POPULATION SIZE AND GENETIC LOAD IN IRRADIATED POPULATIONS OF *DROSOPHILA MELANOGASTER*

HAMPTON L. CARSON

Department of Zoology, Washington University, St. Louis, Missouri

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MUTATIONS are widely accepted as the basic raw materials from which evolutionary adjustments are forged by natural selection. When the gene pool of a competent population faces an environmental challenge, selection may be expected to incorporate any new variants which favorably affect the fitness of their carriers.

Using a design which permits frequent measurement of population size under strictly controlled conditions, SMATHERS (1960), CARSON (1961), and CANNON (1963) have shown that increased adaptation to the existing conditions, as evidenced by increased population size and production, can be observed directly in experimental populations of *Drosophila melanogaster*. In these experiments, genetic variance has been increased by the additions of small amounts of foreign genetic material by outcrossing. The microevolutionary adjustments within the gene pool that follow are evidently based on the quick incorporation of this foreign genetic material into heterotic systems.

The experiments reported in this paper are designed to test the effect of raising the genetic variance in experimental populations in a different way, namely, by the use of large doses of X rays delivered frequently to all the adults in the population. The populations were thus closed ones in which the mutation rate was artificially maintained at a very high level. Under these conditions, the experimental populations represent a sensitive system for the detection of the effects of newly induced genes that cause increase in fitness, whether these effects may be manifested in the heterozygous condition, the homozygous condition, or both. The results clearly show that there is no sustained increase in fitness despite an increased genetic variance as indicated by an observed increase in genetic load. There is thus no evidence for single-gene heterosis or for the induction of new genes having a favorable effect on any of the fitness characters.

MATERIAL AND METHODS

Four replicate base populations were used in these experiments; all flies were homozygous for five third-chromosome recessives: sepia (*se*), spineless (*ss*), ebony-sooty (*e^s*), kidney (*k*) and rough (*ro*). The mode of derivation of these four populations is given in Figure 1 of CARSON (1961). The two control populations used in the present work, and called here Populations A and C, are the same as those designated as C1-g and C-1h in CARSON (1961). The two populations which received X rays, called Populations B and D, are directly descended from the populations labelled E-3 and E-4 in the figure referred to above. Suffice it to repeat here that all flies are ultimately descended from a single mutant strain which has been maintained for many years in the

laboratory and is thus presumably highly inbred. Flies were first introduced into the experimental population system in January 1957, and the four replicate populations used in the present experiments were established in February 1958.

Every detail of the vial population system described by CARSON (1961) has been followed in the maintenance of all four populations. Briefly, the adults were maintained in a food vial to which is taped a cellulose-acetate chamber or "supervial." In these experiments short (95 mm) supervials were used. Adults are changed to a fresh food vial four times a week (e.g. Monday, Wednesday, Friday, and Saturday). Each vial, as it comes off the adult population, is plugged and set aside until flies start hatching. All flies hatching in the back vials are added at regular intervals to the adult population, each time it is opened for changing. The vial which is removed on Saturday, under the above cycle, is designated as "extra" and is either discarded or used for samples. The vials contain measured amounts of food which is kept as uniform as possible (CARSON 1961).

This system permits the following data to be obtained: (1) the weight and number of the flies in the "front-running" adult population, measured on a stated day and hour each week; (2) the number and weight of all young flies hatching in the population ("production"). The experiments were continued for 3½ years. This is a total of 160 weeks or 80 generations. The populations were discarded in June 1961. Weighings were made on a Roller-Smith torsion balance having a capacity of 500 mg.

THE EXPERIMENTS

The control level: Two populations, designated A and C, were maintained as controls without radiation for the entire period of the experiment. They always received identical change treatment and food supply to that of experimental Populations B and D and were always handled at precisely the same time.

The weekly weights of the two control populations (A and C) are shown in Figure 1 for the 160 weeks (80 generations) of the duration of the experiment. The controls have remained close to one another throughout the experiment. During the first 40 weeks, the mean weight is slightly above that found thereafter. This is attributed to slight differences in handling over the years, that is, to environmental factors. For example, during the period between week 25 and week 38 when both controls were elevated, a bias was discovered in the weighing of the yeast, such that all populations, both controls and experimentals, were receiving above the stated amount of 10 mg per vial. When this was corrected, the trend toward increased size was reversed. As all populations were apparently equally biased by this error, the result should not be affected.

The irradiated populations: Two experimental Populations, B and D, were maintained in a fashion exactly parallel to that of the controls except that over the first 2½ years they received 65,000r of X-radiation. Radiation was applied to all adult flies in the population once a week for periods of 20, 10, 10 and 25 weeks (black bars in Figures 2 and 3). The flies were placed in a shallow plastic tube covered with a fine screen of nylon marquisette and were irradiated with a North American Philips Company CT unit No. 11606. During the first three periods of weeks mentioned above, radiation was applied once per week in a single dose of 1000r; during the final 25-week period of radiation, the same amount was given in two doses of 500r each per week. The dose was applied in one minute (for 1000r units) from the unshielded tube at one milliamperere.

