THE EFFECT OF RADIATION ON SMALL COMPETING POPULATIONS OF *DROSOPHILA MELANOGASTER* I. THE ACCUMULATION OF GENETIC DAMAGE

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THE genetic effects of ionizing radiations have now been studied extensively in a wide variety of systems. The study of their cumulative effects in populations has also been given increasing attention in recent years although, because of experimental difficulties, on a more restricted number of organisms. The effect of radiation on a population will be to alter its genetic constitution and, therefore, presumably, to affect the biological fitness. In order to relate these changes in fitness to specific genetic damage three prime conditions must be met. First, the genetic constitution must be exactly known at the commencement of the experiment; second, the accumulation of genetic damage must be closely followed; and third, direct and exact comparisons with control populations must be continuously possible.

These three criteria are satisfied in the following way in the present experiments. The flies are first generation hybrids between two inbred lines and are, therefore, genetically almost identical and at maximum fitness with minimum genetic load. They are homozygous for the second chromosome genes *light* or *straw* and are, therefore, phenotypically distinct, but at each generation heterozygotes between *light* and *straw* are always discarded and the population set up afresh with virgin females and young males. Competition begins at mating, continues through egglaying and larval development and ends with pupation and emergence of the new adult. Competitive abilities are measured by the numbers and proportions emerging and can be followed generation by generation as ancestral irradiation accumulates. The surplus emergent flies at each generation have been tested in various ways for many types of genetic damage.

The whole project is designed, therefore, to provide an accurate assessment of changes in population fitness closely related to genetic damage induced by radiation given at every generation. It also provides a link between theoretical work on genetic changes in small populations and experimental population genetics.

MATERIALS AND METHODS

The present experiments are a continuation of those reported earlier (DYER 1966) and a full description of the experimental methods is given there. The experimental design is shown in Figure 1. Two populations were run as before. In one the *straw* flies were given 1,500r of X rays every generation, in the other neither *light* nor *straw* flies were irradiated.

Detection of second chromosome recessive lethals was by means of the standard *Cy/Bll* test.

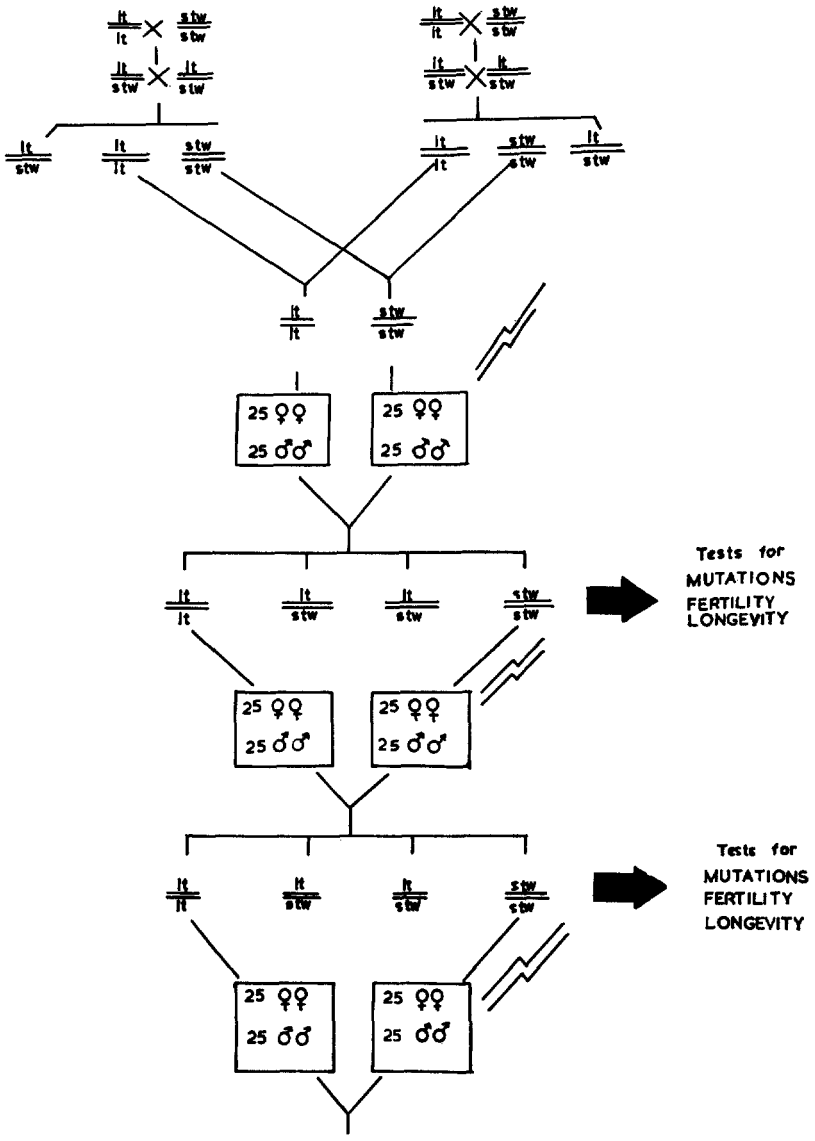

FIGURE 1.—The experimental design.

The presence of sex-linked recessive lethals was determined in single virgin females by the M-5 technique. The incidence of sterility was assayed by singly mating experimental flies to pairs of outbred Oregon K. A fly was deemed sterile when it produced no offspring and semi-sterile when producing less than ten offspring within 16 days at 24°C.

