FITNESS EFFECTS OF EMS-INDUCED MUTATIONS ON THE X CHROMOSOME OF *DROSOPHILA MELANOGASTER*. II. HEMIZYGOUS FITNESS EFFECTS[1]

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ABSTRACT

X chromosomes mutagenized with EMS were tested for their effects on the fitness of hemizygous carriers. The tests were carried out in populations in which treated and untreated X chromosomes segregated from matings between males and attached-X females; the populations were maintained for several generations, during which time changes in the frequencies of the treated and untreated chromosomes were observed. From the rates at which the frequencies changed, the fitness effects of the treated chromosomes were determined. It was found that flies hemizygous for a mutagenized chromosome were 1.7% less fit per mM EMS treatment than those hemizygous for an untreated chromosome. Since the same flies were only 0.5% per mM less viable than their untreated counterparts, the total fitness effect of an X chromosome carrying EMS-induced mutants is three to four times greater than its viability effect. By comparing the heterozygous effect of a mutagenized X chromosome on fitness with the corresponding hemizygous effect, the dominance value for the chromosome is estimated to be about 0.25.

MUTATIONS provide the raw material for evolution. However, most of them are harmful. Their debilitating effects have been studied primarily from the standpoint of viability (MUKAI 1964; MUKAI *et al.* 1972), but they are also known to affect other components of fitness, such as fertility (CHUNG 1962; TEMIN 1966).

We have investigated the total fitness effects of EMS-induced mutations on the X chromosome of *Drosophila melanogaster*. The effects were ascertained by monitoring the frequencies of treated and untreated chromosomes in multigeneration experiments. The induced hemizygous fitness effects are reported in this paper, while the heterozygous fitness effects are reported in the preceding one (MITCHELL 1977). In addition, single generation experiments described by MITCHELL (1977) allowed measurement of the effects of the induced mutants on viability alone.

MATERIALS AND METHODS

The stocks and mutagenesis procedures have been presented in MITCHELL (1977). Here we describe only those procedures peculiar to the determination of the hemizygous fitness effects of the treated chromosomes.

Two types of X chromosomes ($\gamma^1 w^a$ and $\gamma^2 v$) were mutagenized with EMS in such a way that 6 levels of treatment were obtained; in addition, untreated chromosomes of both genotypes were maintained in stock. By mating males carrying treated and untreated X chromosomes to attached-X females $(C(1)DX)$, it was possible to establish populations in which the two kinds of males competed with one another for food, space, and mates. The effects of the treated chromosomes on total fitness could then be determined by monitoring the frequencies of the two male types over time. This strategy worked because when males are mated to attached-X females, they transmit an X chromosome to their sons. This fact permits an analysis of male frequency changes based on a model of selection for an asexual organism; from the analysis, estimates for the fitness effects of the treated chromosomes could be obtained.

To establish the test populations, males carrying X chromosomes of both genotypes were collected from the viability tests described in MITCHELL (1977). These chromosomes were replicated by two generations of matings to $C(1)DX$ females. Then males carrying a chromosome of interest were mated to 50 $C(1)DX$ females in vials (about 25 males $\times$ 25 females per vial, 2 vials per chromosome). After two days, matings between $\gamma^2 v$ males and $C(1)DX$ females, and those between $\gamma^1 w^a$ males and $C(1)DX$ females were mixed in bottles. The mixing was done so that a treated $\gamma^2 v$ chromosome was placed with an untreated $\gamma^1 w^a$ one, and vice-versa. Males carrying these different X chromosomes can easily be distinguished from one another by eye phenotype (vermilion vs. apricot). A total of 360 populations were established, 60 from each level of EMS treatment. The two chromosomes involved in each population were chosen to correspond to the two chromosomes tested for heterozygous fitness effects (see MITCHELL, 1977). The same 60 untreated chromosomes were used as competitors for the 60 treated chromosomes which came from each level of treatment. Half the populations in the experiment were vermilion-treated, the other half were apricot-treated.

To maintain the populations on a schedule of discrete, non-overlapping generations, parents were cleared from each of the population bottles (quarter pint size) after 4 days, and after 11, male progeny were classified and counted. These males, along with their sisters, were then placed in a new bottle to begin another generation of competition. Whenever females carrying free-X chromosomes were spotted, they were eliminated; these troublesome flies originated from paternal nondisjunction in the second meiotic division.