The sizes of the two radiated populations are plotted in Figure 2 (first 80

FIGURE 1. (Top)—Size of two control populations for the duration of the experiment (160 weeks). The time at which the three viability tests were made is indicated. FIGURE 2. (Lower left)—Size of irradiated Populations B and D over the first 80 weeks of experiment. The black bars at the top of the figure indicate periods during which X rays were applied at the rate of 1000r per week. The size of each irradiated population is given in milligrams wet weight above (+) or below (—) the mean level of the controls. FIGURE 3. (Lower right)—Size of irradiated populations B and D during the final 80 weeks of the experiment. The time at which the three viability tests were made is indicated at the lower right.

weeks) and Figure 3 (second 80 weeks). In these graphs, the level of each of the two experimental populations is given in terms of its size in milligrams above (+) or below (−) the mean level of the two control populations at the same time.

Under heavy radiation, the experimental populations are deeply depressed in size (Figures 2 and 3). When radiation is relaxed, both experimentals rise again close to the level of the controls. On two such occasions, however, Population D rose above the other populations and sustained this higher level over a number of generations. Two of these periods are documented in greater detail in Tables 1 and 2.

During the final year of the experiment, three sets of egg samples for viability tests were obtained from each population. Because the "extra" vial described previously was used for these samples; their removal did not affect the size of the populations. The times at which these tests were made are indicated in Figures 1 and 3. On removal from the population, the eggs which had been laid on the food cake were counted and then adults were reared from these eggs under ideal conditions. These data (Table 3 and Figure 4) thus afford a measure of the percent inviability between egg and adult for each period. Irradiated Populations B and D at first show a higher percent inviability than the control populations. During the course of the year, however, this difference from the control populations disappears, so that at the time of the termination of the experiment, controls and experimentals did not differ in this regard.

Heavy continuous irradiation reduces the size of the experimental populations drastically, as can be seen in Figures 2 and 3. During the periods of reduction, there is a striking increase in the size of the flies in the irradiated populations. Figure 5 shows the mean fly size in milligrams wet weight for the experimental populations (solid lines) and controls (dotted lines) over approximately the first

TABLE 1
Mean size of populations during Weeks 49–58 (Figure 2)

Population	Population size mean wet weight (mg/week)	Results of t-tests
A (control)	75.9 ± 1.8	A > B: t = 3.669; P < .01
C (control)	71.8 ± 2.1	C = B: t = 1.852; P > .05
B (irradiated)	66.8 ± 1.7	
D (irradiated)	91.6 ± 1.4	D > A: t = 6.886; P < .001

TABLE 2
Mean size of populations during Weeks 114–145 (Figure 3)

Population	Population size mean wet weight (mg/week)	Results of t-tests
A (control)	74.13 ± 2.39	
C (control)	81.50 ± 2.51	C > A: t = 2.133; P = .02–.05
B (irradiated)	71.47 ± 2.84	
D (irradiated)	100.56 ± 3.15	D > C: t = 4.776; P < .001

TABLE 3

Percent of eggs failing to give adults (inviability) in each population for three periods during the final year

	Population	Total number of eggs	Eggs failing to give adults		Results of chi-square tests
			Number	Percent	
First tests					
Controls	A	2734	1104	40.38	A>C: $\chi^2 = 5.79$; P<.05
	C	1610	591	36.71	
Experimentals	B	1612	821	50.93	D>B: $\chi^2 = 22.93$; P<.01
	D	1797	1062	59.10	
Second tests					
Controls	A	1031	379	36.76	C>A: $\chi^2 = 20.65$; P<.01
	C	1451	666	45.90	
Experimentals	B	802	428	53.37	B>D: $\chi^2 = 6.00$; P<.01
	D	1416	679	47.95	D = C: $\chi^2 = 1.27$; P = .10-.20
Third tests					
Controls	A	94	32	34.04	C>A: $\chi^2 = 5.57$; P<.05
	C	718	337	46.94	
Experimentals	B	277	115	41.52	B = D: $\chi^2 = 3.40$; P>.05
	D	589	284	48.22

FIGURE 4.—Percent eggs failing to give adults in three sets of viability tests made during the last year of the experiment. The control populations (A and C) are represented by open columns and the irradiated populations (B and D) by closed columns.

FIGURE 5.—Mean fly-size in irradiated (solid lines) and control (dotted lines) populations during three periods of radiation treatment.