Both irradiated and control populations were continued for tweny-five generations but at generation 20 a sub-population was set up from that in which *straw* was irradiated. This was continued for 5 generations in exactly the same way, except that the *straw* received no further irradiation.

Estimation of the frequency of recessive lethals in *straw*, *light* and heterozygotes was carried

out in as many generations as was technically manageable. At selected generations a number of the autosomal lethals found were retained and allelism matings were carried out between them to determine the frequency of identical lethals accumulating within the irradiated population. Lethal second chromosomes were maintained balanced by the *Cy* chromosome. Virgin females from one balanced lethal stock were crossed with males from another one. Absence of homozygous *straw* flies among the progeny indicated that the lethals were allelic.

RESULTS

Sterility: Measurement of sterility is important both as an indication of increasing genetic damage and of decreasing population size. Apart from matings for the lethal tests at most generations, special investigations of sterility in both males and females were undertaken at generations 18 and 24. The results are shown in Table 1. The figures indicate a mean level of sterility in the *straw* population of about 20% by generation 18 and slightly more, perhaps 23% by generation 24. This sterility is presumed to be due to the steady accumulation of translocations, complex inversions, and other elements disrupting normal chromosomal behaviour. A proof of this explanation could be obtained by large scale examinations of the salivary gland chromosomes, but this has not been attempted.

Sex-linked lethal mutations: Sex-linked lethal mutations can only be transmitted in females. Their rate of accumulation is, therefore, much slower than that of autosomal lethals and their equilibrium value would only be 25% of that expected of autosomal lethals even if the mutation rates were identical. Since the mutation rate of sex-linked lethals is less than half that of second chromosome lethals, the expected equilibrium value is about 10% of the equilibrium value for second chromosome lethals. This equilibrium value for autosomal lethals seems to be about 70% and, therefore, the expected figure for sex-linked lethals is about 6–7%. The observed values are shown in Table 2 and are in fair agreement with this expected figure. Considering the fairly low values expected and the fluctuations due to the small population size it becomes clear that very extensive data

TABLE 1

The incidence of sterility in generations 18 and 24

Generation	Genotype	Number tested	Number sterile	Percent	Number semisterile	Percent
18	stw ♂	78	20	25.64	0	0.00
24	stw ♂	63	13	20.63	6	9.52
18	stw ♀	34	4	11.76	2	5.88
24	stw ♀	51	11	21.57	3	5.88
18	lt ♂	50	1	2.00	6	12.00
24	lt ♂	70	2	2.86	9	12.86
18	lt ♀	110	8	7.27	30	27.27
24	lt ♀	34	2	5.88	7	20.59
18	het ♂	40	1	2.50	1	2.50
24	het ♂	59	0	0.00	4	6.78
18	het ♀	82	5	6.10	9	10.97
24	het ♀	30	2	6.67	2	6.67

TABLE 2

The accumulation of sex-linked lethals

Generation	Genotype	Number of lethals/Number of males tested	Percent lethals ± S.E.
14	straw	2/54	3.70 ± 0.81
	light	0/65	0.00
16	straw	7/64	10.94 ± 1.28
	light	0/41	0.00
17	heterozygotes	2/116	1.72 ± 0.37
19	straw	3/50	6.00 ± 0.33
	heterozygotes	1/49	2.04 ± 0.34
20	heterozygotes	1/40	2.50 ± 0.78
24	straw	0/33	0.00
25	straw	0/32	0.00
	light	0/46	0.00

would be required to decide, with any accuracy, the true equilibrium value for sex-linked lethals.

Second chromosome lethal mutations: The results obtained by testing for the presence of second chromosome recessive lethals are shown in Table 3 and Figure 2. Since the populations were hybrids from inbred lines, we know that the initial lethal frequency must have been negligible.

a) The irradiated *straw* line: The expected rate of induction of second chromosome recessive lethal mutations under the conditions of the present experiment

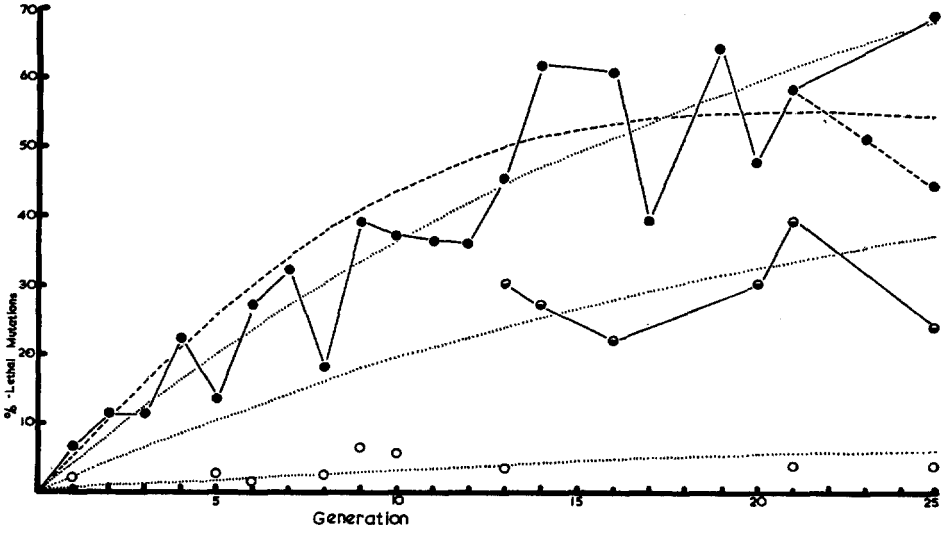

FIGURE 2.—The accumulation of second chromosome recessive lethal mutations: ●———●, the irradiated *straw* population; ● - - - ●, radiation suspended; ○ ○, the non-irradiated light population; ◒———◒, *light/straw* heterozygotes; . . . . . , accumulation curve based on regression of non-lethal chromosomes; – – – –, computer calculated accumulation curve.