The frequencies of the two types of males in a population were monitored over several generations until one type was 90% of the total. Then the population was considered "fixed" and was terminated. The relative fitnesses of the two X chromosomes were sufficiently different that almost all populations were fixed within 6 generations, at which time the entire experiment was ended. A total of 173,638 flies were counted to estimate the hemizygous fitness effects in the 360 populations studied. The standard cornmeal-molasses medium was used throughout this work, and all cultures were raised at 25°.

ANALYSIS

The estimation procedure has been described by SIMMONS, SHELDON and CROW (1978), but we summarize it here briefly. The ratio of competing male types (apricot to vermilion) in a population in generation t (R_t) is a function of the initial ratio and the intensity of selection. Since the ratio of males in generation t differs from that in generation $t-1$ only by the effect of one generation of selection, then $R_t/R_{t-1} = \hat{D}_t$ estimates that effect. Every generation-to-generation transition provides an estimate of the effect, so data collected from a population over many generations can be pooled to obtain an average estimate $(\hat{K}_i)$

for the ith chromosome tested. The pooling procedure involves weighting the log of each $\hat{D}_t$ by the reciprocal of its large sample binomial variance, which in this case is $\hat{W}_t = \hat{N}_t \hat{P}_t (1 - \hat{P}_t)$. In this expression $\hat{N}_t$ is the total number of males counted in generation t, and $\hat{P}_t$ is the proportion which are apricot. The $\hat{K}_i$ are then estimated by the formula

$$\hat{K}_i = \Sigma (\log \hat{D}_t) \hat{W}_t / \Sigma \hat{W}_t \ . \tag{1}$$

We use the log of $\hat{D}_t$ because selection has two postulated components in these experiments, an extrinsic one due to the effects of induced mutants, and an intrinsic one, arising from differences in the tester chromosomes. We assume that these two components act multiplicatively, so that $\log \hat{D}_t$ estimates the sum of the intrinsic and extrinsic components. In the vermilion-treated half of the experiment, the sign of the extrinsic component is opposite that in the apricot-treated half because the induced mutants affect the denominator of the apricot to vermilion ratio, while in the apricot-treated half they affect the numerator. Thus, the extrinsic component estimated in the vermilion-treated part of the experiment is the same size as, but opposite in sign to, that estimated in the apricot-treated part. To calculate the extrinsic component, we obtain an unweighted average of the $\hat{K}_i$ in each half of the experiment; for the apricot-treated populations, we let $\hat{K}_A$ denote this value, and for the vermilion-treated populations, we use $\hat{K}_V$. Then $L = (\hat{K}_V - \hat{K}_A)/2$ estimates the log of the extrinsic effect relative to zero (no effect), and $\hat{u} = e^L - 1$ estimates the extrinsic effect itself, again relative to zero (no effect). Our convention is that negative effects are deleterious, and positive ones are beneficial. To estimate the intrinsic selective effect, we calculate $\hat{M} = (\hat{K}_A + \hat{K}_V)/2$. This estimates the log of the intrinsic effect, so that $\hat{a} = e^L - 1$ estimates the effect itself. By convention, negative values of $\hat{a}$ imply superiority of the vermilion chromosome over the apricot one. The large sample variances of $\hat{u}$ and $\hat{a}$ are obtained from the following formulae:

$$V(\hat{u}) = V(\hat{L}) e^{2\hat{L}} \tag{2}$$

$$V(\hat{a}) = V(\hat{M}) e^{2\hat{M}} \ , \tag{3}$$

where $V(\hat{L})$ and $V(\hat{M})$ are calculated with the formula

$$\tfrac{1}{4} (V(\overline{\hat{K}_V}) + V(\overline{\hat{K}_A})) \ . \tag{4}$$

For statistical analysis, outlying values of the $\hat{K}_i$ were ignored. These were defined as those values in a group deviating from the median of the empirical distribution of the group by more than 2 units, and always came from populations which had "fixed" in one generation.

RESULTS

The selective effects estimated from our data are shown in Table 1. Both intrinsic and extrinsic (induced) effects are given for each level of treatment.

