SOME COMPONENTS OF THE GENETIC LOADS IN IRRADIATED EXPERIMENTAL POPULATIONS OF *DROSOPHILA MELANOGASTER*¹

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POPULATIONS of sexual, diploid, and outbreeding organisms contain concealed genetic loads, which can be greatly increased by radiation exposures. The influence of radiation-induced genetic loads on fitness has been studied in *Drosophila* (WALLACE 1956, 1958; STONE, WHEELER and WILSON 1962; CARSON 1964), swine (COX 1964), mice (GOWEN and STADLER 1964; SEARLE 1964; SPALDING, BROOKS and McWILLIAMS 1964), in *Tribolium* (CRENSHAW 1965) and other organisms. SANKARANARAYANAN (1964, 1965) has examined the effects of X-irradiation on the egg-adult viability in experimental populations of *Drosophila melanogaster* maintained under conditions designed to minimize the natural selection due to larval competition and crowding. Populations in which the males were irradiated generation after generation showed a decline in the egg-adult viability for the first four or five generations, after which equilibria were reached, the levels of which depended on the amount and the history of the radiation exposure. Subpopulations derived from such "chronically irradiated" populations, and then propagated without further irradiation recovered rapidly. Most of them reached 90 to 95% of the egg-adult viability of the Control population in three to seven generations, while others recovered less fully. The present report summarizes the data on the egg-adult viability in three populations which showed incomplete recovery until the time of when the experiments were concluded in July 1965.

MATERIALS AND METHODS

The characteristics of the populations utilized in the present experiments are summarized in Table 1. The populations were maintained in half-pint cultures with cream-of-wheat molasses medium. To minimize the larval competition, the females were allowed to oviposit in 6-inch glass vials with paper spoons containing the cornmeal-molasses-agar medium darkened with charcoal. After 4 to 8 hours, a total of 900 eggs were removed from the spoons and put into 15 culture bottles, 60 per bottle. These numbers of eggs are sufficient to yield between 40 and 60 adults per bottle. The progenies from the bottles were thoroughly mixed in each generation. The total population size was consequently between 600 and 750 adults.

Estimation of the frequencies of lethal and semilethal second chromosomes in the experimental populations was made using the *Cy L/Pm* technique in the earlier generations or the *Cy L* technique in later generations, as described by WALLACE (1956).

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TABLE 1
Description of the experimental populations

Population	Treatment	Start	End
C-1	Control—No radiation	Sept 1962	July 1965
VI-ER-20	Males received 6000r/generation for 20 generations; no radiation later	July 1963	July 1965
IV-ER-30	Males received 4000r/generation for 30 generations; no radiation later	Dec 1963	July 1965
II-EGR-60	Males received 2000r/generation for 60 generations; no radiation later	June 1964	July 1965

Allelism tests of lethal-bearing second chromosomes were carried out for the Control population in a sample taken in the 58th generation, and for population VI-ER-20 taken at generations 19 and 45 after the relaxation of the irradiation. Inter-generation allelism was also determined for population VI-ER-20 by intercrossing the lethal chromosomes of the 19th generation with those of the 45th. The experimental procedure was as follows. Lethal second chromosomes were extracted at the required generation and were maintained as balanced lethal stocks, using the *Cy L* marker chromosomes. Virgin females from one balanced lethal stock were crossed with males from another and absence of wild-type flies among the progeny of this cross was taken as evidence that the lethals involved were alleles. For a series of lethal chromosomes all possible crosses were made i.e., for N lethal chromosomes, the possible number of crosses is $N(N-1)/2$. The crosses were made in creamers, using three virgin females and three males for each cross. Reciprocal crosses were not made.

RESULTS

Egg to adult viability: Figure 1 shows the regression of the egg-adult viability data in the experimental populations. The analysis of this regression includes the contemporaneous generations 29 to 83 in the Control (C1), 5 to 59 in VI-ER-20, 5 to 49 in IV-ER-30, and 5 to 36 in II-EGR-60. In the populations VI-ER-20,

FIGURE 1.—The egg-to-adult viability in the control population (triangles), VI-ER-20 (squares), IV-ER-30 (black circles), and II-EGR-60 (open circles).