TABLE 3

The accumulation of second chromosome recessive lethal mutations

Generation	*Straw*		*Light*	
	Number of lethals/Number of males tested	Observed frequency	Number of lethals/Number of males tested	Observed frequency
1	5/72	6.94 ± 3.00	2/80	2.50 ± 0.17
2	8/68	11.76 ± 3.91	0/58	0.0
3	5/43	11.63 ± 4.88	0/23	0.0
4	9/40	22.50 ± 6.60	0/72	0.0
5	18/132	13.64 ± 2.99	2/75	2.67 ± 0.19
6	23/84	27.38 ± 4.87	1/59	1.69 ± 0.17
7	31/95	32.63 ± 4.81	....	.....
8	6/33	18.18 ± 6.70	2/75	2.67 ± 0.19
9	32/82	39.02 ± 5.38	6/88	6.82 ± 0.28
10	29/77	27.66 ± 5.45	3/52	5.77 ± 0.32
11	25/70	35.71 ± 6.42	....	.....
12	24/68	35.39 ± 7.20	....	.....
13	20/44	45.45 ± 7.50	1/27	3.70 ± 1.1
14	45/71	63.38 ± 5.76	0/54	0.0
15	.....	.....	....	.....
16	81/133	60.90 ± 4.23	....	.....
17	23/59	38.98 ± 6.42	....	.....
18	.....	.....	....	.....
19	25/39	64.10 ± 7.68	....	.....
20	11/23	47.83 ± 10.41	....	.....
21	14/24	58.33 ± 10.06	2/51	3.92 ± 0.11
22	.....	.....	....	.....
23	.....	.....	....	.....
24	.....	.....	....	.....
25	72/105	68.57 ± 3.70	5/120	4.17 ± 0.58

is 6% per generation (DYER 1966). Results from the early generations of the *straw* line are roughly in accord with this. It is evident, however, that while there is a progression to a maximum value of 68% in generation 25 there is also considerable fluctuation in the results. PROUT (1954) reported similar fluctuations in lethal frequencies in his smallest population. The net rate of increase after generation 14 is very low and suggests that equilibrium has nearly been reached in these later generations.

b) The control *light* line: Concentration upon the irradiated *straw* line prevented the accumulation of a large body of data from the control, but it is nevertheless clear from Table 3 that the observed figures are significantly lower than would be expected from the normal spontaneous rate of 0.5% per generation. The marked difference can be ascribed to random sampling effects and selection against the lethals in the heterozygous condition.

c) Lethal frequencies in the heterozygotes: Measurement of the lethal frequency amongst the *light/straw* heterozygotes shows up any heterozygous effects of these lethals undistorted by any elimination as homozygotes. In these tests the observed chromosome was *light* or *straw* or lethal-bearing. Since the lethal-bear-

TABLE 4

The assessment of lethal frequencies among the heterozygotes of the irradiation/non-irradiated lines

Generation	Sample	Overall lethal frequency	Mean frequency of *light* and *straw* lines
13	35 homozygous *light* 8 homozygous *straw* 19 lethals	30.65 ± 5.86	21/71 = 29.58 ± 5.45
14	49 homozygous *light* 17 homozygous *straw* 25 lethals	27.47 ± 4.68	45/125 = 36.00 ± 4.29
16	28 homozygous *light* 17 homozygous *straw* 13 lethals	22.41 ± 5.48	.....
20	10 homozygous *light* 6 homozygous *straw* 7 lethals	30.43 ± 9.59	.....
21	28 homozygous *light* 7 homozygous *straw* 23 lethals	39.66 ± 6.42	16/75 = 21.33 ± 4.73
25	76 homozygous *light* 18 homozygous *straw* 30 lethals	24.19 ± 3.85	77/225 = 34.22 ± 3.16
Mean	117/416	28.12% ± 2.20	159/496 = 32.06 ± 2.10

ing chromosomes are characterized by their absence we cannot tell whether they are *light* or *straw*. If there were any selective advantage or disadvantage associated with heterozygous lethals their frequency would be higher or lower than expected. Tests were therefore carried out in later generations when the lethal frequency had become sufficiently high for such effects to be apparent. The results are shown in Table 4 together with the mean frequency of lethals in the *light* and *straw* lines where this can be calculated since, as already indicated, we cannot make a comparison directly with the *light* or *straw* populations. There is an important difference between the lethal frequencies of the heterozygotes and the mean of the *light* and *straw*. The absolute difference is about 4% which is not itself statistically significant. However, the lethals amongst the *straw* flies have been reduced by an amount corresponding to the allelism frequency, which varies between 15% and 30%, whereas the heterozygotes have suffered no such reduction. When the appropriate adjustment is made to the lethal frequency, i.e., it is supplemented by between 5% and 7% to account for the losses in the *straw*, the difference becomes highly significant. There is, therefore, major selection against these lethal mutations in the heterozygous condition when they are placed on a genetic background markedly different from that of the continuous *straw* population.