TABLE 1

Induced and intrinsic hemizygous fitness effects

EMS dose (mM)	Apricot treated N	Apricot treated J	Vermilion treated N	Vermilion treated J	Induced effect ± standard error	Intrinsic effect ± standard error
2.5	27	1.89	26	2.65	—0.1545 ± 0.0264	—0.6933 ± 0.0096
					—0.1291 ± 0.0682	—0.7089 ± 0.0228
5.0	26	2.27	23	3.39	—0.1503 ± 0.0240	—0.6150 ± 0.0109
					—0.1333 ± 0.0606	—0.6373 ± 0.0253
7.5	24	2.04	23	2.70	—0.1009 ± 0.0298	—0.6756 ± 0.0107
					—0.1030 ± 0.0863	—0.6969 ± 0.0292
10.0	24	1.92	24	2.54	—0.1032 ± 0.0357	—0.6817 ± 0.0127
					—0.0822 ± 0.0831	—0.7084 ± 0.0265
15.0	20	1.75	27	3.52	—0.3795 ± 0.0252	—0.5968 ± 0.0164
					—0.3813 ± 0.0612	—0.6279 ± 0.0368
30.0	21	1.24	24	2.87	—0.4399 ± 0.0252	—0.7389 ± 0.0118
					—0.4197 ± 0.0593	—0.7522 ± 0.0253
					Mean	—0.6669 ± 0.0050
						—0.6886 ± 0.0114

$N =$ number of populations, $J =$ number of generation to generation transitions ("jumps") per population. The first estimate was obtained by the weighted analysis, the second by the unweighted one. Note that the unweighted procedure gives less precise estimates, as explained in the text.

Since the intrinsic selective effects are large, the populations were rapidly fixed for the favored chromosome (which was y^2v). Unweighted estimates of the selective effects, as well as weighted ones, are presented in the table. The unweighted figures were computed by setting the weights in our algorithm equal to one; thus, all observations were counted equally. This had the effect of using the difference between the log of the last ratio and that of the first, divided by the number of cycles of selection, to estimate the selective effect of a chromosome. The weighted and unweighted estimates turn out to be very similar, probably because most populations were monitored for less than 3 cycles of selection ("jumps" in Table 1). Nevertheless, the unweighted estimates have larger standard errors, since they take no account of the information provided by the intermediate data points which are included in the weighted analysis.

The overall weighted estimate for the intrinsic effect is —0.6669 ± 0.0050; the corresponding unweighted estimate is —0.6886 ± 0.0114. These imply that the relative fitnesses of the apricot and vermilion males are about 0.32 (apricot) and 1.00 (vermilion).

Since the extrinsic effects generally increase with the level of EMS treatment, we have modeled the increase with a regression line constrained to pass through zero. The model, $Y = B_1X$ ($Y =$ extrinsic effect, $X =$ level of treatment) was chosen over a purely quadratic one ($Y = B_2X^2$) and over a combination of the two ($Y = B_1X + B_2X^2$), because it adequately explains the data. Based on the weighted values, B_1 is estimated to be —0.0166 ± 0.0026, which is significantly different from zero. This implies that flies carrying a chromosome treated with a 1 mM dose of EMS are about 1.7% less fit than their untreated counterparts.

Thus, at the highest treatment level (30 mM) the intrinsic fitness of the tested chromosomes was reduced by about 50%. The unweighted points give a value of -0.0159 ± 0.0025 for B_1, again significantly different from zero and quite similar to the weighted estimate. The regression lines based on the weighted and unweighted data are shown in Figure 1.

