FITNESS EFFECTS OF EMS-INDUCED MUTATIONS ON THE *X* CHROMOSOME OF *DROSOPHILA MELANOGASTER*. I. VIABILITY EFFECTS AND HETEROZYGOUS FITNESS EFFECTS[1]

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ABSTRACT

Drosophila melanogaster X chromosomes were mutagenized by feeding males sucrose solutions containing ethyl methanesulfonate (EMS); the concentrations of EMS in the food were 2.5 mM, 5.0 mM, and 10.0 mM. Chromosomes were exposed to the mutagen up to three times by treating males in succeeding generations. After treatment, the effective exposures were 2.5, 5.0, 7.5, 10.0, 15.0, and 30.0 mM EMS. *X* chromosomes treated in this manner were tested for effects on fitness in both hemizygous and heterozygous conditions, and for effects on viability in hemizygous and homozygous conditions. In addition, untreated *X* chromosomes were available for study. The viability and heterozygous fitness effects are presented in this paper, and the hemizygous fitness effects are discussed in the accompanying one (MITCHELL and SIMMONS 1977). Hemizygous and homozygous viability effects were measured by segregation tests in vial cultures. For hemizygous males, viability was reduced 0.5 percent per mM EMS treatment; for homozygous females, it was reduced 0.7 percent per mM treatment. The decline in viability appeared to be a linear function of EMS dose. The viabilities of males and females were strongly correlated. Heterozygous fitness effects were measured by monitoring changes in the frequencies of treated and untreated *X* chromosomes in discrete generation populations which, through the use of an *X-Y* translocation, maintained them only in heterozygous condition. Flies that were heterozygous for a treated chromosome were found to be 0.4 percent less fit per mM EMS than flies heterozygous for an untreated one.

MUTATION is the source of new variation in a population. Since the vast majority of new mutants first appear in heterozygous condition, their ultimate fate depends on the fitness effects they have in that state. Although viability and fertility are both major components of fitness, most attempts to measure the effects of new mutations on fitness in Drosophila have concentrated on the viability component. For example, MUKAI and coworkers (MUKAI 1964; MUKAI *et al.* 1972) determined the homozygous and heterozygous effects on viability of newly arising spontaneous mutations. TEMIN (in preparation),

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MUKAI (1970), and OHNISHI (1974, 1977) studied the homozygous and heterozygous viability effects of mutations induced with ethyl methanesulfonate (EMS). Other workers have studied the viability effects of mutations induced with radiation (WALLACE 1958; FRIEDMAN 1964; SIMMONS 1976).

Recently, techniques have been developed to measure the effects of new mutations on total fitness. SIMMONS, SHELDON and CROW (1978) have studied the total heterozygous effects of mutants induced with EMS on the second chromosome of *Drosophila melanogaster*, but these effects were ascertained in males only.

The present report is concerned with the heterozygous fitness effects of EMS-treated X chromosomes in females. These effects were studied by using an X-Y translocation ($\overline{XY}$) with multiple inversions on the X, which permits free X chromosomes to be carried in heterozygous females without recombinational breakup. The frequencies of two competing translocation heterozygotes (XY/X_u and $\overline{XY}/X_t$) were followed in populations maintained with translocation males (XY). The free X chromosome of one heterozygote carried EMS-induced mutations (X_t), while the other heterozygote carried an untreated X (X_u). In these populations, changes in the frequencies of the treated chromosomes were used to assess the fitness effects of the induced mutations. In addition, the homozygous and hemizygous viability effects of the experimental chromosomes were determined. Data concerning the hemizygous fitness effects of the treated X chromosomes are presented in the accompanying paper (MITCHELL and SIMMONS 1977).

MATERIALS AND METHODS

A. *Stocks*

Detailed information concerning the chromosomes and markers used in these experiments can be found in LINDSLEY and GRELL (1968). The stocks that were used, along with brief descriptions, follow (see also Figure 1):

1. *C(1)DX/f^{s}su(f)^{ts67g}/Y*. This stock was used for collecting attached-X virgin females. *C(1)DX* is an attached-X chromosome marked with the mutants *y* (yellow body) and *f* (forked bristles). The free-X chromosome of the stock has a temperature-sensitive larval lethal; when cultures are raised at 29°, all the males die; emerging females are therefore automatically virgin. In practice, virgin females were collected by allowing parental flies to mate and lay eggs for 3 days at 25°. Then the parents were discarded, and the cultures were placed at 29° for the next 3 days, after which they were restored to 25°. When hatching begins, there is a period of 3 to 4 days when absolutely no males emerge.