IV-ER-30 and II-EGR-60 generations 0 to 4 have been excluded and not shown in Figure 1, since it is during this period that the viabilities increased to near plateau levels of 70 to 75% (FIGURE 1, SANKARANARAYANAN 1965). In VI-ER-20, IV-ER-30 and II-EGR-60, generation 0 is the last generation in which radiation was administered and consequently, generation 1 is the first generation after the cessation of the irradiation. The regression estimates are as follows:

$$\begin{aligned}\text{C-1: } Y &= 89.6 - 0.018X; \sigma_b = 0.012 \\ \text{VI-ER-20: } Y &= 76.6 - 0.040X; \sigma_b = 0.017 \\ \text{IV-ER-30: } Y &= 69.0 + 0.103X; \sigma_b = 0.033 \\ \text{II-EGR-60: } Y &= 71.5 - 0.003X; \sigma_b = 0.030\end{aligned}$$

The regression coefficients of the irradiated populations can be compared with those of the Control using the Student's *t* test. The results are:

$$\begin{aligned}\text{C-1 and VI-ER-20: } t &= 1.08, \text{ d.f. } 106, P \text{ between } 0.20 \text{ and } 0.30 \\ \text{C-1 and IV-ER-30: } t &= 3.90, \text{ d.f. } 96, P < 0.001 \\ \text{C-1 and II-EGR-60: } t &= 0.49, \text{ d.f. } 83, P \text{ between } 0.60 \text{ and } 0.70\end{aligned}$$

Only in the population IV-ER-30 has there been a significant improvement of the egg-to-adult viability during the period covered by the observations, and this seemed to occur chiefly between the 16th and the 19th generations.

Lethal and semilethal second chromosomes: Table 2 reports the frequencies of lethal and semilethal second chromosomes observed in the populations, with lethals and semilethals defined as in SANKARANARAYANAN (1965). It should be pointed out that the lethal frequency in the Control, 11.6%, is low in comparison with the author's previous finding (1965) of 17.8% in generations 14 and 20, and with findings of several other investigators on populations also derived from WALLACE's population 3 (see for example SPIESS and ALLEN [1961]; GREENBERG and CROW [1960]). In addition, the value is about one half of that ordinarily found for natural populations of *D. melanogaster*. This is probably due to the possible reduction in population number in these experiments, and the approach to some new equilibrium.

TABLE 2

*Frequencies of lethal and semilethal second chromosomes in the experimental populations.
The irradiation was discontinued in the generation marked 0*

Population	Generation	Chromosomes tested	Lethal	Semilethal	Percent lethal and semilethal
C-1 (Control)	58	439	51	..	11.6 $\pm$ 1.5
VI-ER-20	0	108	90	5	87.9 $\pm$ 3.1
	19	145	110	..	75.9 $\pm$ 3.6
	45	142	56	1	40.1 $\pm$ 4.1
IV-ER-30	0	103	87	6	90.3 $\pm$ 2.9
	19	112	67	..	59.8 $\pm$ 4.6
	45	137	63	..	46.0 $\pm$ 4.3
II-EGR-60	0	107	61	..	57.0 $\pm$ 4.8
	19	110	44	1	40.9 $\pm$ 4.7

The populations which received 120,000r during 20 and during 30 generations (VI-ER-20 and IV-ER-30) had, at the time the radiation was relaxed, about 90% of the second chromosomes lethal or semilethal in double dose. The population II-EGR-60 which received the same total amount but in 60 generations, had significantly fewer lethals. This is consistent with the view that many radiation-induced lethals are deleterious in the heterozygotes, and that in the population receiving less radiation per generation, some of the lethals were being eliminated even before the radiation was relaxed, while new lethals were arising through radiation-induced mutation.

After the radiation is relaxed the frequencies of lethal and semilethal chromosomes repaidly decrease in all populations with radiation histories as Table 2 clearly shows. It is, however, equally clear that the decline in the frequencies of the lethal and semilethal chromosomees has not reached the Control level, $11.6 \pm 1.5\%$. In VI-ER-20 and IV-ER-30, the drop was in 45 generations from 88 or 90% to 40.1 ± 4.1 and $46.0 \pm 4.3\%$ respectively. In II-EGR-60 it was from 57 to 40.9% in 19 generations.

