RECESSIVE LETHALS IN SECOND CHROMOSOMES OF *DROSOPHILA MELANOGASTER* WITH RADIATION HISTORIES[1]

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IN the preceding article, SANKARANARAYANAN (1967) reviews the histories of four experimental populations of *Drosophila melanogaster*. The populations were started in 1962; one of them (population A) was an unirradiated control, and the others (B, C, and D) received 120,000r of X rays, given in 60, 30, and 20 generations respectively. After the irradiation was discontinued, the populations lived under conditions of minimal larval competition for 31 (B), 44 (C), and 54 (D) generations. In January 1965 the populations were transferred to laboratory population cages, and lived since then under conditions of acute crowding and competition for food. In December 1965, the present author started a study of the genetic loads of viability variants in the second chromosomes of these populations. The results are reported in the following pages.

MATERIALS AND METHODS

The standard *Cy L/Pm* method (WALLACE 1956) has been used to determine the frequencies of the lethal and semilethal chromosomes found in samples of males taken from the experimental populations. Cultures which produced no wild-type flies in the test generation were considered to carry lethal second chromosomes; those which produced more than zero but less than 15% wild-type flies were regarded as semilethal. For the studies on allelism only complete lethals were used; 50 lethal chromosomes were studied from each of the populations B, C, and D, and 45 chromosomes from the control population A. The lethal chromosomes were kept for further experiments in strains balanced over *Cy L*. All experimental cultures were kept in a constant-temperature room at 25°C. The extraction of the lethals was made between December 1965 and September 1966; the study of the allelism was made thereafter.

RESULTS

The numbers of second chromosomes tested, and the percentages which were lethal or semilethal in double dose, are shown in Table 1. It is instructive to compare these data with previous analyses of SANKARANARAYANAN (1966). The control population showed a statistically nonsignificant increase (13.3 *vs.* 11.6%, lethals and semilethals combined). No significant frequency changes occurred in the populations C (48.1 *vs.* 46.0%) and D (45.6 *vs.* 40.1%). Only in the population B was there a decline of the frequency of lethal and semilethal chromosomes (29.8 *vs.* 40.9%). This result is not unexpected, since the last test of B by SANKARANARAYANAN was made only 19 generations after the irradiation was

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TABLE 1

Frequencies of lethal and semilethal second chromosomes in the experimental populations in September 1966

Population	Chromosomes tested	Percent lethal	Percent semilethal
A	451	11.1	2.2
B	373	26.3	3.5
C	310	45.2	2.9
D	327	42.5	3.1

discontinued, while C and D were tested 45 generations after the irradiation was stopped. In other words, C and D had reached an equilibrium frequency of lethal chromosomes, while in B this frequency was still declining in the interval between SANKARANARAYANAN's tests and mine.

The results of the intercrosses between the strains carrying lethals are represented graphically in Figures 1 and 2. The total numbers of the crosses made, and the numbers of crosses which manifested allelism of lethals, are reported in Table 2. An at first sight curious result is that high frequencies of allelism are found in the control and in the most heavily irradiated population D. As shown below, this is due to the lethals at certain loci having attained high frequencies in these populations. The data in Table 2 can be compared with those in Table 4 of SANKARANARAYANAN (1966). He found 3.9% of the crosses between the lethal-containing stocks of population A to be allelic, and 10.4 to 14.1% in population D. The increase of the frequency of alleles in the Control population is significant.

From the data in Figures 1 and 2, one can deduce the numbers of allelic lethals present in samples of 50 chromosomes from each of the populations B, C, and D, or of 45 chromosomes from the control population A. The results are shown in Table 3. Among the 45 lethal chromosomes sampled in the Control, 18 lethals were represented each only once (in other words, no alleles of these lethals were found). There was however one lethal found in 6 chromosomes, another in 8 chromosomes, and a third in 15 chromosomes. In the populations B, C, and D there were 14, 6, and 10 lethals respectively which were singletons among samples of 50 lethal chromosomes. The other lethals were found twice or more times within a population; seven lethals were found nine or ten times each. The lethals found frequently within a population (1 in A, 4 in B, 4 in C, and 6 in D) were

TABLE 2

Numbers of intercrosses between lethal-carrying strains which show the presence of allelic lethals