Allelism of second chromosome lethals: All the previous data have concerned the rate of induction and accumulation of mutations. The rate of elimination is

TABLE 5

The degree of allelism among the recovered lethal mutations

Generation	Number of chromosomes	Number of crosses	Number of times loci represented 1	2	3	4	5	>5	Total alleles	Percent allelism	Rate of elimination percent
14	44	946	3	1	0	1	1	4	297	31.40 ± 6.25	12.82
16	23	253	3	2	2	0	0	2	48	18.97 ± 6.40	6.66
21	14	91	2	4	0	0	0	1	19	20.88 ± 8.53	7.10
25	43	903	1	11	1	2	1	4	136	15.06 ± 1.66	7.34
3*	25	300	0	0	2	2	0	2	86	28.67 ± 4.21	7.50
5*	49	1176	0	2	1	1	2	5	289	24.57 ± 3.37	4.89

* Generations after the suspension of irradiation.

also of considerable importance and this can be determined from the degree of allelism of the lethals within each generation. Samples of lethals at generations 14, 16, 21, 25 and at generations 3 and 5 after suspension of irradiation were, therefore, retained and allelism matings were carried out between them. The results are shown in Table 5. The degree of allelism obtaining at each of these generations was very high indeed. This represents a high degree of identity of these mutations and therefore a very high rate of elimination—much higher in all cases than the rate of induction. This is convincing evidence that many of these mutations must have been preferentially selected as heterozygotes. The standard errors quoted in Table 5 have been calculated by the method outlined in CURNOW, CRITCHLEY and DYER (1969) and take into account the restricted number of different lethal mutations and the distribution of lethals on the chromosomes. These authors give two different methods for obtaining a true rate of allelism in these circumstances and show why the straightforward binomial standard error $\sqrt{pq/n}$ is not appropriate.

On the suspension of irradiation: The lethal frequencies found in the population after the suspension of irradiation are shown in Table 6. There is no significant reduction in the frequency of lethals at either the third or fifth generation after the suspension of irradiation. However, as shown in Table 5, the actual losses due to homozygosity would lead to a loss of recessive lethals much greater than that observed. The fact that the observed figures do not show a very large loss is further evidence for a net favourable selective effect of some lethals in the

TABLE 6

The changing lethal frequencies on suspension of irradiation

Generation		Number of lethals	Observed frequency
0	(20)	11/23	47.83 ± 10.41
1	(21)		
2	(22)		
3	(23)	67/131	51.14 ± 4.36
4	(24)		
5	(25)	83/186	44.62 ± 3.64

heterozygous condition with this integrated genetic background. The continued high level of allelism which is shown in Table 5 emphasizes the particular selective advantages of a limited number of lethals. Although it might be objected that part of these high values might be due to the effects of random genetic drift, of the 12 mutations present in generation S5, 2 were found first in generation 14, 1 in generation 16 and 2 in generation S3. Thus at least 5/12 lethals contributing 2/3rds of the lethal frequency show evidence of preservation by selection.

CALCULATIONS

The information presented above can now be used to calculate the rate of accumulation of genetic damage and likely equilibrium levels. Sex-linked lethals cannot unfortunately be included in this since, as indicated, there are not really enough data to warrant it. To determine an equilibrium value for autosomal lethals the rate of accumulation must be compared with the rate of elimination at each generation. Equilibrium is reached when accumulation and elimination are equal.

The rate of accumulation of lethal chromosomes depends on three things: firstly, the radiation dose which is delivered to a given germ cell stage, which in these experiments remains constant; secondly the saturation effect as larger proportions of the chromosome become lethal; and thirdly any heterozygous advantage that might exist to preferentially select some chromosomes.

The rate of elimination depends primarily on the rate of elimination due to homozygosity. The likelihood of homozygosity in turn depends on effective population size and the likelihood of allelism of lethal chromosomes. Any heterozygous disadvantage would also tend to accelerate elimination.

The nominal population size at each generation was 50. This would lead to an increased likelihood of identity by descent of about 1% at each generation. However, the mean figure of about 20% sterility in the irradiated *straw* line indicates an effective population size reduced to about 40 by generation 18 and slightly fewer by generation 24. The population size is, therefore, decreasing by about 1 every other generation and there is consequently a slight increase in the rate of approach to homozygosity on this account.

The likelihood of allelism of independently arising lethal genes is 0.25% (IVES 1945; WALLACE 1950; PROUT 1954). In the present populations there was a tendency for the accumulation of more than one lethal per chromosome, which therefore affected the likelihood of allelism of lethal chromosomes. In accordance with DOBZHANSKY and WRIGHT (1941) and all following workers in this field, the distribution of lethals on chromosomes is assumed to be Poisson. Data to be presented in the second paper of this series (DYER 1969b) show that this is not strictly accurate, nevertheless the errors are not thought seriously to disturb the results. Table 7 shows minimum estimates of the mean number of lethals per lethal chromosome and, calculated from this, the likelihood of allelism of lethal chromosome at each generation in the irradiated *straw* line. Table 7 also shows

TABLE 7

The elimination of second chromosome recessive lethal mutations in the irradiated straw *line*