<div align="center">DISCUSSION</div>

A. *Hemizygous fitness effects*

Drosophila X chromosomes mutagenized with EMS seem to reduce the fitness of their hemizygous carriers by an amount which is proportional to the dose of the mutagen; the average fitness reduction per mM EMS is estimated to be 1.7%, using a mutation-free chromosome as a standard. From the data we have collected, there is no evidence for non-linearity in the dose-effect relationship; this agrees with the observations made in the preceding paper, namely that the viabilities of hemizygotes and homozygotes decline linearly with increasing EMS dose. However, the power of the statistical analysis is not great. To the extent the data permit, this suggests that there are no load-reducing interactions among the induced mutations. However, such interactions were detected among spontaneous
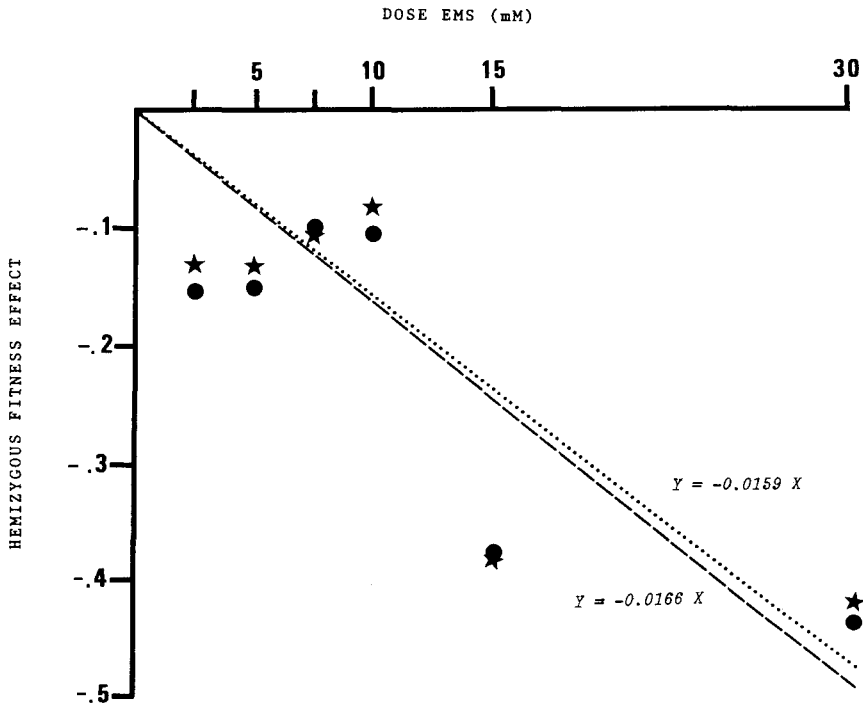

FIGURE 1.—Induced hemizygous fitness effects as a function of EMS dose. Observed points and estimated regression lines are shown, with the circles and dashed lines referring to the weighted analysis and the stars and dotted lines referring to the unweighted analysis. Negative effects are, by convention, deleterious.

mutants by MUKAI (1969), and among mutations from an equilibrium population by TEMIN *et al.* (1969), but in both cases their effects were slight. We suspect that chromosomes with many mutants, among which interactions might have occurred, were lost during our experiment, so their effects were never recognized. As was mentioned in the previous paper, the mutations in these experiments were accumulated on unsheltered X chromosomes, which were subject to selection, so we think our final sample of treated chromosomes was a biased one; it did not represent the total array of debilitating mutations induced by the treatment. However, since the main purpose of this research was to compare fitness effects and viability effects of mild mutants, the loss of the more drastic mutants is not a major concern.

B. *Fitness effects and viability effects*

The decline in hemizygous viability reported in the preceding paper amounts to 0.5% per mM EMS, while the decline in hemizygous fitness reported here is 1.7%. This implies that the fitness effects of EMS-induced mutations are more than 3 times larger than their corresponding viability effects. In these experiments, the intrinsic hemizygous viability of the apricot chromosome was about 18% less than that of the vermilion one. However, the intrinsic hemizygous fitness of the apricot chromosome was 68% less than that of the vermilion one. Thus, the intrinsic effects of these chromosomes on fitness are about 4 times larger than their effects on viability.

These results indicate that there is much more to fitness than just viability. KNIGHT and ROBERTSON (1957), SVED (1971) and SVED and AYALA (1970) suggested that fertility makes a more important contribution to overall fitness than viability. From our data, the fertility component would seem to be 3–4 times as large as the viability one. However, this relationship could be distorted in favor of the viability component, since chromosomes with large fitness effects were fixed before the first population counts. For this reason, the fitness effects are probably underestimated, relative to the viability ones, so the relationship between total fitness and its viability component is biased. This seems to justify the statement that the fertility component of fitness is more than 3–4 times the viability component.