Population	Crosses made	Alleles found	Percent alleles
A	990	136	13.7
B	1225	78	6.4
C	1225	105	8.6
D	1225	159	13.0

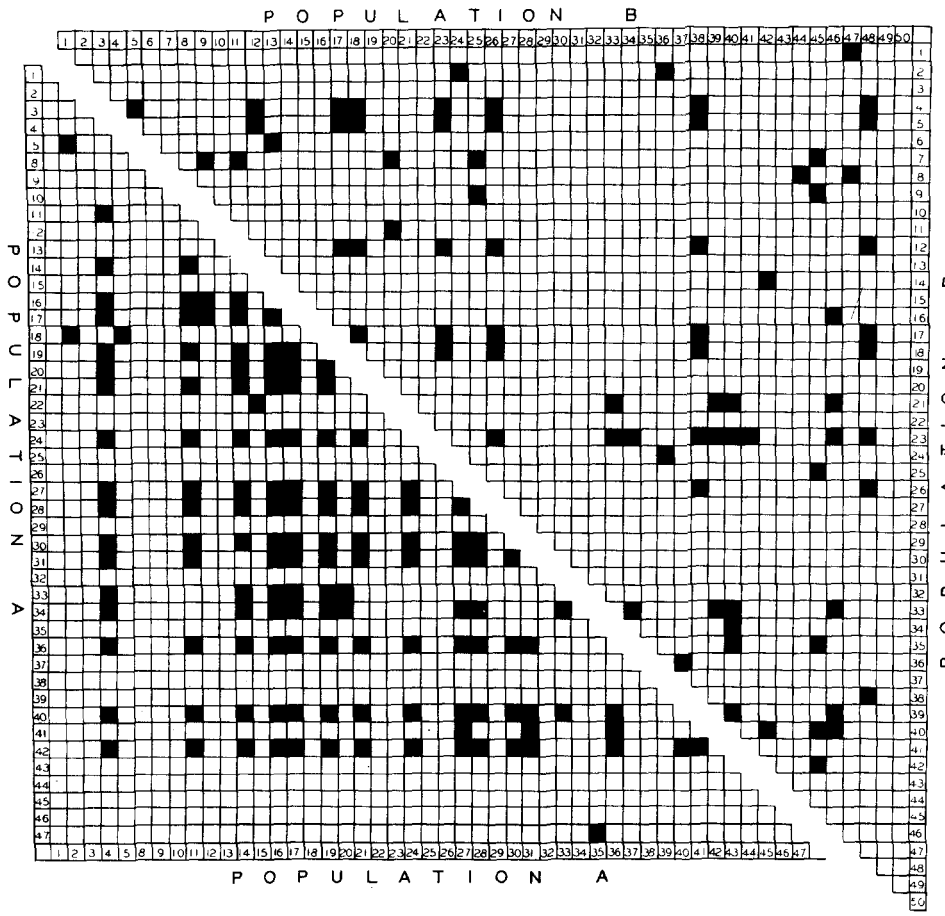

FIGURE 1.—Distribution of the alleles in populations A and B. (Black square indicates allelic combination.)

intercrossed to discover whether these lethals occurred in more than a single population. No inter-populational allelisms were found.

The data in Figures 1 and 2 may also be used to estimate the numbers of lethal

TABLE 3

Numbers of the lethals found once, twice, or several times in the experimental populations

Population	Times found										Lethal loci
	1	2	3	4	5	6	7	8	9	10 . . . 15	
A	18	2	2	.	.	1	.	1	.	. 1	25
B	14	8	4	2	.	1	.	.	1	. .	30
C	6	12	2	1	1	.	.	.	1	1 .	24
D	10	7	7	1	2	1	1	.	2	2 .	33

