changes in random (A) and inbreeding (B) loads, and in the frequency of lethal second chromosomes in irradiated population.

TABLE 6

Frequencies of lethal second chromosomes in the irradiated and nonirradiated populations

Irradiation			Control	
Accumulated dose of X rays (r)	Number of chromosomes tested	% lethals	Number of chromosomes tested	% lethals
10,000	101	33.7 ± 4.70	104	17.3 ± 3.71
25,000	97	50.5 ± 5.07	101	16.8 ± 3.72
50,000	99	66.7 ± 4.74	103	19.4 ± 3.90
75,000	90	71.1 ± 4.78	119	17.7 ± 3.50
			Mean = 17.8 ± 1.85	

genetic loads as shown in the same figure, and also to the result obtained in the WALLACE's (1956) populations 5 and 6.

DISCUSSION

It has been well known that the egg-to-adult survival is strongly affected by the degree of larval crowding in cultures. To see the effect of larval crowding on survival, the correlation coefficient between the percent survival and the number of eggs per culture vial counted was computed using the original data for the control population. The correlation coefficients at $F = 0, 1/16, 1/8$ and $1/4$ were, respectively, 0.062, 0.020, 0.035 and 0.056, none of which was significantly different from zero. This result indicates that the effect of larval crowding on egg-to-adult survival can be ignored in the present case. For the irradiated population the correlations between the percent survival and the number of eggs per vial were found not to be significant in all cases. With 10kr irradiation, however, the percentage of survival was higher at $F = 1/16$ than $F = 1/8$. That might result from the fact that at $F = 1/16$ with 10kr the number of eggs per vial, about 150, was greater than those in the other cases.

It is of interest to compare the B-value in the control population with those reported by others. The most extensive data are those by SANKARANARAYANAN (1964) with *D. melanogaster*. In his control population, C-1, the B-value has been estimated to be 0.1810 ± 0.015 , while it is 0.6269 ± 0.042 in our population. The difference between the two values is clearly significant. One of the causes of this difference may be considered to be the different way of maintaining the experimental population. The population was kept to a total 2,000 flies in a plastic cage ($40 \times 30 \times 13$ cm) in our case, while to a total 600–850 flies in 10–15 culture bottles in his case. On the other hand, the B-value in this study is quite comparable to that in the experiment of DOBZHANSKY, SPASSKY and TIDWELL (1963), in which the range of the B-value was 0.74 to 0.47 with a mean of 0.66. Furthermore, the B/A ratio obtained in our study is also nearly the same as that in this study, i.e., 4.27 and 4.41, respectively.

The present study has shown that irradiation with X rays greatly increases the genetic loads, especially the inbreeding load, B. This is supported by the studies of TORROJA (1964) and SANKARANARAYANAN (1964). TORROJA's experiment with *D. pseudoobscura* has shown that the A- and B-values are both significantly higher in the population two generations after irradiation with 16,000r than in the nonirradiated population. However, in his irradiated population the A-value, 1.157 ± 0.076 , is almost twice as large as the B-value, 0.609 ± 0.434 , while in our study the former is always much smaller than the latter at all levels of accumulated doses tested. This discrepancy may be caused by extremely low percentage of egg-to-adult survival at $F = 0$ in TORROJA's "R" population relative to that in our irradiated population. In the experiment by SANKARANARAYANAN of chronically irradiated *D. melanogaster*, the egg-to-adult viability dropped sharply in the very first generation, but a few generations after the radiation was discontinued, this viability returned rapidly to almost the control level.

MORTON, CROW and MULLER (1956) gave theoretical reasons for utilizing the face value of the B/A ratio to discriminate between genetic loads of mutational and balanced kinds. On the basis of their theory, the face value of B/A obtained in our control population favors the conclusion of predominant mutational loads. However, it is quite dubious to utilize the B/A ratio for discriminating between two kinds of genetic loads since there are several objections, such as synergistic interaction between loci concerned, non-genetic deaths, small effective size of population, and maternal and/or paternal effect on egg hatchability (LEVENE 1963; DOBZHANSKY *et al.* 1963; MALOGOLOWKIN *et al.* 1964; METTLER, MOYER and KOJIMA 1966).