2. *C(1)DX/FM7,sn^{X2}B/Y*. The free X chromosome (*FM7,sn^{X2}B*) of this stock was used as a balancer in the viability tests.

3. *y+Y^{S}FM7.Y^{L}B^{S}, y w^{a} v^{Of}/Bld*. The $\overline{XY}$ translocation, developed by CRAYMER (1974) with marker modifications for this project, contains the multiply inverted X chromosome *FM7* with the recessive mutants yellow (*y*), the apricot allele of white (*w^{a}*), and the Offermann allele of vermilion (*v^{Of}*). The short arm of the Y is translocated to one end of the X; the long arm of the Y is translocated to the other end. The tips of the X-Y translocation are marked with *y+* and *B^{S}*, which aid in detecting breakdown. The translocation is symbolized $\overline{XY}$ (read, "translocation X,Y").

This complex chromosome contains all the male fertility factors, as well as the *FM7* balancer, an efficient suppressor of crossing over. Females in the stock carry this translocation chromosome along with a free X, the Blond (*Bld*) chromosome. The only fertile males in the stock possess

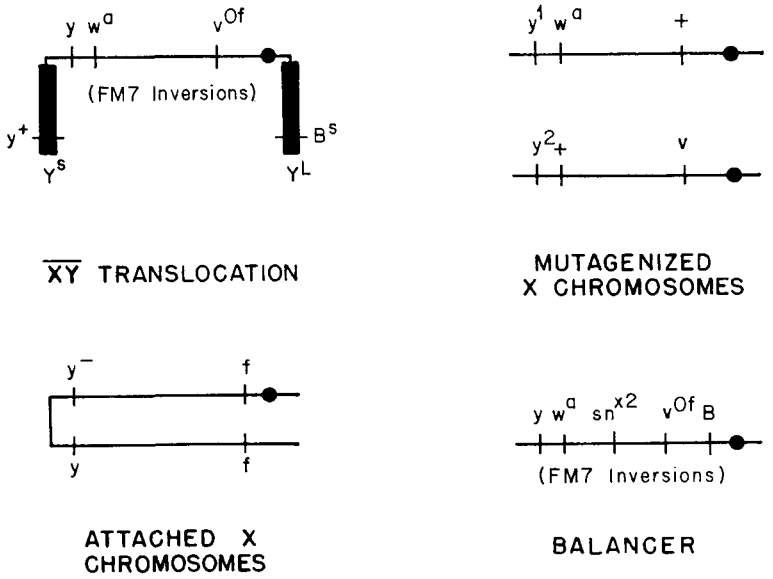

FIGURE 1.—*X* chromosomes used in the experiments.

the translocation chromosome; sterile *XO* males are also present. Females homozygous for the translocation do survive, but have much reduced fertility and viability.

There is a tendency for the translocation chromosome to pair with itself during meiosis; crossing over in heterochromatic regions can result in reconstitution of a free-*Y* chromosome containing all the necessary fertility factors.

4. y^2v/Y. The markers are yellow body (y^2) and vermilion eyes (v).

5. y^1w^a/Y. The markers are yellow body (y^1) and white-apricot eyes (w^a). These two *X*-chromosome types were treated with the mutagen EMS. The markers were chosen so that females in the heterozygous fitness experiment could be easily distinguished. Females in that experiment were $\overline{XY}/y^1w^a$, phenotypically yellow-1, apricot and Bar, and $\overline{XY}/y^2v$, phenotypically yellow-2, vermilion and Bar.