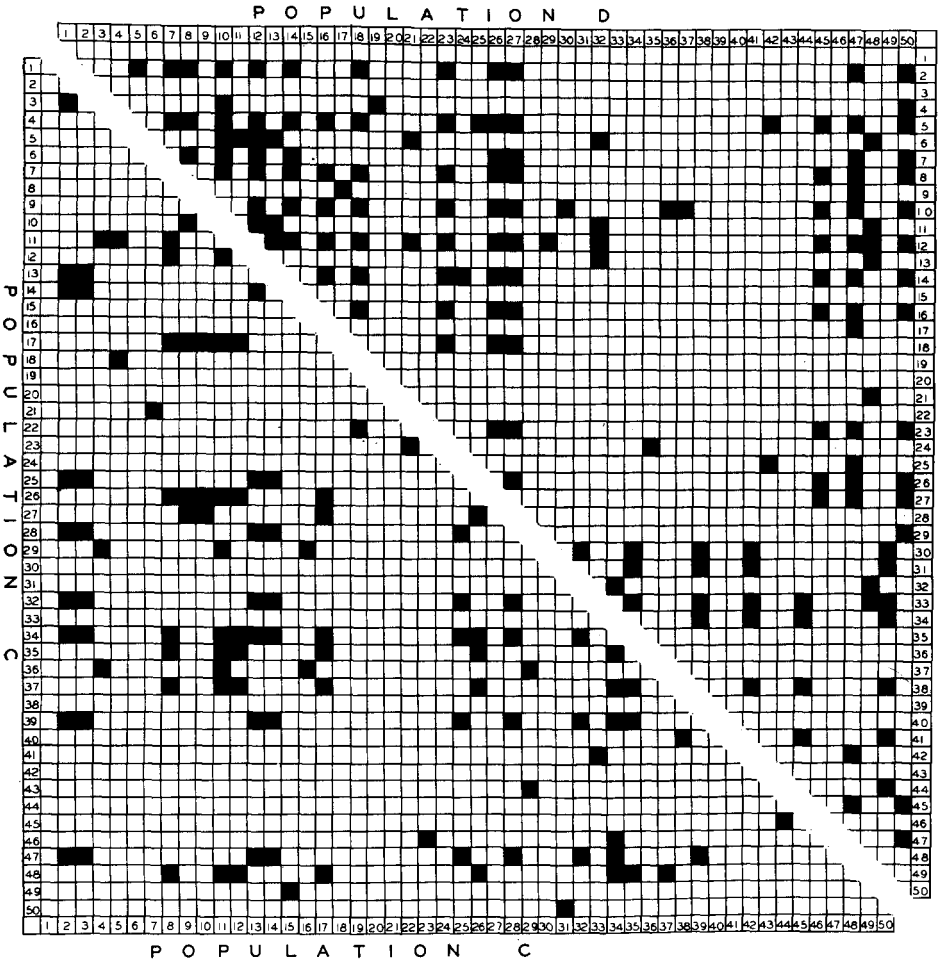

FIGURE 2.—Distribution of the alleles in populations C and D. (Black square indicates allelic combination.)

TABLE 4

Numbers of chromosomes carrying at least one, two, or more lethals

Population	Numbers of lethals								
	1	2	3	4	5	6	7	8	9
A	36	7	2	.	.	.	.	.	.
B	40	7	2	1	.	.	.	.	.
C	40	7	2	1	.	.	.	.	.
D	28	8	5	4	1	2	.	1	1

loci in the tested chromosomes. These numbers are minimal estimates; finding that a certain chromosome, A, has a lethal allelic with that in another chromosome, B, does not exclude the possibility that each of these chromosomes contains two allelic lethals, or also other lethals the alleles of which were not found among the chromosomes sampled. The estimates obtained are shown in Table 4. In the Control population, most of the chromosomes tested showed evidence of a single lethal. By contrast, in population D at least 22 of the 50 chromosomes appeared to carry two or more lethals, and two chromosomes carried as many as 8 and 9 lethals respectively.

The data on which these estimates are based are not quite self-consistent. For example, chromosome No. 34 in population A (Figure 1) contains a lethal allelic with Nos. 4, 14, 16, 17, 19, 20, 27, 28, and 33. The chromosomes Nos. 4, 14, 16, 17, 19, 20 and 33 all have a lethal in common, since all the crosses involving these chromosomes show evidences of allelism. However, these seven chromosomes show no allelism with Nos. 27 and 28, while these latter are allelic to Nos. 11, 21, 24, 30, 31, 36, 40 and 42, to which No. 34 is not allelic. All such "inconsistencies" are real ones, since they were checked by repeated crosses. No instances were found in which there was any question as to whether the lethals were or were not allelic. Such seeming inconsistencies have been encountered also by other investigators studying the allelism of lethals (a recent example is GOLUBOVSKY (1966) who studied a cluster of ten lethal second chromosomes from a natural population of *Drosophila melanogaster* in Russia).