As mentioned previously, the rate of increase in genetic loads and lethal chromosome frequency all suggest that the radiation-induced mutations are deleterious in the heterozygous condition. Let us now estimate the amount of reduction in fitness of the mutant heterozygotes. Let the initial gene frequency at $t = 0$ be q_0 , and assume that the mutation rate is increased by radiation by K times the spontaneous rate, u , the frequency of the mutant genes in the first generation after irradiation is given by $q_1 = q_0 (1 - h) + Ku$ if $h \gg \sqrt{su}$, where h stands for the degree of deleterious effects of the mutant genes in the heterozygous condition. If the population is successively irradiated for t generations the frequency becomes

$$\begin{aligned} q_t &= q_0 + q_0(K - 1) [1 - (1 - h)^t] \\ &\approx q_0 + q_0(K - 1)(1 - e^{-ht}) \end{aligned} \quad (1)$$

However, we must take into account the further selection which was directed against the mutant genes during the process of the genetic tests which were performed after X irradiation was discontinued. Such a selection is expected to have taken place for three generations in the test of genetic loads and for one generation in the lethal test. Taking account of this situation, the above equation (1) can be modified as follows:

$$q_t = q_0 + q_0(K - 1)(1 - e^{-ht})e^{-3h} \quad (2)$$

for the frequency of the mutant genes which contribute the genetic loads, and

$$q_t = q_0 + q_0(K - 1)(1 - e^{-ht})e^{-h} \quad (3)$$

for the frequency of lethal genes.

According to the theoretical analysis of MORTON, CROW and MULLER (1956), the random load, A , is expressed by $A = \Sigma x + \Sigma q^2s + 2 \Sigma q(1 - q)h$, where s refers to the selection coefficient of the homozygotes for the detrimental gene, and the summation is the over all loci having the genes in question. If $\Sigma x = 0$, this quantity can be approximated to $A = 2 \Sigma qh$, neglecting the second order terms in q . Then, the random load in the t th generation in the irradiated population is given by

$$A_t = 2 \Sigma q_t h \quad (4)$$

If we substitute from (2) into (4), assuming that h is the same for all loci we have

$$A_t = A_0 + A_0(K - 1)(1 - e^{-ht})e^{-3h} \quad (5)$$

where $A_0 = 2 \Sigma q_0 h$ (0.1469 in the present case). This equation is now applicable to estimate h using the data given in Table 5. Calculating the ratio (R) of

$(A_t - A_0)$ to $(A_{t'} - A_0)$ which is expressed as $R = (A_t - A_0)/(A_{t'} - A_0) = (1 - e^{-ht})/(1 - e^{-ht'})$, we estimated h yielding the closest value to the calculated ratio, R . For example, $(A_5 - A_0)/(A_{10} - A_0) = (1 - e^{-5h})/(1 - e^{-10h}) = 0.7841$ and then h is estimated to be 0.258. The average value of h is estimated to be 0.296 ± 0.109 . Inserting this h value in equation (5), K is estimated to be 7.03. Thus, the equation showing the relationship between random load, A , and generation, t , can be given by

$$A_t = 0.147 + 0.365(1 - e^{-0.296t}) \quad (6)$$

The theoretical curve expected by the above equation is presented in Figure 3. It is obvious that the observations are well fitted to the curve and the approach to the equilibrium value of A is rather rapid. The equilibrium value of A is estimated to be 0.512, which is about 3.5 times the A value in the nonirradiated population.

The inbreeding load, B , is expressed by $B = \sum q_s - \sum q^2s - 2 \sum q(1 - q)h$. This quantity can be approximated to $B = \sum q_s - 2 \sum qh$, neglecting the second order terms in q . Then, the inbreeding load in the t th generation in the irradiated population is given by

$$B_t = \sum q_t s - 2 \sum q_t h \quad (7)$$

If we substitute from (2) into (7), assuming that h is the same for all loci we have

$$B_t = B_0 + B_0(K - 1)(1 - e^{-ht})e^{-3h} \quad (8)$$

where $B_0 = \sum q_0 s - 2 \sum q_0 h$ (0.6269 in the present case). Using the data given in Table 5, the value of h was estimated by the same way as that in the random

FIGURE 3.—Expected changes in the random load, A , and the inbreeding load, B . The curvilinear lines represent the theoretical curves by the equations, $A_t = 0.147 + 0.365(1 - e^{-0.296t})$ and $B_t = 0.627 + 1.937(1 - e^{-0.126t})$. $\circ$: Observations.

load, A. The value of h was estimated to be 0.126 ± 0.035 on the average. Inserting this h value into equation (8), K is estimated to be 5.49. Thus, the equation showing the relation of inbreeding load, B , to generation, t , can be given by

$$B_t = 0.627 + 1.937(1 - e^{-1.26t}) \quad (9)$$

Figure 3 shows that the observations are well fitted to the theoretical curve expected by equation (9) and that the inbreeding load reaches equilibrium. The equilibrium value of B is estimated to be 1.56, this being about 4 times the magnitude of inbreeding load in the control population. Comparing the h value estimated in random load with that in inbreeding load, the former is extremely higher than the latter. The high estimate of h in random load might probably reflect that many, or most of the mutations which contributed to the random load were chromosome aberrations.