B. *Mutagenesis*

Two types of *X* chromosomes (y^2v and y^1w^a) were treated with different concentrations of EMS, once or three times. Single y^2v and y^1w^a chromosomes were replicated many times by crossing males to attached-*X* females *[C(1)DX]* for two generations. Then males of each type were collected and mutagenized with EMS solutions according to the procedure of LEWIS and BACHER (1968). The solutions were prepared in concentrations of 2.5 mM, 5.0 mM, and 10.0 mM in 1% sucrose; for treatment, approximately 2.5 ml of each solution was injected onto tissue paper wadded at the bottom of a 25mm × 90 mm vial. Flies were allowed to feed on the soaked tissue for 24 hours; 20 males, 10 of each chromosome type, were treated together in a vial. Flies carrying chromosomes not scheduled for treatment in a given generation were placed in vials containing tissue soaked only with the sucrose solution. In each of these generations, treated and untreated males were individually mated to *C(1)DX* females, and male progeny were collected. Some chromosomes were treated once, some three times, and some not at all. For the three different concentrations of mutagen, this gave cumulative levels of 0.0, 2.5, 5.0, 7.5, 10.0, 15.0, and 30.0 mM EMS. Mating males to attached-*X* females automatically eliminated lethal mutations induced by the treatment. Lines of treated and untreated chromosomes were established by individually mating males recovered from the last generation of treatment with *C(1)DX* females.

C. *Viability tests*

The viabilities of the X chromosomes were determined by observing the segregation of homozygous females and hemizygous males in cultures with heterozygous females used as a standard. The heterozygous females carried the chromosome of interest X_i = y^2v or y^1w^a), and the balancer *FM7*. The tests were initiated by crossing single males from each chromosome line to $\overline{XY}$/*Bld* females. Three daughters of the constitution $\overline{XY}/X_i$ were then mated to *FM7* males to produce *FM7*/X_i females and X_i/Y males, which were then sib-mated. For this last cross, 3 vial cultures of 2 pairs each were established at 25°. Four days after the cross, the parents were transferred to a fresh vial. The progeny segregating from these crosses were classified and counted on the 13th day after starting them. Four classes emerged: (1) *FM7*/X_i females, (2) X_i/X_i females, (3) X_i/Y males, and (4)*FM7*/Y males. The first class was chosen as a standard because the last, which would have been the best, turned out to have poor viability itself; many cultures had very few *FM7* males. Though counts of the original culture and its transfer were recorded separately, they were pooled for the statistical analysis. Indices for the homozygous female viability and hemizygous male viability were computed by dividing the respective totals in classes (2) and (3) by the total in class (1). Indices for each chromosome were obtained by averaging the results of the three replicate tests.

The viabilities of 615 chromosomes, with three replicates of each, were determined. However, not all of these were included in the statistical analysis. The reasons for excluding a chromosome from the analysis follow:

1. The number of flies in any one of the three replicate cultures was less than or equal to 50 (18 chromosomes eliminated).

2. The number of flies in any of the three categories of interest (heterozygous standard females, treated hemizygotes, and treated homozygotes) was less than or equal to one (4 chromosomes eliminated).

3. The chromosome index was less than 0.45 for either males or females. Preliminary analysis indicated such chromosomes were outliers since they contained drastic mutations with large effects (19 chromosomes eliminated).

After culling, 574 chromosomes (286 vermilion and 288 apricot) remained. These contained polygenic mutations with individually small effects on viability. A total of 368,172 flies were counted for the viability tests.

D. *Heterozygous fitness tests*

The heterozygous fitness effects on the treated X chromosomes were determined by monitoring changes in the frequencies of treated X chromosomes in competition with untreated ones. During competition, treated and untreated chromosomes were kept heterozygous by using the X-Y translocation. 360 competitive populations were established, each containing a different treated X chromosome. 60 chromosomes from each level of EMS treatment were selected to start these populations; within each group, half were y^2v; the other half were y^1w^a. The same set of 60 untreated chromosomes was used to provide competitors for the chromosomes of each treated group. 50 females carrying a treated $X(X_t)$ of one marker type and 50 females carrying an untreated $X(X_u)$ of the other were used to start each population. These females were produced by crossing single males taken from a chromosome line (either X_t/Y or X_u/Y) to $\overline{XY}$/*Bld* females. Thus, the relevant females in the populations were of the constitutions $\overline{XY}/X_t$ and $\overline{XY}/X_u$. The fertile males were $\overline{XY}$. X_t and X_u males, having no Y chromosome, were sterile. $\overline{XY}/\overline{XY}$ females were present, but rare, since they had greatly reduced fertility and viability. The $\overline{XY}$ translocation contains a multiply-inverted X chromosome to prevent crossing over in heterozygous females, so the integrity of the treated and untreated free-X chromosomes was preserved. In heterozygous females, the free-X chromosome was transmitted only to daughters, while the translocation was passed on to sons. Thus, the populations reproduced in such a way that the progeny were exactly like their parents.

