EVOLUTION OF EXPERIMENTAL "MUTATOR" POPULATIONS OF *DROSOPHILA MELANOGASTER*

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Manuscript received May 27, 1975
Revised copy received September 2, 1975

ABSTRACT

The theory of evolution predicts that the rate of adaptation of a population is a function of the amount of genetic variation present in the population. This has been experimentally demonstrated in Drosophila populations in which genetic variability was increased either by mass hybridization of two gene pools, or by X-irradiation.—Mutator genes increase the spontaneous mutation rates of their carriers. We have now studied the effects of a third-chromosome mutator gene, *mt*, on the rate of adaptation of laboratory populations. Initially, experimental and control populations had similar genetic constitutions except for the presence or absence of the *mt* gene. The populations were maintained for 20–25 generations by "serial transfer" under conditions of very intense selection.—The number of flies produced per unit time remained constant throughout the experiment in the experimental as well as in the control populations. However, in the mutator-carrying populations the average longevity of the flies (and consequently the average population size) gradually decreased. Under the experimental conditions natural selection is unable to counteract completely the increased input of deleterious mutations due to the *mt* gene.

GENETIC variation is a necessary condition for natural selection to occur. It seems intuitively obvious that the larger the amount of genetic variation in a population, the greater the opportunity for natural selection to occur. AYALA (1968a, 1969a, and references therein) has shown experimentally that populations with greater initial stores of genetic variation may increase their adaptation to the environment at a faster rate than populations with lesser initial amounts of genetic variation. Genetic variability was increased in two ways: (1) by mass hybridization between flies collected in two different natural populations (AYALA 1965a, b) by subjecting large numbers of flies to high doses of X-rays for three generations prior to the start of the experimental populations (AYALA, 1966a, 1969b). However, CARSON (1964) did not observe adaptive increases in experimental populations of Drosophila that were subject to irradiation at several intermittent periods throughout many generations.

We report here a study of experimental populations with genetic variability increased in still another way, i.e., by the presence of a mutator gene. A mutator gene may be simply defined as a gene that increases the spontaneous mutation rates of its carriers. Mutator genes have been found in a variety of organisms.

They have been identified in several species of Drosophila (DEMEREC 1937; NEEL 1942; MAMPEL 1943; IVES 1950; KIDWELL, KIDWELL and NEI 1973; MUKAI *et al.* 1974; a review in GREEN 1973). Mutator genes are also known in microorganisms, such as *Escherichia coli* (COX 1973), *Salmonella typhimurium* (MIKAYE 1960), *Saccharomyces cerevisiae* (VON BORSTEL *et al.* 1973), *schizosaccharomyces pombe* (LOPRIENO 1973), and in T4 bacteriophage (DRAKE 1973).

The adaptive role of mutator genes is far from understood, notwithstanding their being apparently common in natural populations. Cox and GIBSON (1974) have found that mutator strains of *Escherichia coli* are better competitors than their mutator-free counterparts. We have undertaken to study the effects of a mutator gene on the adaptation of populations of a multicellular organism, *Drosophila melanogaster*. We wanted to test whether increased mutation rates in mutator-carrying strains would result in increased rates of adaptation to an experimental environment.

MATERIALS AND METHODS

Strains: The mutator gene used in these experiments is the third-chromosome gene *mt* (III, 57–58) in *Drosophila melanogaster* (GREEN 1970). Some of its properties are: (1) it appears to function only in females; (2) it is semidominant; (3) it increases primary nondisjunction; (4) it decreases crossing over in the *X* chromosome; and (5) homozygous *mt/mt* females exhibit approximately a threefold increase in the frequency of sex-linked recessive lethal mutation rates (GREEN 1970; GREEN and LEFEVRE 1972; GOLD and GREEN 1974).