Estimates of the average number of lethal genes per chromosome: The data in Table 2 make it possible to compute the average number of lethal genes per chromosome on the assumption that the lethal genes have a Poisson distribution on the chromosomes. This is given by the formula $n\bar{q} = -\ln(1-Q)$ Where Q is the frequency of lethal chromosomes, n the number of loci on the second chromosome which regularly mutate to recessive lethal alleles and $\bar{q}$ the mean frequency of lethal alleles in the population per lethal producing locus. Such estimates are given in Table 3. Populations VI-ER-20 and IV-ER-30 at generation 0 had, respectively, 1.79 and 1.86 lethal genes per chromosome, while population II-EGR-60 had only 0.85 lethal genes per chromosome. These frequencies are much larger than that in the Control, 0.12. After the irradiation was discontinued, the frequencies of lethal chromosomes as well as the average number of lethal genes per chromosome decreased. It is to be expected that those lethals with the greatest deleterious effects as heterozygotes will be eliminated by natural selection most rapidly; persisting longer will be those with mild or neutral effects and those that

TABLE 3

Calculated mean number of lethal genes per chromosome in the experimental populations

Population	Generation	$n\bar{q}$
C-1 (Control)	58	0.12
VI-ER-20	0	1.79
	19	1.42
	45	0.50
IV-ER-30	0	1.86
	19	0.91
	45	0.62
II-EGR-60	0	0.85
	19	0.51

TABLE 4

Allelism frequency in the experimental populations, showing the generations from which lethal chromosomes were sampled, the number of the homozygous lethal chromosomes in the sample, the number of crosses made among these chromosomes, and the numbers of lethals found to be represented once or more times in the sample

Population	Genera- tion	No. homo- lethal chromo- somes	No. of crosses	Times represented in sample											Total alleles	Percent alleles
				1	2	3	4	5	6	7	8	9	10	>10		
C-1 (Control)	58	50	1225	21	3	1	2		2						48	3.9 ± 0.55
VI-ER-20	19	82	3321	18	6	4		2		1			2	2	344	10.4 ± 0.53
													(13,16)			
VI-ER-20	45	50	1225	13	4		1		1			1	1	(15)	173	14.1 ± 0.99

are heterotic. The decrease in lethal frequencies might also be based in part on the same process that has probably occurred in the Control: reduction in effective population size.

Allelism of the lethals on the second chromosome: Some insight into the nature of the lethals retained in the populations under the relaxed natural selection may be obtained from allelism tests between lethal second chromosomes extracted from the experimental populations. Such tests have been made for the Control population at generation 58, and for population VI-ER-20 at generations 19 and 45. These tests were modelled after similar tests by WALLACE (1950), PROUT (1954), OSHIMA (1962) and BAND and IVES (1963). The results are presented in Table 4. The time when the samples were taken (in generations from the start of the populations), the numbers of the chromosomes sampled, and the numbers of crosses made are shown. Also shown are the numbers of lethals represented once, twice, three times, etc., among the lethal chromosomes sampled. Finally, the total numbers of the alleles observed and the frequencies of allelism are reported. In the column headed "1" are shown the numbers of lethal chromosomes which were found to be allelic to no other lethal chromosomes in the sample.

It can be seen that the frequency of allelism among lethals is, among our populations, the lowest in Control, being $3.9 \pm 0.55\%$. This is, however, a higher frequency than found by PROUT (1954) in the population No. 3 of WALLACE, which served as Control in the experiments of the latter author. The environment was, however, more stringent, and the population size greater in the experiments of WALLACE than in the present ones. In the population VI-ER-20 in the generation 19, the frequency of allelism was $10.4 \pm 0.5\%$, and several lethals were found to be common, two of them being represented in 13 and 16 chromosomes respectively (Table 4). In generation 45, the allelism frequency was increased farther— $14.1 \pm 0.99\%$. This increase has occurred despite the fact that the number of lethal genes per lethal chromosome has declined considerably, as shown in Table 3. It seems that the elimination of the lethals did not occur entirely at random; some lethals were more likely to be eliminated than others.