To regulate reproduction, the populations were kept on a schedule of discrete, non-overlapping generations in quarter pint bottles at 25°. Every 11 days adults were etherized, classified and

counted. Then they were transferred to a fresh bottle, from which they were removed after four days so that parents and progeny would not mix. The frequencies of the two types of heterozygous females (apricot and vermilion) in each population were determined every generation until one type was at least 90% of the total. The population was then considered "fixed" and was terminated. The experiment was continued for 14 generations during which time the majority of the populations had gone to fixation for one of the competing chromosomes. Those populations which fixed in one or two generations were not included in the analyses since founder effects were probably more important than actual fitness differences. A total of 296,219 female flies were counted for the heterozygous fitness tests.

For all stocks and experimental cultures, the standard cornmeal-molasses medium was used.

RESULTS

A. *Viability effects*

The effects on viability of EMS-induced mutations were determined by computing viability indices for each of the chromosomes tested; these were related to the corresponding levels of EMS treatment. If β_0 is the viability index of an untreated chromosome and X is the cumulative dose of EMS (in mM) with which a chromosome has been treated, then the viability of a chromosome treated with X mM of EMS can be written as

$$Y = \beta_0 (1 + s)^X \ . \tag{1}$$

In (1), s is the selective effect on viability of mutations induced by 1 mM EMS; the mutants are assumed to act multiplicatively to lower the viability of the flies which carry them. By convention, negative values of s mean that the mutants are harmful; positive values mean that they are beneficial. Since for s small, $1 + s \approx e^s$, the viability can be written as

$$Y = \beta_0 e^{sX} \ , \tag{2a}$$

$$\text{so } Y' = \beta_0' + sX \ , \tag{2b}$$

where Y' is the natural logarithm of the viability index, and β_0' is the natural logarithm of the intrinsic viability of a chromosome, *i.e.*, the logarithm of the viability of an untreated chromosome.

Table 1 gives the means and standard deviations of the transformed and untransformed viability indices separately for males (hemizygotes) and females (homozygotes) for each level of EMS treatment, for both apricot and vermilion chromosomes. There is an obvious decrease in the mean index as EMS dose increases. It is also apparent that the apricot chromosomes have a lower intrinsic viability than the vermilion ones.

To estimate s, a linear regression was performed on log transformed viability indices, using EMS dose as the independent variable; the three replicates of each chromosome were averaged (after transformation) to give a viability value representative of that chromosome. These values were used for the dependent variable in the regression analysis. The slope of the regression line directly estimates the selective effect on viability; male and female indices were analyzed separately. The regression lines are shown in Figures 2a and 2b. The slope of

each line is significantly different from zero, indicating definite treatment effects.

Since all the chromosome indices were utilized for the regression analysis, repeats at each level of EMS treatment were available. It was therefore possible to check for significant lack of fit in the regression model. No evidence for lack of fit was found for any of the regression analyses.

The average viability effect (s) induced by 1 mM of EMS was estimated by averaging the slopes of the regression lines of the apricot and vermilion chromosomes; its variance was obtained from the regression analysis. For male (hemizygote) viability, $\hat{s} = -0.0054 \pm 0.0009$; for female (homozygote) viability, $\hat{s} = -0.0070 \pm 0.0008$. This means that mutants induced on the X chromosome