Generation	Mean number of lethals per lethal chromosome	Likelihood of allelism: a) Under random mating	Likelihood of allelism: b) Due to small, falling population size	Likelihood of allelism: c) Net* result	Calculated net rate of elimination
1	1.00		1.00	0.25	0.00
2	1.12		1.01	1.26	0.01
3	1.12	0.25	1.02	2.28	0.02
4	1.28		1.03	3.31	0.05
5	1.14		1.04	4.35	0.12
6	1.35		1.05	5.40	0.19
7	1.43		1.06	6.46	0.30
8	1.21	0.27	1.07	7.53	0.43
9	1.54		1.09	8.62	0.58
10	1.52		1.10	9.72	0.76
11	1.48		1.11	10.83	0.95
12	1.48		1.12	11.95	1.15
13	1.66	0.29	1.14	13.09	1.37
14	2.00		1.15	14.24	1.58
15	. . .		1.16	15.40	1.79
16	1.98		1.18	16.58	1.99
17	1.55		1.19	17.77	2.17
18	. . .	0.31	1.20	18.98	2.34
19	2.05		1.22	20.20	2.49
20	1.71		1.23	21.44	2.62
21	1.92		1.25	22.69	2.73
22	. . .		1.28	23.95	2.82
23	. . .	0.33	1.30	25.23	2.89
24	. . .		1.30	26.53	2.94
25	2.16		1.32	27.85	2.99

* i.e. likelihood of allelism of mutations induced at generation 1. It is composed of the random component together with the cumulative sum of increase due to the small and falling population size.

the increasing likelihood of allelism due to restricted and decreasing population size. Table 8 shows similar estimates for the suspended population.

Given these simple parameters, i.e., 6% induction of lethal chromosomes every generation and a low but gradually increasing elimination rate, one can construct a theoretical accumulation curve and check its correspondence with the observed data. The factors for inbreeding and increasing homozygosity must be applied separately to each generation's influx of mutations which provide separate starting points. Each separate influx of mutations can be considered to have been inbred for different numbers of generations both with itself and with every other generation's influx. The likelihood of allelism of lethal genes which provides the appropriate elimination factor is, therefore, different for each case. This set of

TABLE 8

The elimination of second chromosome recessive lethal mutations in the suspended straw *line*

Generation	Mean number of lethals per lethal chromosome	Likelihood of allelism			Calculated net rate of elimination
		a) Under random mating	b) Due to small but increasing population size	c) Net* result	
0 (20)	1.71	0.31	1.23	21.44	2.62
1 (21)	. . .	0.31	1.22	22.66	2.71
2 (22)	. . .	0.31	1.20	23.86	2.73
3 (23)	1.24	0.31	1.19	25.05	2.71
4 (24)	. . .	0.31	1.18	25.23	2.65
5 (25)	1.45	0.31	1.16	26.39	2.56

* i.e. likelihood of allelism of mutations induced at generation 1. This is composed of the random component and the cumulative sum from all previous generations of the inbreeding component.

calculations was carried out for the irradiated *straw* population after being programmed for the Atlas computer by Mr. D. G. PAPWORTH. The methods used and the results obtained are described more fully in the APPENDIX to this paper. The figures for accumulation of second chromosome lethals are shown in column 1 of Table 9. The maximum frequency is reached at generation 20 and thereafter there is a slight decline due to the effects of inbreeding. This peak is an indication of equilibrium but it occurs at a level more than 10% lower than that suggested by the observed results. Calculations were carried out on the suspended population in exactly the same way, except that the effective population number was considered to increase slightly over those 5 generations as the factors causing sterility were eliminated. The rate of elimination was slightly greater than that observed although the differences at neither the third or fifth generation after suspension were significant. The results are shown in column 2 of Table 9.

As well as furnishing figures for the net accumulation at each generation, net rates of elimination of pre-existing lethals at each generation can be determined from the results of these calculations. These two sets of figures for accumulation and elimination are plotted in Figure 3 together with the experimental data on the rate of elimination derived from allelism matings. There is fairly good agreement between observed and calculated data on the rate of accumulation apart from the discrepancy of maximum values previously noted. On the other hand there is a very marked difference between the observed and calculated rates of elimination. Not only is the observed rate of elimination considerably greater but, between generation 14 and 25, there is no trend towards an increase in this rate—rather the reverse in fact.

The problem of assessing the accumulation and equilibrium levels of lethal genes is a very complex one. We can only observe the accumulation of lethal chromosomes and must also consider such variables as population size, degree of crossing over and selection on the heterozygotes of lethal genes.

The calculations presented here take no account of possible advantageous or disadvantageous heterozygous effects and we see that, in consequence, they cannot give a detailed picture of the processes actually occurring. NEI (1968) has made

TABLE 9

The accumulation of second chromosome recessive lethal mutations

Generation	Frequency based on 6% rate of induction and varying elimination due to allelism	Frequency based on regression of log-frequency of non-lethal chromosomes on generation
1	6.00	4.44
2	11.63	8.68
3	16.91	12.72
4	21.84	16.60
5	26.41	20.30
6	30.64	23.83
7	34.50	27.20
8	28.01	30.44
9	41.15	33.53
10	43.92	36.47
11	46.32	39.29
12	48.41	41.47
13	50.14	44.55
14	51.55	47.03
15	52.67	49.37
16	53.52	51.60
17	54.14	53.75
18	54.55	55.80
19	54.78	57.76
20	54.87	59.64
21 (S1)	54.85 (52.17)	61.43
22 (S2)	54.75 (49.43)	63.13
23 (S3)	54.58 (46.71)	64.77
24 (S4)	54.36 (44.06)	66.35
25 (S5)	54.11 (41.50)	67.84

some progress in devising means of measuring some of the possible heterozygous effects in these circumstances. The calculations also ignore the effects of recombination of lethal genes which modifies the effective levels of accumulation and elimination of lethal chromosomes (ALAN ROBERTSON, personal communication). The magnitude of the difference between observed and calculated rates of elimination of lethal chromosomes and, therefore, of the selective advantage of lethal genes may thus be slightly exaggerated.