In this study as well as in the other investigations we can determine not the frequencies of individual lethal genes, but the frequency of chromosomes carrying a lethal gene or genes in a population. If we make the transformation $Q_1 = -\log_e(1 - Q)$, where Q is the frequency of lethal-bearing chromosomes, then Q_1 becomes a sum of gene frequency, q , at the individual loci. Therefore, by using equation (3), Q_1 in the t th generation in the irradiated population is given by

$$Q_{1(t)} = Q_{1(0)} + Q_{1(0)}(K - 1)(1 - e^{-ht})e^{-h} \quad (10)$$

This equation was fitted to the experimental data presented in Table 6 and we estimated the h value by means of the same procedure as described before. The average value of h is 0.073 ± 0.015 . Inserting this value into equation (10), K is estimated to be 9.9. Thus, the equation showing the relationship between the frequency of lethal genes and the generation during which the population has been irradiated is given by

$$Q_{1(t)} = 0.198 + 1.638(1 - e^{-0.073t}) \quad (11)$$

In this case, the observations are well fitted to the curve expected by equation (11), as shown in Figure 4. The equilibrium frequency of lethal genes is estimated to be 1.83, which corresponds to 84% lethal-bearing chromosomes.

WALLACE (1956) performed a most penetrating study on the changes in lethal chromosome frequency in irradiated populations of *D. melanogaster* kept in laboratory population cages. His experiment has shown that the frequency of lethal second chromosomes was logarithmically increased in Populations 5 and 6, which were irradiated with 5r/hr (or approximately 2,000r/generation) of gamma rays in every generation. The equilibrium frequency of lethal chromosomes was estimated to be 85.7% in Population 5 and 86.5% in Population 6 (PROUT 1954). According to methods devised by WRIGHT (DOBZHANSKY and WRIGHT 1941; WRIGHT, DOBZHANSKY and HOVANITZ 1942), PROUT (1954) estimated h to be 0.022 and 0.017 for Populations 5 and 6, respectively. Recently NEI (1969) developed some mathematical formulas applicable to the experimental data on the changes in frequency of lethal genes and discussed their heterozygous effects. His estimate of h was 0.021 for Population 5 and 0.023 for Population 6, being close to PROUT's estimates mentioned above. These estimates are of the same order as those obtained for lethal genes in various natural populations

FIGURE 4.—Expected change in the frequency of lethal second chromosomes. The curvilinear line represents the theoretical curve expected by $Q_{1(t)} = 0.198 + 1.638(1 - e^{-0.073t})$. ○: Observations.

of *Drosophila* (CROW and TEMIN 1964). Moreover, NEI used his mathematical formulas to analyze DYER's (1966) experimental data on the accumulation of lethal genes in extremely small populations of *D. melanogaster* irradiated with an acute dose of 1,500 rad of X rays, and he estimated h to be 0.058.

The estimates of h obtained for our irradiated population and for DYER's are higher than the estimated h 's for WALLACE's irradiated populations. This suggests that there might be differences between lethals in the chronically irradiated population and in the acutely irradiated one; that is, the high estimate of h might probably reflect that the rate of induction of chromosome aberrations such as translocations was higher in the acutely irradiated population than in the chronically irradiated one. In any case, we may conclude that the lethal genes induced by radiation are on the average slightly deleterious in the heterozygous condition.

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SUMMARY

The random load, A , and the inbreeding load, B , proposed by MORTON, CROW

and MULLER (1956), and the frequency of lethal second chromosomes in a population of *D. melanogaster*, which was irradiated with a dose of 5,000r of X rays in every generation, were examined. The random load, A, was significantly increased by irradiation. It first increased rapidly and then gradually approached the equilibrium value, which was estimated to be 0.512. The inbreeding load, B, was strongly affected by radiation and increased with an increase in dose, although the rate of increase in B-value tended to decrease as the dose increased. According to the theoretical analysis of the data, the equilibrium value of B was estimated to be 1.56, being about four times the magnitude of the inbreeding load in the nonirradiated population. The heterozygous effects of radiation-induced mutations, which contributed to the random load and inbreeding load, are also estimated to be 0.296 and 0.126. It was clearly shown that X irradiation brought about a fairly rapid increase in the frequency of lethal second chromosomes. However, the relationship between the frequency and the accumulated dose of X rays was obviously curvilinear similar to that for genetic loads. On the basis of the theoretical analysis of the data, the heterozygous effect of lethal genes induced by radiation was estimated to be 0.073. The results obtained in this study indicate that most of the mutations induced by ionizing radiation are deleterious on the average in the heterozygous condition.

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