We initially had two strains of *D. melanogaster* thought to be of similar genetic constitutions except for the presence of the *mt* gene or of its wild-type, *mt*+, allele. A series of crosses was nevertheless carried out to homogenize the genetic background of the mutator and mutator-free experimental strains, as follows (see LINDSLEY and GRELL 1967, for details about the markers):

(1) $(mt)/(mt)$ mutator strain, *M*.

$$\mathrm{P}\ \varphi\varphi\ \frac{(mt)}{(mt)} \times \frac{ry\ Sb}{ry^{X1}Ubx^{130}}\ \delta\delta$$

$$\mathrm{F}_1\ \varphi\varphi\ \frac{(mt)}{ry\ Sb} \times \frac{ry\ Sb}{ry^{X1}Ubx^{130}}\ \delta\delta$$

$$\vdots$$

$$\mathrm{F}_5\ \varphi\varphi\ \frac{(mt)}{ry\ Sb} \times \frac{(mt)}{ry\ Sb}\ \delta\delta$$

$$\mathrm{F}_6\ \varphi\varphi\ \frac{(mt)}{(mt)} \times \frac{(mt)}{(mt)}\ \delta\delta$$

$$\downarrow$$

$(mt)/(mt)$ strain

(2) $(mt+)/(mt+)$ wild-type strain, *W*.

The crosses were the same as for (1), except that $(mt+)$ should be replaced wherever (mt) appears.

Ubx^{130} is a third chromosome balancer. The gene *mt* maps very near the location of *Sb*, and no crossing-over has been detected between the two; in any case, tests described below confirm the presence of the *mt* gene in the mutator populations used in the experiment. The *M* (mutator) and *W* (wild-type) strains recovered at the end of the series of crosses are genetically similar to each other except for a small chromosomal segment containing the *mt* locus. Elsewhere, the

genetic constitution of the *M* and *W* strains has been derived from the *ry Sb/ry*^{XI} *Ubx*^{130} stock. in the crosses, the *mt* and *mt*+ genes are shown in parentheses to indicate that they cannot be visibly differentiated.

Test for detecting the mutator gene: The test to verify the presence of the mutator is based on a property of the *mt* gene, namely that it increases primary nondisjunction in females. The females (wild in phenotype) to be tested are crossed with males *y Hw w/B*^{S}*Y*, i.e., ♀ ♀ +/+ × *y Hw w/B*^{S}*Y*. The "exceptional" offspring are sorted out as follows:

	Genotype	Phenotype
Female nondisjunction:	+/+*B*^{s}*Y*	*B* female
	0/*y Hw w*	*y Hw w* male
	+/*y Hw w/B*^{s}*Y*	*Hw B* female
Male nondisjunction:	+/0	+ male

The *y Hw w* ♂ ♂ and *B* ♀ ♀ should be present in higher frequencies in the progenies of mutator strains than in the controls. Our wild and mutator strains were crossed as above and the numbers of "exceptional" males (*y Hw w* ♂ ♂) were compared by means of a 2 × 2 contingency table (see RESULTS).

Experimental design: Two experimental mutator populations (*Ma* and *Mb*) and two wild populations (*Wa* and *Wb*) were established. Each population was started with 500 pairs of adult flies. The experimental populations were kept in half-pint bottles at 25°, using the serial transfer technique (CARSON 1958; AYALA 1965a). The bottles contain measured amounts of cornmeal and molasses Drosophila medium, with a double piece of Kleenex tissue, 5 × 18 cm, partially pressed into the medium to provide additional resting surfaces for the adult flies and for pupation. The adult flies were introduced in a bottle and allowed to oviposit for three days, after which they were transferred without etherization to a new bottle; after four days, the adult flies were transferred again to a fresh bottle. For each experimental population, this cycle was repeated throughout the experiment: the adult flies in the population were transferred to a new culture twice a week, at alternating periods of three and four days. When emergence of adult flies began in the cultures where eggs had been laid, the young flies were collected twice a week, on the same days in which the adult population was transferred to a new bottle with fresh food. These young flies were etherized, counted and added to the adult population immediately after its transfer to a new bottle. The adult ovipositing flies were thus always in a single bottle with fresh food, whereas 10 additional bottles in each series contained eggs, larvae and newly hatched adults. Every week, after the four-day interval, before adding the newborn flies emerged during the previous period, the adult population was etherized, counted and then transferred to a fresh bottle. Flies began to emerge around the 11th day after oviposition and mass emergence occurred between the 14th and 17th day; thereafter it fell off rather quickly. The bottles were discarded on the 35th day.