The calculations given here indicate the most important areas where further theoretical and experimental work in this field is needed to construct models of greater generality (ALAN ROBERTSON, M. NEI, personal communications).

An alternative approach is to construct a net accumulation curve from the observed data. This can be done by determining the regression of the log-frequency of non-lethal chromosomes on generation. The results for the *straw* population are given in the third column of Table 9. The mean rate of accumulation turns out to be 4.44 ± 0.29% per generation over 25 generations compared with 4.546 over the first 12 generations (DYER 1966). The curve constructed from these figures does not provide a good fit to the observed results ($\chi^2 = 40.27$; P =

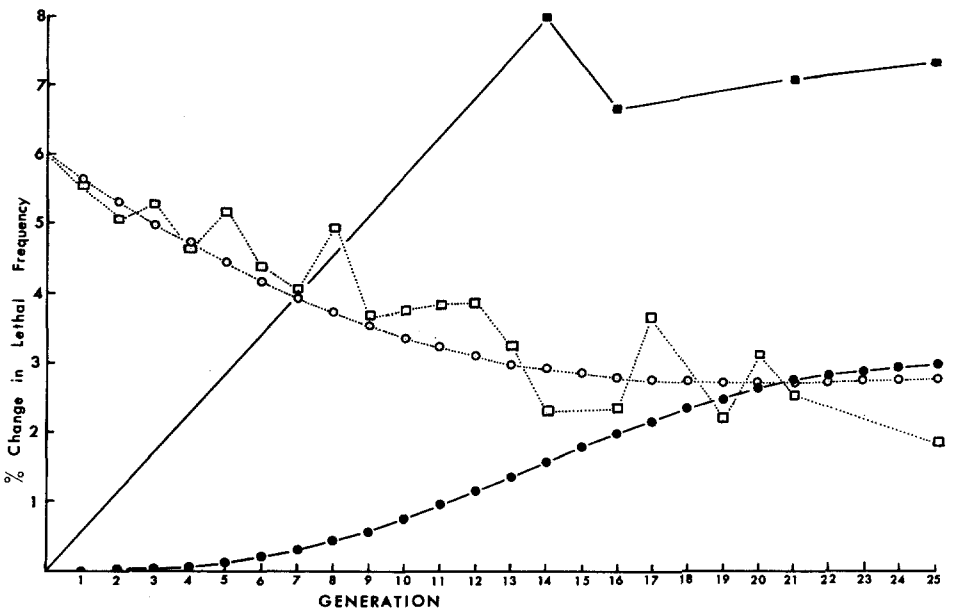

FIGURE 3.—Comparison of rate of accumulation and elimination of second chromosome recessive lethals: ○———○, calculated rate of accumulation; ●———●, calculated rate of elimination; □———□, observed rate of accumulation; ■———■, observed rate of elimination.

0.003). This provides confirmation of the fact, already noted, that random fluctuations from generation to generation play a very important part in determining the lethal frequencies.

When the same method is applied to the data from the control *light* line the mean accumulation rate turns out to be 0.29% per generation instead of the expected normal spontaneous rate of 0.5% per generation. The marked difference can be ascribed to random sampling effects and selection against the lethals in the heterozygous condition.

In applying these methods to the heterozygote population the mutation rate can be assumed to be 50% of that of the *straw* line. The observations for the heterozygotes, however, differ significantly from the above regression with a rate of accumulation 50% of the *straw* line, $\mu = 2.22\%$ ($P = 0.014$). These accumulation curves are plotted in Figure 2 using the accumulation figures of 4.44% and 0.29% for the *straw* and *light*, respectively, and the mean of these for the heterozygotes.

DISCUSSION

One of the most important problems currently under investigation in Drosophila populations is the relative magnitude of the balanced and mutational loads. The present experiments impose a heavy mutational load on the populations, but the conditions of severe competition also enhance the selective differentials contributing to the balanced load.

Considering first the irradiated *straw* line with its integrated gene pool, the very high levels of allelism found and the very high frequencies attained by some lethals suggest the importance of certain overdominant loci and, therefore, the balanced load. This is especially true after the suspension of irradiation when the proportion of frequently occurring loci is increased. Various other figures for allelism among lethals of both natural and irradiated populations have been obtained by several workers (DOBZHANSKY and WRIGHT 1941; IVES 1945; DUBININ 1946; PROUT 1954; BAND 1964; MERRELL 1965; TOBARI 1965; OSHIMA 1965; SANKARANARAYANAN 1966). In samples from a fairly small population of around 1,000, PROUT obtained figures of over 10% allelism partly attributable to an heterotic lethal. TOBARI, from a population of 8,000, demonstrated allelism of 7.5% and this he attributed to the presence of overdominant loci. SANKARANARAYANAN also postulated overdominant loci to explain his figures of 10% and 14% from populations of only a few hundred. OSHIMA (1965) measured the allelism frequency among 4 natural populations in Japan and found it varied between 3.58% and 2.35%. He then showed that some recessive lethals had been maintained for long periods of time in the same population and that the formation of gene complexes and heterotic inversions was important in their preservation. The present results, however, show a greater degree of allelism than any previously reported and emphasize the effects of severe competition which magnifies the selective advantages of a few overdominant loci. There is no indication of any increase in the degree of allelism during the time which elapses between generation 14 and 25 neither is there any correlation between lethal frequency and degree of allelism. There is not, therefore, always a simple relationship between inbreeding, lethal frequency and allelism as has sometimes been suggested (OSHIMA 1965; TOBARI 1965).