Further light on the situation comes from the data on the frequency of alleles

TABLE 5

*The estimated frequencies of lethal alleles in the generations 19 and 45
in the population VI-ER-20*

Allele	Frequency	
	Generation 19	Generation 45
x_1	0.1480	0.0079
x_2	0.0093	0.0473
x_3	0.1203	0.1183
x_4	0.0278	0.0158
x_5	0.0093	0.0079
x_6	0.0278	0.0158
x_7	0.0463	0.0079
x_8	0.0185	0.0710
x_9	0.0093	0.0079
x_{10}	0.0093	0.0079
x_{11}	0.0463	
x_{12}	0.0926	
x_{13}	0.0926	
x_{14}	0.0648	
x_{15}		0.0316
x_{16}		0.0158
x_{17}		0.0158

between the lethals taken in the 19th and the 45th generations. A total of 4100 intercrosses (82 lethals from the 19th $\times$ 50 lethals from the 45th generation) gave 254, or $6.2 \pm 0.37\%$. The question that presents itself is whether the lethals which were frequent in the 19th generation persisted until the 45th generation, and if so, with what frequencies.

In all, ten lethal loci were found to be represented in the samples taken both in the 19th and in the 45th generations. Estimates of their gametic frequencies in these populations are given in Table 5. It can be seen that the frequencies of some of the lethals have remained fairly constant (x_3 , x_4 , x_5 , x_6 , x_9 and x_{10}) during the time interval between these generations. Two lethals (x_2 and x_8) have shown approximately threefold increases; two others (x_1 and x_7) have decreased in frequency; four lethals (x_{11} , x_{12} , x_{13} and x_{14}) were found in the earlier but not in the later sample; and three lethals (x_{15} , x_{16} and x_{17}) were found in the later but not in the earlier sample.

It can be plausibly assumed that almost every lethal present in our population appeared by mutation originally in a single chromosome. Why are some lethals lost and some preserved and multiplied? Some eliminations are a matter of chance; the deleterious effects of the lethals in heterozygous condition make, however, the losses more probable than they would be otherwise. On the other hand, some lethals persist and even increase in frequencies. The lethals x_2 and x_8 are examples. This may be due to random genetic drift; the small effective population sizes as evidenced by the high allelism rates and the decreases in lethal frequencies might permit random drift to be an effective force. More likely, these lethals cause

heterosis on the genetic background and under the environmental conditions in the experimental populations. Natural selection may be expected to increase the frequencies of such lethals by favoring the heterozygotes. An indirect check of this hypothesis can be had from the calculations of the average selection coefficients for the radiation-induced lethals under the environmental conditions of the experiments.

Such estimates were obtained for population VI-ER-20 between generations 0 and 19, and also between 19 and 45. The necessary mathematical formulae have been worked out by DOBZHANSKY and WRIGHT (1941), and an improvement has been suggested by PROFESSOR LEWONTIN (personal communication), specifically for the present data.

$$S = \frac{\left[V - \frac{\Delta\bar{Q}}{1-\bar{Q}} \right] - A\bar{Q}^2 \left[1 + V - \frac{\Delta\bar{Q}}{1-\bar{Q}} \right]}{\bar{Q} \left[1 + 2V - 2 \frac{\Delta\bar{Q}}{1-\bar{Q}} \right] - 2 A\bar{Q}^2 \left[1 + V - \frac{\Delta\bar{Q}}{1-\bar{Q}} \right]}$$

and

$$s = S\bar{Q} / -\ln(1-\bar{Q}),$$

where S and s are the disadvantages caused by the lethal chromosomes, or the lethal genes respectively, in heterozygous condition, V the rate of appearance of lethal chromosomes by mutation, Q the frequency of lethal chromosomes, A the frequency of allelism, and ΔQ the change in frequency of lethal chromosomes per generation.

WALLACE (1951) found the rate of mutation for lethal chromosomes in his control (unirradiated) population No. 3 to be $V = 0.45\%$. This figure can be used for our calculations as the best available approximation. The value of S turns out to be -0.0628 between the generations 0 and 19, and -0.0212 between the 19th and 45th generations. This suggests, *for these particular lethals*, a certain degree of overdominance under the conditions of the experiments. While the absolute values of S as computed above are subject to sampling errors of unknown magnitude, it is highly probable that at least some heterotic lethals are present. This agrees with the findings of other authors (see WALLACE 1958 and references therein).