The flies were individually counted during the first 25 weeks of the experiment. Thereafter, samples with 200–300 flies of either adult or newborn flies were counted and weighed, and the rest were weighed; the total number of flies was then estimated by a simple proportion. The weighings were made in a balance with a 0.1 mg precision. The cultures were kept in a constant temperature incubator at 25 ± 0.5°, but were exposed to room temperature for no more than three hours per week, while being counted and weighed. The four populations were started all on the same day in October 1972 and ended in March 1974.

RESULTS

The experiment has been designed to study the effect of the third-chromosome mutator gene in homozygous condition (*mt/mt*) upon the adaptedness of *D. melanogaster* populations carrying it. The null hypothesis is that no such effect exists. Homozygous *mt*$^{+}$/*mt*$^{+}$ *D. melanogaster* lines were used as controls. Four

variates are measured in each population: *productivity*, i.e., number of flies produced per week; *survivorship*, i.e., number of flies surviving from the previous transfer; *population size*, which is simply the number of adults surviving from the previous transfer plus the productivity during the previous week; and *longevity* of the flies in the adult population, estimated according to the method given below.

The weekly counts for productivity, survivorship, and population size are given in Figure 1 for the experimental population *Ma*, and in Figure 2 for the control population *Wa*. The data are very similar for populations *Ma* and *Mb*, and also for populations *Wa* and *Wb*. Very rapidly, all four populations reached productivities of about 700 flies per week, survivorships somewhat above 1,000 flies, and population sizes of 1,700 or higher. Means and regression coefficients from week seven to the end of the experiment (week 70) are given for the various parameters in Tables 1 and 2. Standard errors have been computed using the regression mean square error (SOKAL and ROHLF 1969). The regression lines have been drawn in the figures.

The experimental and the control populations behave differently in various respects. In the experimental populations, *Ma* and *Mb*, the mean productivity per week is about 710 flies, and remains fairly constant throughout the experiment. The regression coefficients for productivity (-0.12 ± 1.01, and -1.40 ± 1.11 flies per week, for populations *Ma* and *Mb*, respectively) are not significantly different from zero. The survivorship and population size of the two ex-

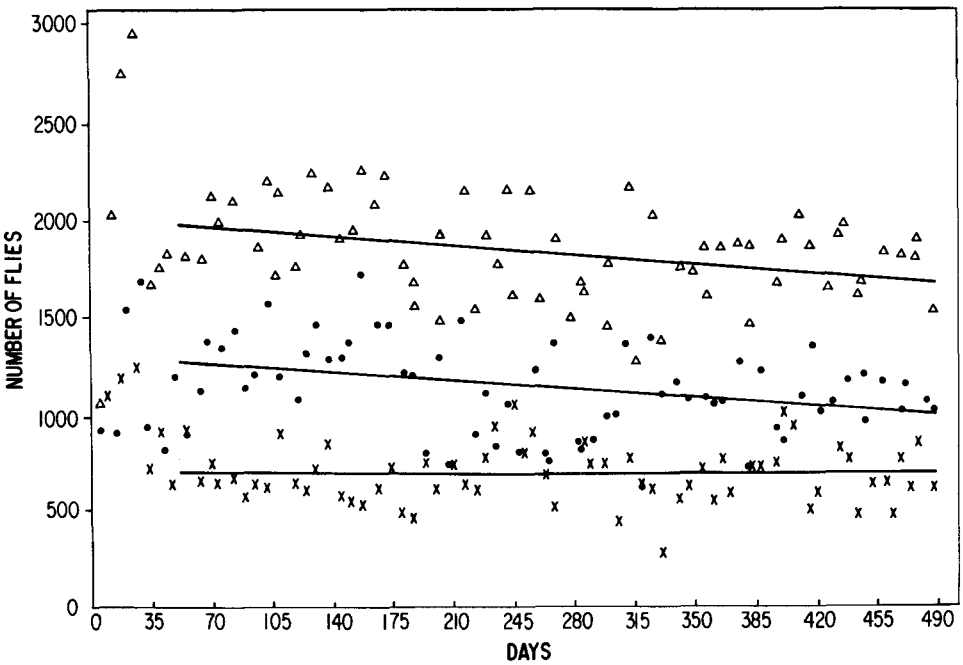

FIGURE 1.—Weekly counts for productivity (crosses) survivorship (circles), and population size (triangles) in the mutator-carrying population *Ma*. The regression lines for the measurements between weeks 7 to 70 have been drawn.