Direct extrapolation of these results to the *light/straw* heterozygotes would lead one to expect a frequency of lethals around 10% higher than the mean of those occurring in the *light* and *straw* populations individually. This is because the 20% or so elimination of lethals as homozygotes which occurs in the *straw* line cannot take place in the heterozygotes. The actual figure of about 4% less is important evidence of the difference that genetic background can make to the nature of the selection on various mutations. Preferential selection in the *straw* line with its integrated gene pool and moderate level of heterozygosity is matched by adverse selection in the heterozygotes in which the gene pool is discontinuous and at a maximum level of heterozygosity. WALLACE (1958; 1963) OSHIMA and KITAGAWA (1961) MUKAI, CHIGUSA and YOSHIKAWA (1965) and DYER (1966) have presented evidence of different kinds showing broadly that selection coefficients against adverse genetic changes increase as the level of heterozygosity of the gene pool increases. The present evidence can be interpreted in the same way.

There is, then, in this heavily irradiated population a considerable buildup of genetic damage. Under strong natural selection, however, many mutations can be modified into useful components of the gene pool, can become very numerous and can form a balanced genetic load. Where this modification and incorporation cannot occur, i.e., in non-integrated gene pools, the full deleterious effects of a

heavy mutational load are made manifest. The changing flux of genes and gene frequencies under these two differing circumstances is demonstrated in the second paper of this series.

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SUMMARY

Two populations of *Drosophila melanogaster*, genetically similar but phenotypically distinguishable, were allowed to compete in the same population cages for a number of successive generations. The populations were set up with 50 adults each at every generation. At every generation after the first, those flies in excess of 50 which hatched from each population and all the heterozygotes between the populations were used to test for the accumulation of genetic damage. This genetic damage was induced by irradiating, at the commencement of each generation, all the adults from one of the populations with 1500r X rays.—Under these conditions sterility increased at approximately 1% per generation and there was a slow but definite accumulation of sex-linked lethals. Induction of second chromosome recessive lethals was about 6% per generation but the net rate of accumulation appeared to be about 4.4%. By generation 25 equilibrium was almost reached at just below 70% recessive lethal chromosomes, whereas a set of calculations using some extracted population parameters suggested equilibrium might be reached at about 55%. Further studies were confined to this class of genetic changes. Allelism matings were carried out between selections of lethals retained at each generation and these showed a high degree of identity of lethals, with a rate of elimination considerably in excess of the rate of induction. It was concluded that some lethal mutations must have marked heterozygous advantage to account for these two discrepancies. These conclusions were supported by results in a population where radiation was suspended. Among the heterozygotes between the two populations, however, the number of lethals was lower than expected and in this case it is suggested that the discontinuous genetic background resulted in stronger selection against nearly all lethals.

APPENDIX

At the commencement of the experiment the frequency of lethal mutants is zero, 6% lethal mutations are, therefore, induced at the gametic stage, which is the stage irradiated.

With the formation of zygotes 6% × 6% will carry 2 lethals. The random likelihood that these two mutations are at the same locus and are therefore eliminated is, according to previous work, 0.25% (IVES 1945; WALLACE 1950; PROUT 1954). At the next irradiation slightly fewer new mutations are induced, i.e., 6% of non-lethal chromosomes which are at a frequency of just over 94% i.e., 100%—[6%(6% × 6% × 0.25%)] With the formation of zygotes the mutations from the first generation come together and are eliminated at the rate of $[0.06-(0.06^2 \times 0.0025)]^2 \times 0.0126$ since they have been inbred for 1 generation together. They are also eliminated because they are occasionally at the same loci as those induced in the second generation. The chance of this is, of course, the purely random one of 0.25%. After the third irradiation the mutations left from the first generation have a likelihood of 2.28% of being allelic among themselves, those of the second a 1.27% chance. Mutations from the third generation have a 0.25%

chance both of being allelic among themselves and with mutations from either the first or second generation but mutations from the first and second generations have now been inbred together for one generation and have a 1.27% likelihood of allelism. The same processes are deemed to occur throughout the course of 25 generations. The calculations get no more complex only considerably longer.

The two flow diagrams constructed by Mr. D. G. PAPWORTH to cover this situation and that for the last 5 generations when irradiations are suspended, and, therefore, no new mutations occur, are given below. (Figures 4 and 5). A programme based on these flow diagrams was