Perhaps not unexpectedly, complications of this kind are especially prevalent in population D. Chromosome No. 10 (Figure 2) seems to contain at least nine "simple" lethals, as shown by its allelic relationships. It has given allelisms in 19 out of the 49 crosses to the other chromosomes. It bridges the following clusters of lethal chromosomes, which may be supposed to carry different lethals.

Lethal 1—Nos. 2, 5, 8, 10, 12, 14, 23, 26, 50
Lethal 2—Nos. 5, 10, 12, 14, 23, 26, 27, 47, 50
Lethal 3—Nos. 2, 5, 7, 8, 10, 12, 14, 27, 47, 50
Lethal 4—Nos. 5, 8, 10, 12, 14, 16, 18, 23, 26, 27
Lethal 5—Nos. 4, 10, 50
Lethal 6—Nos. 6, 10
Lethal 7—Nos. 10, 30
Lethal 8—Nos. 10, 36
Lethal 9—Nos. 10, 37

Chromosome No. 12 in the same population D (Figure 2) contains eight lethals. Four of them, lethal-1, lethal-2, lethal-3, and lethal-4, are the same as in the chromosome No. 10 described above. The other four lethals are shared with the following chromosomes:

Lethal 10—Nos. 12, 14, 16, 27, 45, 50
Lethal 11—Nos. 12, 32, 48
Lethal 12—Nos. 12, 21, 48
Lethal 13—Nos. 12, 29, 50

Complex "complementation" systems of this kind may, of course, have expla-

nations other than the presence of so many lethals in the same chromosome. We may be dealing with a variety of alleles at the same locus, some combinations of which are lethal and others are viable. If this is the case, it would follow that some of the lethal loci are represented in the experimental populations more times than the numbers in Table 3 indicate. We may also be dealing with synthetic lethals of the kind described by WALLACE *et al.* (1953) in *D. melanogaster*, DOBZHANSKY (1946) and SPASSKY, LEVENE and DOBZHANSKY (1958) in *D. pseudoobscura*, and MAGALHAES *et al.* (1965) in *D. willistoni*. The information now available does not permit discrimination between these explanations, which are not mutually exclusive. Experiments to test them are being planned.

In March, and again in May 1967, the egg-to-adult viability was studied in the populations A, B and D (C was accidentally lost). The same technique was employed as that used by SANKARANARAYANAN: 30 samples of 50 eggs each deposited by the flies from the populations were placed in half-pint bottles, and allowed to develop to the adult stage. The results are shown in Table 5. The viability in population A is only slightly if at all lower than observed by SANKARANARAYANAN (1967, Table 2) between 0 and 180 days after the start of the population cages. A quite different result was obtained in the populations B and D. SANKARANARAYANAN (1967, Table 2) observed an increase in the viability from 72–73% when the populations were started, to about 87% 180 days later; the data in Table 5 show that by March-May 1967, when the populations had been living in the population cages for approximately 26–28 months, the viability decreased down to or below the levels observed when the population cages were started by SANKARANARAYANAN. The differences in the viability between population A on one hand and B and D on the other are statistically highly significant, and so are the differences between the viabilities of B and D observed in 1967 and those found by SANKARANARAYANAN two years earlier. The natural selection under the conditions of intense competition in the population cage has led to a higher, instead of lower, egg-to-adult mortality. A probable explanation of this apparent paradox is given in the DISCUSSION.