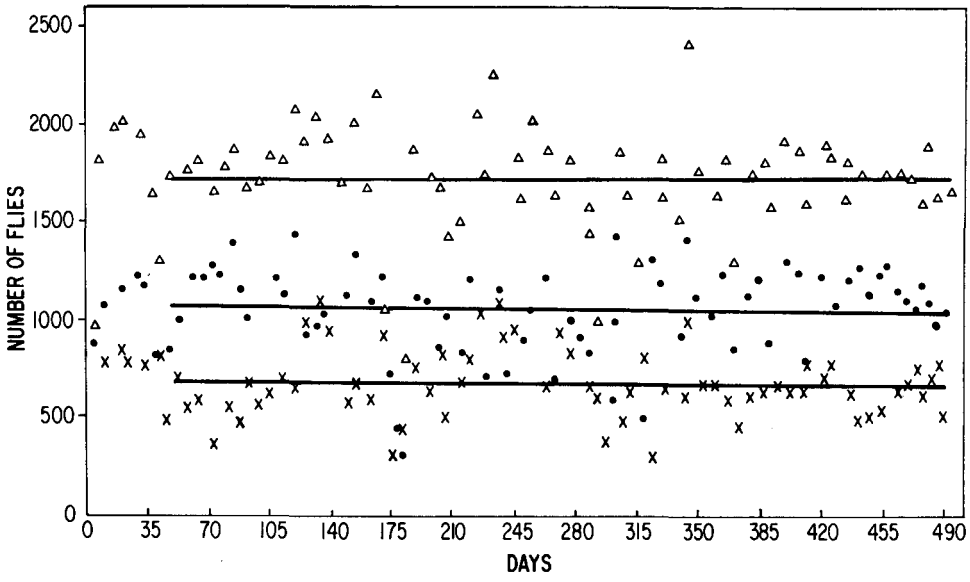

FIGURE 2.—Weekly counts for productivity (crosses) survivorship (circles), and population size (triangles) in the control population *Wa*. The regression lines for the measurement between weeks 7 to 70 have been drawn.

perimental populations gradually decreased throughout the experimental period. In the early generations, survivorship is about 1200–1300 flies, but it decreases to about 1000 by week 70. The regression coefficients for survivorship are significantly negative: -4.16 ± 1.49 and -3.32 ± 1.40 flies per week for populations *Ma* and *Mb* respectively. That is, from week 7 to 70 the average number of survivor flies decreases by 262 and 209 flies (or about 20% of the mean) in *Ma* and *Mb* respectively. The mean population sizes of the experimental populations are about 1800 flies, but again there is a statistically significant gradual decrease in numbers. The regression coefficients are significantly negative (-4.38 ± 1.49 and -4.72 ± 1.74 for *Ma* and *Mb* respectively); the average

TABLE 1

Mean productivity, mean survivorship, mean population size and regression coefficients (b) *of productivity, survivorship and population size on time for mutator populations* Ma *and* Mb *at 25°*

	Ma		*Mb*	
	Mean±S.E.	b±S.E.	Mean±S.E.	b±S.E.
Productivity	701.8±18.5	−0.12±1.01	718.0±20.5	−1.40±1.11
Survivorship	1142.1±27.4	−4.16±1.49**	1088.6±25.8	−3.32±1.40*
Population size	1843.9±27.5	−4.38±1.49***	1806.6±32.1	−4.72±1.74**

All the statistics are based on 64 observations (weeks 7 to 70). Units for *b* are flies/week. S.E. is the standard error.

* $0.01 \leq P \leq 0.025$
** $0.005 \leq P \leq 0.001$
*** $P \leq 0.001$

TABLE 2

Mean productivity, mean survivorship, mean population size and regression coefficients (b) of productivity, survivorship and population size on time for wild populations Wa *and* Wb *at 25°*

	Wa		*Wb*	
	Mean±S.E.	b±S.E.	Mean±S.E.	b±S.E.
Productivity	664.8±23.3	−0.57±1.27	674.7±24.5	−0.05±1.34
Survivorship	1047.4±28.2	−0.26±1.56	1055.2±27.8	−1.01±1.51
Population size	1712.2±33.8	−0.83±1.84	1729.8±30.5	−1.05±1.66

All the statistics are based on 64 observations (weeks 7 to 70). Units for b are flies/week. None of the regression coefficients is statistically significant. S.E. is the standard error.

numbers of flies decreased by 276 and 297 flies (or 15% and 16% of the mean) between weeks seven and 70. Standard tests for homogeneity indicate that the two experimental populations behave similarly to each other.