DISCUSSION

Populations of *Drosophila* irradiated over a number of generations may accumulate very heavy genetic loads. The expressed genetic loads are eliminated rather rapidly. In the populations studied in the present experiments there was, following the cessation of the irradiation, a rapid recovery of the egg to adult viability for about four generations. This was presumably due to the elimination of the gross chromosomal aberrations and of other components of genetic loads having pronounced deleterious dominant or semidominant effects. The egg to adult viability has not, however, returned to the control level. The viability in the populations with irradiation histories continues, for 30 to 50 generations, to

be 15 to 20% lower than in the control. Natural selection is evidently operating in these populations to eliminate some poorly viable zygotes. What is, however, the genetic nature of these zygotes being eliminated?

The results presented above indicate an interesting but puzzling situation. Table 2 shows that the proportions of the chromosomes with lethals and semilethals have declined sharply after the cessation of the irradiation, although by no means down to the Control level. Many, presumably a majority, of the radiation-induced lethals are deleterious in heterozygous condition, and they are eliminated rather efficiently by natural selection, even when larval competition is minimized as in our experimental environments. If this were the whole story, the egg to adult viability should be increasing hand in hand with the decline of the frequencies of the homozygous-lethal chromosomes. Figure 1 shows, however, that this is not the case. The egg to adult viability has shown no improvement in two populations and only a slight improvement in the third, and that chiefly in the earlier generations. Does this mean that natural selection has ceased working? This is evidently not so, since the egg to adult viability in the populations with radiation histories remains 15 to 20% below the Control level (Figure 1). Why do these 15 or 20% of the zygotes fail to reach the adult stage?

The radiation treatment has made possible mutational events, including occasional highly heterotic variants which are lethal, semilethal. Every once in a while in some generation in some population these arise and start their slow climb to high frequencies and to ultimate production of a substantial balanced load in its population. It is significant that the population chosen for this particular allelism study was one which did not recover to the Control level of viability for the entire period covered by this report. This choice perhaps, increased the chance of finding heterotic lethals. Formation of homozygotes for such lethals in every generation would cause, when a genetic equilibrium is reached, a constant and uniform mortality of the zygotes, as actually observed. It would, however, be a rather extraordinary coincidence if the reduction of the viability owing to the elimination of the variants which are deleterious in the heterozygotes were exactly balanced by the increasing mortality of the homozygotes for the heterotic variants on the way towards their equilibrium frequencies.

Another possibility that might be considered is that the populations are in a temporary state of linkage disequilibrium, which might simulate overdominance and require a long time to break down. Some of the lethals may have arisen in chromosomes of otherwise high viability or maybe associated with inversions or other chromosome aberrations, which are not unlikely with the high total radiation dose received. Perhaps some type of balanced polymorphism has been set up, whereby the lethals could be retained without themselves being truly heterotic. If that were true, then the period covered by the present observations is not extensive enough for a verification of this hypothesis.

The chromosome analysis reveals that the frequencies of lethal and semilethal second chromosomes in the populations with radiation histories have dropped considerably following the cessation of the irradiation. These frequencies have not, however, reached the Control level. Populations that have similar egg-to-

adult viabilities can carry either small or large concealed genetic loads consisting of lethal and semilethal chromosomes. The magnitude of these concealed genetic loads is not *per se* an index of the fitness of the population. More attention must be given to the less drastic components of the genetic loads—the subvital chromosomes. This we plan to do in another publication.

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SUMMARY

Three experimental populations, kept under conditions designed to minimize the larval competition and crowding, received each 120,000r of X rays during 20, 30 and 60 generations respectively. The egg-to-adult viability decreased sharply, but, after the radiation was discontinued, the egg-to-adult viability recovered rapidly for several generations and then reached a plateau. This plateau is, however, 15 to 20% below that in the Control, unirradiated, population.—The frequencies of the second chromosomes which were lethal or semilethal when homozygous declined after the cessation of the radiation, without however in any case reaching the Control level. The frequency of alleles among the lethals has increased. Some particular lethal chromosomes have become more frequent, probably owing to heterotic effects under the conditions obtained in these experiments.

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