TABLE 1

Mean viabilities of treated chromosomes ± standard errors of the means

EMS dose (mM)	Number of chromosomes	Mean untransformed viability index		Mean transformed viability index	
		Females	Males	Females	Males
Vermilion					
0.0	43	1.1846	1.2717	0.1508	0.2043
		±0.0233	±0.0401	±0.0185	±0.0280
2.5	45	1.1844	1.3093	0.1481	0.2363
		±0.0248	±0.0369	±0.0197	±0.0255
5.0	47	1.1660	1.2909	0.1302	0.2310
		±0.0222	±0.0278	±0.0200	±0.0211
7.5	37	1.1190	1.2434	0.0798	0.1560
		±0.0315	±0.0403	±0.0344	±0.0455
10.0	43	1.1377	1.2782	0.1090	0.2173
		±0.0165	±0.0284	±0.0148	±0.0208
15.0	35	1.0468	1.1571	0.0102	0.1127
		±0.0332	±0.0373	±0.0363	±0.0370
30.0	36	0.9677	1.0785	−0.0633	0.0379
		±0.0271	±0.0354	±0.0322	±0.0352
Apricot					
0.0	45	1.1459	1.0496	0.1156	0.0196
		±0.0200	±0.0239	±0.0176	±0.0235
2.5	49	1.1182	1.0596	0.0947	0.0342
		±0.0161	±0.0176	±0.0146	±0.0173
5.0	47	1.1508	1.0511	0.1221	0.0255
		±0.0197	±0.0192	±0.0181	±0.0187
7.5	39	1.1045	1.0566	0.0681	0.0087
		±0.0277	±0.0350	±0.0280	±0.0420
10.0	43	1.1180	1.0514	0.0923	0.0293
		±0.0190	±0.0199	±0.0166	±0.0182
15.0	32	0.9973	0.9526	−0.0590	−0.0940
		±0.0416	±0.0344	±0.0559	±0.0437
30.0	33	0.9919	0.9588	−0.0444	−0.0728
		±0.0331	±0.0281	±0.0402	±0.0319

See text for the method of calculating the viability index; individual viability indices were logarithmically transformed.

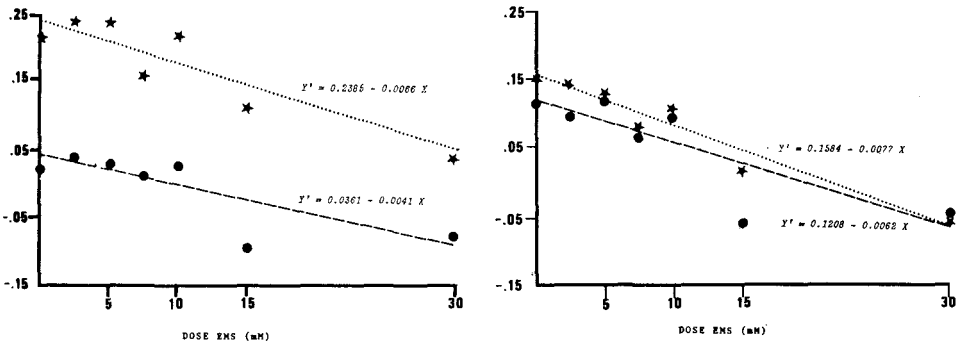

FIGURE 2.—(a, left) Male viability as a function of EMS dose. (b, right) Female viability as a function of EMS dose. Observed points and estimated regression lines are shown: stars and dotted lines refer to the vermilion chromosomes, circles and dashed lines to the apricot chromosomes. The transformation is logarithmic.

with 1 mM EMS reduce hemizygous viability by roughly 0.5%; they reduce homozygous viability by a slightly greater amount, about 0.7%.

The constant terms of the estimated regression lines were used to estimate the intrinsic viability disadvantage (α) of the apricot chromosomes relative to the vermilion chromosomes. Letting $\hat{\beta}_0'(w^a)$ and $\hat{\beta}_0'(v)$ be the constants in the regression equations for apricot and vermilion chromosomes, respectively,

$$\hat{\alpha} = 1 - \exp\{\hat{\beta}_0'(w^a) - \hat{\beta}_0'(v)\} \ . \tag{3}$$

The maximum likelihood variance estimate of α was obtained by calculating

$$V(\hat{\alpha}) = \{V(\hat{\beta}_0'(v)) + V(\hat{\beta}_0'(w^a))\} \exp\{2(\hat{\beta}_0'(w^a) - \hat{\beta}_0'(v))\} \ . \tag{4}$$

For male viability, $\hat{\alpha} = 0.1832 \pm 0.0181$, implying that the apricot males had an intrinsic viability which was about 18% less than that of the vermilion males. Therefore, the relative viabilities of apricot and vermilion males carrying untreated chromosomes were 0.817 (apricot) to 1.00 (vermilion). For female viability, $\hat{\alpha} = 0.0370 \pm 0.0191$. Thus the females carrying untreated apricot chromosomes were about 96% as viable as females carrying untreated vermilion chromosomes, and their relative viabilities were 0.963 (apricot) to 1.00 (vermilion).