As in the experimental populations, the productivity of the control populations remains constant throughout the experiment. The mean numbers of flies produced per week are 665 and 675, and the regression coefficients -0.57 ± 1.27 and -0.05 ± 1.34, respectively for *Wa* and *Wb*. However, the survivorship and population size of the two control populations also remain constant throughout the experiment, rather than gradually decreasing as was the case for the mutator populations. In populations *Wa* and *Wb*, the mean survivorship is about 1,050 flies, and the mean population size about 1720; the regression coefficients for survivorship and for production size are for both populations small and not significantly different from zero. The two control populations, *Wa* and *Wb*, are not significantly different from each other with respect to any of the parameters measured.

The analyses just given indicate that survivorship and population size decrease at a statistically significant rate through time in the mutator populations, but not in the controls. We have tested this difference in another way. For each week the counts of *Wa* and *Wb* are added together and their total is subtracted from the total of *Ma* plus *Mb*. That is, we have obtained a new statistic, d, expressing the difference in numbers between the experimental and the control populations: $d = (Ma + Mb) - (Wa + Wb)$. The regression coefficient of d (in flies per week) is, for population size -7.25 ± 2.85 ($t_{62} = 2.57$, $P < 0.02$), and for survivorship -6.21 ± 2.49 ($t_{62} = 2.49$, $P < 0.02$). Thus, the mutator populations are decreasing in numbers at a rate significantly greater than the control populations. The mean value of d is, for population size 211 ± 54 ($t_{63} = 3.89$, $P < 0.001$), and for survivorship 128 ± 48 ($t_{63} = 2.67$, $P < 0.01$). Thus the mean number of flies is significantly greater in the mutator than in the control populations. The positive mean difference in numbers may be due to differences in genetic background not completely eliminated by the series of backcrosses carried out just before starting the experimental populations.

The mean longevity of the flies in the adult populations was estimated using the method given in AYALA (1965a; see also DOBZHANSKY and PAVLOVSKY 1961). If birth and death occur continuously at a constant rate, the mean longevity, m, is equal to the mean number of individuals, N, divided by the mean number, B, born per unit time. In the present case the death process is approximately continuous. The birth process, however, is essentially discrete. For the present purpose, "birth" is not the time of the biological birth but the time at which an individual enters the population. This takes place after intervals of three or four days in the experiment. Therefore, a correction factor is necessary to account for the discrete character of the "births" and $a = N/B$ is only a crude estimate of longevity. Assuming that the distribution of time of death is exponential, it can be shown that (see DOBZHANSKY and PAVLOVSKY 1961, Appendix):

$$a = \frac{N}{B} = \frac{4e^{-7/m} + 3e^{-4/m}}{1 - e^{-7/m}} .$$

Corrected estimates of longevity, m, were worked out for the "wild" and "mutator" strains and can be found in Table 3. For the "mutator" strains two values of m are given, one at week seven and another at the end of the experiment, i.e. week 70. The appropriate regression equations were used to predict the mean adult population sizes at those times. It can be seen that the mean longevity of adult flies decreased, during that interval, from 14.10 days to 11.17 days and from 13.72 days to 10.92 days for *Ma* and *Mb*, respectively. This is roughly equivalent to a decrease of 0.14 and 0.13 days per generation (assuming three weeks per generation).