Our results agree with SVED's (1971) observation that the cumulative effect on fitness of the mutants carried by a chromosome taken from a natural population is greater than the cumulative viability effect. SIMMONS, SHELDON and CROW (1978) also suggested that the fitness effect of a chromosome carrying mutants, in this case EMS-induced ones, was larger than the viability effect. That the fitness effects of mutants are larger than their viability effects suggests that most mutations should be rapidly eliminated from a natural population if they are partially dominant. This seems to be the case for lethals (CROW and TEMIN 1964), and may also be so for detrimentals. An empirical prediction stemming from the relationship between total fitness and its viability component is that the total inbreeding depression obtained by making Drosophila chromosomes homozygous must be quite large, at least 3–4 times larger than that esti-

mated by studying the viabilities of homozygous flies. SVED (1971) suggested that this is indeed the case; he found that homozygosis for a second chromosome taken from nature lowers fitness by 85%. The comparable viability reduction is about 10–15% (TEMIN 1966; TEMIN *et al.* 1969).

C. *Dominance estimates*

By comparing the estimates for the heterozygous fitness effects of the induced mutants (found in MITCHELL, 1977) with the corresponding hemizygous fitness effects (found in this paper), an average value for the dominance of the mutants can be obtained. This average is weighted by the selective effects of the mutants and by their mutation rates. The weight is, therefore, a measure of the total impact each mutation has on the population. Formally, the total X chromosome *heterozygous fitness effect* is

$$\Sigma \, u \, w \, h \, ,$$

where u is the mutation rate at a particular locus, w is the selective effect of a mutant at that locus, and h is its measure of dominance. Accordingly, the total X chromosome *hemizygous fitness effect* is

$$\Sigma \, u \, w \, .$$

Using the weighted estimates of this paper and MITCHELL (1977), we obtain

$$\begin{aligned}\hat{\bar{h}} &= \Sigma \, uwh \, / \, \Sigma \, uw \\ &= -0.0041 \, / -0.0166 \\ &= 0.247 \pm 0.072 \, .\end{aligned} \tag{5}$$

The unweighted estimates give

$$\begin{aligned}\hat{\bar{h}} &= -0.0044 \ -0.0159 \\ &= 0.277 \pm 0.082 \, .\end{aligned}$$

Maximum likelihood estimates of the variances of these values were obtained by the formula

$$V(\hat{\bar{h}}) = \{V(\Sigma uwh) + V(\Sigma uw) \, h^2\} \, / (\Sigma uw)^2 \, . \tag{6}$$

Since the numerator and denominator of $\hat{\bar{h}}$ are correlated, this formula should include a covariance term. However, the covariance cannot be calculated from the data, so we regard the estimated variance of $\hat{\bar{h}}$ as an upper limit; the covariance term would necessarily reduce it.

Thus the average dominance value with respect to fitness for EMS-induced mutations on the X chromosome is about 0.25. This value neglects major differences between the effects of mutations in males and females.

The dominance value for fitness agrees remarkably well with estimates which come from the viability studies of TEMIN (0.20, in preparation) and OHNISHI

(0.27, 1977); both examined the viability-reducing effects of EMS-induced detrimentals on the Drosophila second chromosome. Our estimate for the average dominance of EMS-induced mutations, as well as those of TEMIN and OHNISHI, is lower than estimates obtained for spontaneous mutants (0.40, MUKAI *et al.* 1972; 0.49, OHNISHI 1977). This seems to indicate that EMS-induced mutants have a different character than spontaneous ones. For Drosophila, the molecular nature of both types of lesions is obscure. However, we do know that the distribution of effects of EMS-induced mutants, at least with respect to viability, is different from that of spontaneous ones (OHNISHI 1974, 1977): EMS-induced mutants have a larger mean effect, or a larger variance of the effect, or both. What we find interesting is that the dominance value for fitness for EMS-induced mutants is essentially the same as the dominance value for viability. No one has estimated the dominance value for fitness for spontaneous mutants, but the value for viability has been measured. In light of the results with EMS-induced mutants, it is not unreasonable to speculate that the dominance for fitness and that for viability are the same.

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