60 weeks of the experiment. Whereas the size of the individuals in the control populations remains close to .600 mg, the size of the flies in the irradiated populations is much greater, being frequently above .750 mg. This effect appears to be due to the fact that competition for the limited food supply is much less when the population size is reduced by irradiation. Surviving flies, therefore, are able to grow to a much larger size than in the crowded control populations.

DISCUSSION

The sizes of the control populations have shown little if any significant long-term increases or decreases for the 80 generations of this experiment. A similar result was observed for comparable control populations by CARSON (1961) and CANNON (1963). When varying amounts of foreign genetic material are introduced into such inbred, stable populations, however, an immediate large increase in population size is observed. This is sustained for many generations although some decline in size has been observed in some long-term experiments (CARSON 1961). Population size increase is not only immediate but may occur even when recombination is essentially inhibited by the use of inversions. Balanced polymorphism for certain oligogene markers has been observed in many experiments. These facts have led to the conclusion (CANNON 1963) that these effects are due to a luxuriant type of heterosis which is rapidly produced, sustained over a period of generations and does not appear to involve any period of evolutionary coadaptation. The heterosis appears to be based on the heterotic effects of polygene blocks rather than on the oligogene loci themselves (SMATHERS 1960).

The present experiments provide no evidence to indicate that irradiation may provide genetic variation through mutation on which similar heterotic adjustment systems may be built. Thus, despite the very large number of genetic

variants undoubtedly introduced into the population by the nearly continuous radiation, controls and experimentals did not differ at the termination of the experiment. As a source of genetic diversity upon which fitness changes may be built, therefore, hybridization appears to be far superior to radiation induced mutations.

One of the experimental populations (D, Figures 2 and 3) showed several significant but transitory rises in population size. These were sustained for a number of generations; the second of these is especially striking and requires explanation. The possibility that newly induced heterotic genes were temporarily serving to increase fitness cannot be excluded. The writer considers it more likely that the effect is a spurious one, that is, unrelated to fitness. It is possible, for instance, that the size of the population might be temporarily swelled by long-lived sterile flies of one or both sexes.

WALLACE (1952) found that the adaptive values for several characteristics of flies from an irradiated population were above those of a corresponding control population. He suggested that artificially induced genetic variants had been included in the adaptive fabric of this population. The same author (1958, 1963) has presented evidence which he interprets as indicating that newly induced mutations, tested in the heterozygous condition, increase the average viability of otherwise highly homozygous individuals. WALLACE's interpretations of the data, however, have been questioned (e.g. MULLER and FALK 1961; FALK 1961). The present experiments provide a favorable system for the detection of the effects that any such new genetic variability might have on fitness. The absence of any sustained increases in fitness in the irradiated populations, therefore, indicates that increased viability effects of newly induced mutations, if they indeed are real, cannot be easily incorporated into the gene pool and affect fitness. Accordingly, the present data provide no support for WALLACE's conclusions.

In the present experiments, 65,000r were delivered to each of two populations homozygous for five third chromosome recessives. The appearance of an induced reversion could be relatively easily detected. In fact, the design of the experiments was specifically chosen and periodic examination of the phenotypes in the populations was made with this possibility in mind. If a mutation rate constant of 2×10^{-8} per r per locus is assumed, then the cumulative radiation induced mutation rate would be on the order of $2 \times 10^{-8} \times 65,000$, or 1.3×10^{-3} . A mutation rate of such magnitude should have produced some reversions and one might expect natural selection to favor such alleles. No such result, however, was obtained.

A few generations following the cessation of radiation, both experimental populations show a greater amount of inviability between egg and adult than the controls. This is ascribed to a genetic load of lethal or viability-reducing genes induced by the radiation. Tests have shown, however, that the experimental populations quickly divest themselves of this load so that after one year their genetic load, due to this type of gene, is not different from the controls. This is attributed to the efficiency with which natural selection combined with random drift in small populations is able to rid such a population of deleterious genes.

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SUMMARY

Two experimental populations of the sepia-spineless-rough stock of *Drosophila melanogaster* each received 65,000r of X rays over a period of 2½ years. Two control populations were handled in an identical manner but received no radiation. All four populations were discarded one year after the cessation of irradiation of the experimentals. The method of handling permitted weekly measurements of population size of all populations, affording a measure of fitness of the populations.

As expected, the size of the experimental populations declines under radiation. Population size is recovered rapidly when radiation is suspended. Although one of the experimental populations maintained an elevated fitness level at several times for a number of generations, these effects were reversed and at the end of the experiment, controls and experimentals did not differ from one another. Following the cessation of the irradiation, the genetic loads of the irradiated populations were found to be elevated relative to the controls. These increased loads, however, disappeared within a year.

Newly induced mutations do not appear to provide an important source of genetic diversity whereby fitness can be improved in experimental population systems where natural selection is operating.

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