Test for detecting the mutator gene: At week 70, virgin females were collected from *Ma* and *Wa* and then crossed with $y\ Hw\ w/B^{s}Y$ males, as indicated in the MATERIALS AND METHODS section. "Exceptional" $y\ Hw\ w$ males were scored among their male offspring with the following results:

	"exceptional" ♂♂	"normal" ♂♂	"exceptional"/total
Ma:	24	8735	0.0027
Wa:	9	9824	0.0009

TABLE 3

Estimated mean longevity, in days, of the adult flies in the various populations

Population	Mean longevity		
	a	$m—a$	m
Wa	11.03	2.09	13.12
Wb	10.95	2.08	13.03
Ma (week seven)	12.00	2.10	14.10
Ma (week 70)	9.10	2.07	11.17
Mb (week seven)	11.63	2.09	13.72
Mb (week 70)	8.86	2.06	10.92

a, crude estimate of longevity; $m—a$, correction factor; m, corrected mean longevity.

A χ^2 test showed that *Ma* and *Wa* differed significantly in their *y Mw w* male outputs ($\chi^2_1 = 7.78$, $P < 0.01$). The *mt* gene is known to increase the frequency of nondisjunction by a factor of three; that is exactly what we observe here. This confirms the presence of the mutator gene, *mt*, in the *Ma* experimental population. Given that the performances of *Ma* and *Mb* are identical, it can be safely assumed that the mutator *mt* was also present in *Mb*.

DISCUSSION

In terms of the rate of sex-linked lethal mutations, the effects of the third-chromosome mutator gene, *mt*, used in the present experiments, are equivalent to a dose of 100r of X-rays applied to the males per generation. Our experiments are different from those of AYALA (1966a, 1969a, b) and CARSON (1964) in various respects, including the fact that the mutagenic effects of the mutator gene are present throughout the experiment, while AYALA applied irradiation prior to starting the experimental populations, and CARSON allowed for periods of recovery between radiations. Our results are also different. We did not observe any significant changes in the number of flies produced per week, but survivorship and population size gradually decreased. With respect to the latter two parameters, natural selection was apparently unable to counteract completely the increased input of deleterious mutations in the mutator-carrying populations.

Productivity and survivorship are not necessarily correlated since they measure two different properties of a population, and may be limited by different factors—productivity by food, and survivorship by living space in the case of serial transfer populations (AYALA 1966b). It has been repeatedly observed in experimental populations (e.g., AYALA 1965b, 1968b) that survivorship responds more readily than productivity to the joint effects of selection and mutation. Such is the case also in our experiments. AYALA (1968a, b) has pointed out that Drosophila larvae have often been selected for competition in their natural environments, while the adults face quite a novel situation under crowded experimental conditions. We suggest that the lack of response of the mutator-carrying populations with respect to productivity may be the case because natural selection with respect to productivity is primarily "soft" selection (WALLACE 1967). Under a serial transfer regime, the limited amount of food available allows fewer than one per cent of the eggs laid to develop into adults. The zygotes carrying mutator-induced deleterious genotypes are included among the more than 99% that fail to reach adulthood whether the mutator gene is present or not. This hypothesis is supported by the lack of difference between the productivities of the mutator and the control populations. Since no significant differences in productivity exist between *Ma* and *Mb*, or between *Wa* and *Wb*, we have pooled each set of data (*Ma* + *Mb*, *Wa* + *Wb*). A *t* test indicates that the productivities of the two sets are not statistically different at the five percent level of significance.

With respect to the adult flies the situation is quite different. The average number of adults in the populations is above 1,000 flies, all living in a volume less than one half-pint; the mean longevity of an adult fly ranges between 11 and 14 days (Table 3). The genetic variants induced by the mutator gene have

deleterious effects that are not compensated by natural selection acting on the increased levels of variability. The net effect of the mutator gene is a cumulative reduction of the average longeivity of the flies, at a rate of about 0.13–0.14 days per generation.

In conclusion, the third-chromosome mutator gene, *mt*, decreases the average survivorship and population size of experimental populations of *D. melanogaster* maintained by serial transfer. Whether other mutator genes would have similar effects remains conjectual. The *mt* gene increases the frequency of deletions, besides being a segregation distorter. Mutator strains of *Escherichia coli* have been shown to be better competitors in a chemostat than strains lacking the mutator (Cox and GIBSON 1974). Furthermore, the superiority of the mutator strains correlates strongly with instabilities in the chemostat environment. The adaptive effects of the *mt* gene in *D. melanogaster* might conceivably be different in environments more heterogeneous at any one time, and more variable through time, than the laboratory environments used in our experiments.

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