An additive model of gene action was also postulated. The estimate for the unit viability effect in males was $-.0048 \pm 0.0007$, and in females it was $-.0058 \pm 0.0007$. These estimates are not very different from the analogous results of the multiplicative model. Thus, whether the new mutations reduce viability in a multiplicative or additive fashion, the estimated unit effects are very similar. Further information is contained in MITCHELL (1976).

For each chromosome the average viability indices were used to find the correlation between male and female viability (Table 2). The standard formula was used to compute the correlation coefficient. There were no significant trends over levels of treatment, and no differences in the mean correlation coefficient between

TABLE 2

Correlations between male and female viabilities

EMS dose (mM)	Vermilion Number of chromosomes	Vermilion Correlations	Apricot Number of chromosomes	Apricot Correlations
0.0	43	0.6915 ± 0.1128	45	0.4489 ± 0.1363
2.5	45	0.3990 ± 0.1398	49	0.2153 ± 0.1424*
5.0	47	0.6709 ± 0.1105	47	0.4196 ± 0.1353
7.5	37	0.5182 ± 0.1446	39	0.6969 ± 0.1179
10.0	43	0.3851 ± 0.1441	43	0.2516 ± 0.1512
15.0	35	0.9017 ± 0.0752	32	0.8705 ± 0.0899
30.0	36	0.8026 ± 0.1023	33	0.8037 ± 0.1069
	Mean	0.6241 ± 0.0457	Mean	0.5295 ± 0.0481

All correlations *except* those marked with an asterisk are significantly larger than zero.

apricot and vermilion chromosomes. Significantly positive average correlations of 0.6241 ± 0.0457 for the vermilion chromosomes, and 0.5295 ± 0.0481 for the apricot chromosomes were obtained.

B. *Heterozygous fitness effects*

The ratio of apricot females to vermilion females in each generation was used to estimate the EMS-induced fitness effects and the difference between the intrinsic fitnesses of the apricot and vermilion chromosomes. The estimation procedure is described in detail by SIMMONS, SHELDON and CROW (1978) and is summarized in the accompanying paper (MITCHELL and SIMMONS 1977). In essence, it makes use of changes in the ratio of apricot to vermilion females in a population during the course of the experiment; the rate of change of this ratio (after logarithmic transformation) is a function of the intensity of selection. In half the populations of the experiment, apricot females were heterozygous for EMS-induced mutations; in the other half, vermilion females were heterozygous for the mutants. The rate of change of the transformed ratio in the apricot treated half of the experiment should equal the difference between the intrinsic fitnesses of the two chromosomes plus the effects of the induced mutants carried on the apricot chromosome. In the vermilion treated half, the rate of change should equal the intrinsic fitness difference minus the effects of the mutants. These relationships have been used to estimate the intrinsic fitness difference between the apricot and vermilion chromosomes, and the fitness effects of the induced mutants. Weighted and unweighted estimates of the mean effects of the mutants induced at each level of EMS treatment have been calculated; these are given in Table 3, along with estimates for the intrinsic fitness difference between the apricot and vermilion chromosomes. The weighted estimates in the table make use of a statistical procedure for counting each datum in the calculation in proportion to its information content [the procedure is discussed in the accompanying paper (MITCHELL and SIMMONS 1977)]; the unweighted estimates are based on calculations in which all the data are counted equally. Though both procedures give comparable estimates, the weighted procedure gives more precise ones.