DISCUSSION

Some 50 to 75 generations after the irradiation was discontinued, the populations with radiation histories have remained genetically different from the control

TABLE 5

Egg-to-adult viability in the experimental populations in March and May 1967

Population and date	Adults from 1500 eggs	Percent adults
A, March	1263	84.20 ± 1.16
A, May	1279	85.27 ± 1.06
B, March	1030	68.67 ± 1.38
B, May	872	58.13 ± 1.04
D, March	986	65.73 ± 1.62
D, May	977	65.13 ± 2.12

population. In the populations with radiation histories, the frequencies of second chromosomes which are lethal or semilethal in double dose are double to triple the frequencies in the control. These higher frequencies of lethal chromosomes are not accounted for simply by greater numbers of different lethals represented; more important is the fact that some lethal loci are found in many chromosomes in the populations with radiation histories. Table 3 shows that 25 different lethals were found in a sample of 45 lethal chromosomes in the control population, and 30, 24 and 33 different lethals in samples of 50 lethal chromosomes from each of the three irradiated populations. In the control population, 18 of the 25 lethals were found only once in a sample of 45, but one lethal was found 15 times. In the irradiated populations, 14, 6 and 10 among 30, 24, and 33 lethal loci respectively were found only once in samples of 50 lethal chromosomes, and consequently more than half of the lethals were found two or more times.

What is the nature of the genetic load revealed by our experiments in the control and in the irradiated populations? The salient fact is, of course, that continued natural selection did not equalize the loads in the control and in the irradiated populations. Most of the mutational changes originally induced by the radiation were deleterious, both when homozygous and when heterozygous. This is evidenced by the rapid improvement of the egg-to-adult viability, and by the decline in the frequencies of the chromosomes lethal in double dose following the discontinuation of the irradiation. However, both the viability and the lethal frequencies in the populations with radiation histories eventually reached plateau levels different from the control. The egg-to-adult viability was closest to the control about half a year after the populations were exposed to sharply competitive conditions in the population cages; but further selection has led to declines of the viability of the progenies of the flies from the population cages, despite the intense competition to which these populations were continuously subjected. Some of the genetic variants induced by the irradiation have evidently been not only retained but multiplied in the experimental populations. Indeed, some of the second-chromosome lethals, which arose presumably by single mutations in single individuals, have now reached considerable frequencies, being present in many chromosomes in the populations. Theoretically, this could have taken place either owing to random genetic drift, or because of the heterotic effects which these mutants produce in the heterozygotes. The genetic drift hypothesis is not a plausible explanation, since the populations were never small. Heterosis is by far the most likely explanation.

The observation that the egg-to-adult viability in the populations first rose after they were placed in population cages, and then fell again, deserves comment. The environments in which these populations existed before and after they were transferred to the laboratory cages were quite different—minimal larval competition at first and very intense competition in the cages. It is, then, reasonable to suppose that some of the variants which were heterotic under relaxed competition became deleterious in the cages, and some which were neutral or even slightly deleterious became heterotic. The gene pool of each population was reconstructed accordingly, and at some intermediate stage of the reconstruction the variants

deleterious in the heterozygotes were eliminated before the heterotic ones (but deleterious in homozygotes) reached higher frequencies. The genetic system then became stabilized, giving a high average fitness despite the elimination of ill-adapted homozygotes in each generation. To be sure, the elimination of the lethals alone could account for only a part of the observed egg-to-adult mortality. Knowing the frequencies and the rates of allelism of the complete lethals in the second chromosomes, it is easy to compute the percent mortality expected from this source. The estimates are: Population A—0.2%; Population B—0.6%; Population C—2.0%; and Population D—2.7%.

The third chromosomes in our populations have probably at least as many lethals as the second chromosome; the mortality estimates due to homozygosis for lethals must, accordingly, be doubled. Even so, the observed egg-to-adult mortality is several times greater. Some fraction of this mortality is doubtless of environmental rather than genetic origin, but the much greater mortality in the populations with radiation histories than in the control shows that the genetic component is by no means negligible. There must be some mortality due to chromosomes which are subvital rather than lethal or semilethal when homozygous. The adaptive system arisen in the populations with radiation histories appears to rely heavily on balanced polymorphism and heterosis.

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SUMMARY

Experimental populations with radiation histories, and a control population, were kept in laboratory population cages under conditions of extreme crowding and intense larval competition. The frequencies of second chromosomes which were lethal or semilethal when homozygous remained at levels appreciably higher than in the control, while the egg-to-adult viability declined below the control level in the populations with radiation histories. The rates of allelism of the lethals in experimental populations are fairly high, owing to some lethal loci being present in many chromosomes. These lethals are maintained in the populations by natural selection because of their heterotic effects in heterozygous individuals.

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