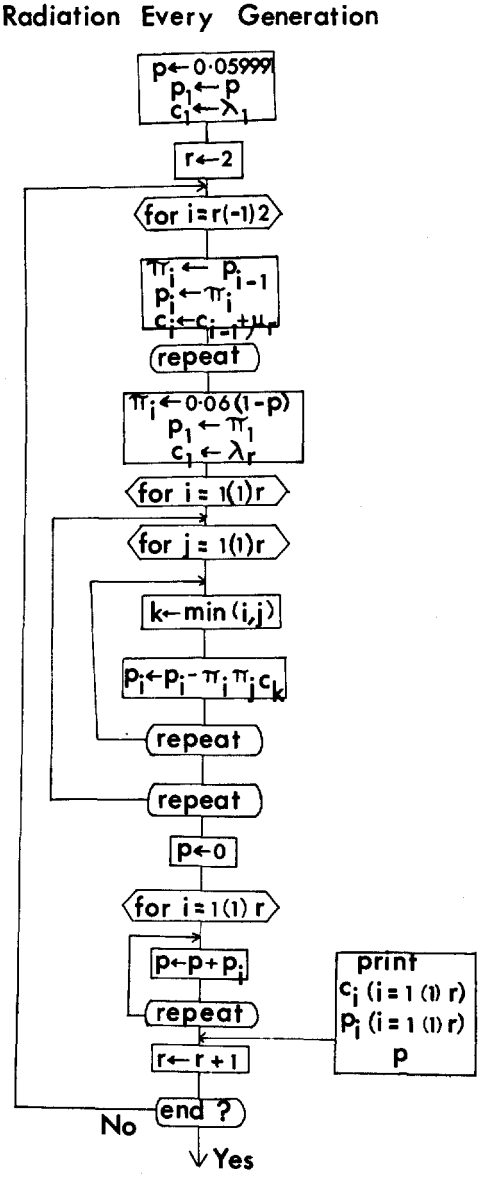

FIGURE 4.—Flow diagram for calculations of the accumulation of recessive lethal chromosomes given radiation every generation.

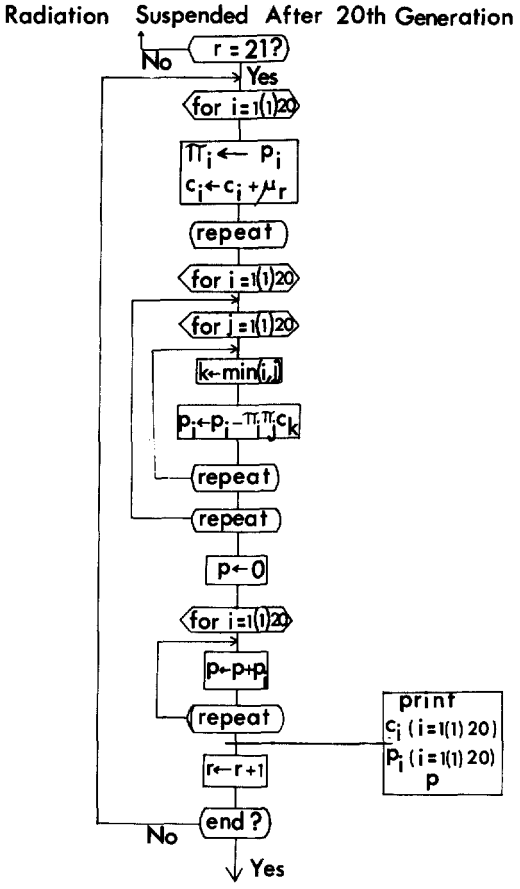

FIGURE 5.—Flow diagram for calculation of the change in frequency of recessive lethal chromosomes after the suspension of irradiation.

Notation for flow diagrams

π_i = proportion of lethals at *beginning* of generation r that were induced in generation r — i + 1. P_i = same π_i, but at *end* of generation r. c_i = elimination factor during generation r for lethals that were induced in generation r — i + 1. p = overall proportion of lethals at end of generation r. r = current generation number. μr = proportion inbreeding in r. r = likelihood of allelism of chromosomes in generation r. i,j,k = are running indices.

written for the Atlas computer. The results to which these schemes give rise are far too bulky to be given in full but an extract is given showing the progress of mutations induced at irradiations 1, 5, 10, 15 and 20 under regular irradiation. Alongside this are shown the behaviour of the same groups of lethals supposing the last five generations to be without further irradiation.

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TABLE 10

The fate of lethals induced at different generations i.e. percent induced and their subsequent reduction

Generation	1		5		10		15		20	
	Irradiated	Suspended	Irradiated	Suspended	Irradiated	Suspended	Irradiated	Suspended	Irradiated	Suspended
1	6.00	...	...	...	...	...	...	...	...	...
2	5.99	...	...	...	...	...	...	...	...	...
3	5.98	...	...	...	...	...	...	...	...	...
4	5.96	...	...	...	...	...	...	...	...	...
5	5.92	...	4.69	...	...	...	...	...	...	...
6	5.86	...	4.67	...	...	...	...	...	...	...
7	5.79	...	4.64	...	...	...	...	...	...	...
8	5.69	...	4.59	...	...	...	...	...	...	...
9	5.58	...	4.52	...	...	...	...	...	...	...
10	5.44	...	4.43	...	3.53	...	...	...	...	...
11	5.28	...	4.33	...	3.51	...	...	...	...	...
12	5.11	...	4.21	...	3.47	...	...	...	...	...
13	4.91	...	4.07	...	3.41	...	...	...	...	...
14	4.70	...	3.91	...	3.33	...	...	...	...	...
15	4.48	...	3.74	...	3.24	...	2.90	...	...	...
16	4.25	...	3.57	...	3.14	...	2.88	...	...	...
17	4.01	...	3.38	...	3.02	...	2.84	...	...	...
18	3.77	...	3.19	...	2.89	...	2.78	...	...	...
19	3.53	...	3.00	...	2.75	...	2.71	...	...	...
20	3.29	...	2.81	...	2.61	...	2.63	...	2.71	...
21	3.06	3.06	2.62	2.62	2.46	2.46	2.53	2.53	2.68	2.69
22	2.84	2.84	2.44	2.44	2.32	2.32	2.42	2.43	2.64	2.65
23	2.62	2.63	2.26	2.27	2.17	2.18	2.31	2.32	2.59	2.60
24	2.42	2.25	2.09	2.11	2.02	2.04	2.19	2.21	2.52	2.53
25	2.22	2.25	1.93	1.95	1.88	1.91	2.07	2.10	2.43	2.46

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