TABLE 3

Induced and intrinsic heterozygous 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	9.22	28	7.79	−0.0311 ± 0.0141	−0.0139 ± 0.0144
					−0.0217 ± 0.0467	−0.0151 ± 0.0470
5.0	29	8.90	27	9.78	−0.0640 ± 0.0123	−0.0734 ± 0.0122
					−0.0774 ± 0.0363	−0.0723 ± 0.0365
7.5	27	8.59	28	8.57	−0.0687 ± 0.0130	0.0039 ± 0.0140
					−0.0802 ± 0.0409	0.0356 ± 0.0461
10.0	27	7.63	25	7.76	−0.0114 ± 0.0140	0.0536 ± 0.0149
					−0.0253 ± 0.0458	0.0524 ± 0.0495
15.0	25	10.04	28	8.68	−0.0963 ± 0.0114	−0.0868 ± 0.0116
					−0.0999 ± 0.0368	−0.0915 ± 0.0372
30.0	28	8.36	25	9.32	−0.0974 ± 0.0125	−0.0910 ± 0.0126
					−0.0979 ± 0.0447	−0.1146 ± 0.0439
					Mean	−0.0346 ± 0.0054
						−0.0343 ± 0.0178

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 procedure. Note that the unweighted estimates are less precise, as explained in the text.

The intrinsic difference between the heterozygous fitnesses of the apricot and vermilion chromosomes was estimated to be about 3.5%. Thus, the fitness of the apricot chromosome, relative to the vermilion chromosome (arbitrarily given a fitness of 1.0) was 0.965.

The fitness effects of the induced mutants obviously increase with the level of EMS treatment. To relate these fitness effects to the corresponding levels of treatment, linear regression were performed with the level of treatment as the independent variable, and the fitness effect as the dependent variable. The regression equations were constrained to pass through zero since no treatment implies no effect. The model $Y = \beta_1 X$ was found to adequately explain the data, where Y is the induced fitness effect, and X is the level of treatment. Based on the regression of the weighted fitness estimates on the level of treatment, $\hat{\beta}_1 = -0.0041 \pm 0.0010$. The unweighted fitness estimates give a value of $\hat{\beta}_1 = -0.0044 \pm 0.0011$. The regression lines are shown in Figure 3. Estimates for $\hat{\beta}_1$ appear to be significantly different from zero; they imply that flies which are heterozygous carriers of a chromosome treated with a 1 mM solution of EMS are about 0.4% less fit than flies which do not carry such a chromosome.

DISCUSSION

A. *Viability effects*

The results show that treatment of *D. melanogaster* males with a solution of EMS induces polygenic mutations on the X chromosome, the cumulative effect of which is to reduce viability. The reduction for hemizygotes (males) amounts

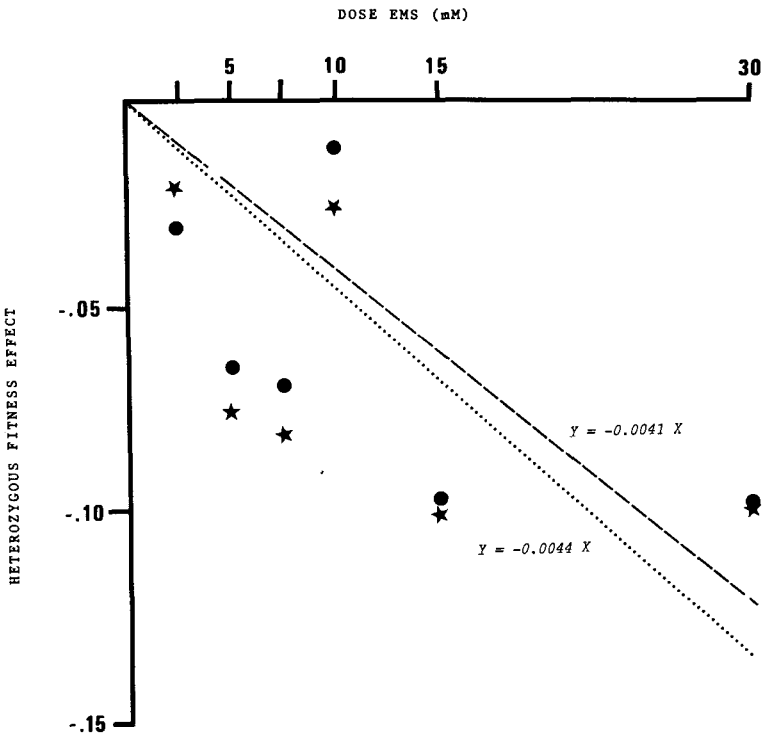

FIGURE 3.—Induced heterozygous fitness effects as a function of EMS dose. Observed points and estimated regression lines are shown: stars and dotted lines refer to the unweighted analysis, circles and dashed lines to the weighted analysis. By convention, negative effects are deleterious.

to 0.5% per treatment with 1 mM EMS; that for homozygotes (females) is slightly greater, 0.7%. The difference between these two is not statistically significant, but it could be justified on *a priori* grounds. During the experiment there was selection on male viability, but not on female viability. Because mutations were induced on unsheltered *X* chromosomes in males, mutants specifically detrimental to females could accumulate with impunity. Of course, the effects of such female-specific mutations would be revealed in viability tests designed to make *X* chromosomes homozygous. Perhaps the slightly larger decline in viability seen for homozygotes can be attributed to such mutations. However, the measurement errors associated with these viability reductions are large enough that the case cannot be pressed.

In this connection it should be noted that a significantly positive correlation was found between the viabilities of males and females at all levels of EMS treatment. This must mean that most viability-reducing mutations have basically the same effects in males and females, not an unreasonable conclusion in light of the fact that flies of both sexes share a multitude of vital functions influenced by sex-linked genes.

The most extensive study of the viability-reducing effects of EMS-induced

mutations is that of OHNISHI (1974, 1977); for experimental material he utilized *D. melanogaster* second chromosomes mutagenized over many generations with various concentrations of EMS (0.1 mM to 5 mM). Using the quasinormal (63% to 100% normal homozygous viability) chromosomes, OHNISHI found a proportional viability reduction of 0.0139 per mM EMS per generation (calculated by performing a weighted regression of the proportional viability decrease due to this class of chromosomes on EMS dose, where the weight for each decrease is the reciprocal of its variance). For the quasinormal and deleterious (32% to 63% normal) chromosomes combined, the analagous figure is 0.0269. Since the *X* chromosome is roughly half the size of the second chromosome, these figures translate into 0.007 (quasinormals only) and 0.0134 (quasinormals + deleterious), in *X* chromosome equivalents. The *X* chromosomes studied here were all at least 39% of normal viability, since for both males and females the minimum viability index was 0.45, and the normal viability was 1.15. The values of the proportional viability decline found here for males and females (0.005 and 0.007) are close to those predicted by the OHNISHI data, but they seem to be on the low side. A likely explanation for this is that the most deleterious mutants induced by the mutagen did not survive the course of the experiment. However, since the main purpose of this study was to investigate the heterozygous fitness effects of mild mutants and to compare these with the effects on viability in homozygotes and hemizygotes, the loss of the more drastic mutants is not a problem.

B. *Heterozygous fitness effects*

Regression analysis of the induced heterozygous fitness effects on EMS dose indicates that fitness declines linearly with increasing concentrations of EMS. The data do show some curvature, but forcing the regression line through the origin makes this not significant. The number of fitted points is too small for a firm conclusion to be drawn, but since the decline in viability seen with increasing concentrations of EMS is nicely linear, the claim that fitness declines in a linear fashion is not outlandish.

Under the assumption of linearity, the average heterozygous fitness reduction caused by a chromosome carrying EMS-induced mutants is about 0.4% per mM EMS. There is no indication from these experiments that newly induced mutations are overdominant; rather, on the average, they are partially dominant. The degree of dominance is estimated in the accompanying paper (MITCHELL and SIMMONS 1977).

It is interesting to note that the *homozygous viability effect* observed in females in these experiments (induced, 0.7%; intrinsic, 4%) is about equal to the *heterozygous fitness effect*, also observed in females (induced, 0.4% intrinsic, 4%). SIMMONS, SHELDON and CROW (1978) found that the average homozygous viability effect of a mutant-bearing second chromosome (observed in both males and females, without distinction) was roughly the same as the average heterozygous fitness effect (observed only in males). The equivalence of homozygous viability effects and heterozygous fitness effects was predicted by MUKAI *et al.*

(1972) through an analysis of the persistence of new mutants in natural populations.

All of the *viability* studies previously cited have indicated that new mutations, whether induced or spontaneous, make their greatest impact upon a population by deleterious effects upon heterozygous carriers. The conclusions from these experiments on *total fitness* further substantiate those claims and indicate that future experiments should concentrate upon the total fitness effects for greater resolving power and more information relevant to the study of effects of mutation on the population.

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