

SPONTANEOUS AND ETHYL METHANESULFONATE-INDUCED
MUTATIONS CONTROLLING VIABILITY IN
DROSOPHILA MELANOGASTER.

II. HOMOZYGOUS EFFECT OF POLYGENIC MUTATIONS¹

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ABSTRACT

Polygenic mutations affecting viability were accumulated on the second chromosome of *Drosophila melanogaster* by treating flies with EMS in successive generations. The treated chromosomes were later made homozygous and tested for their effects on viability by comparison of the frequency of such homozygotes with that of other genotypes in the same culture. The treated wild-type chromosomes were kept heterozygous in *Pm/+* males by mating individual males in successive generations to *Cy/Pm* females. The number of generations of accumulation was 1 to 30 generations, depending on the concentration of EMS. A similar experiment for spontaneous polygenic mutations was also conducted by accumulating mutations for 40 generations. The lower limit of the spontaneous mutation rate of viability polygenes is estimated to be 0.06 per second chromosome per generation, which is about 12 times as high as the spontaneous recessive lethal mutation rate, 0.005. EMS-induced polygenic mutations increase linearly with the number of treated generations and with the concentration of EMS. The minimum mutation rate of viability polygenes is about 0.017 per 10^{-4} M, which is only slightly larger than the lethal rate of 0.013 per 10^{-4} M. The maximum estimate of the viability reduction of a single mutant is about 6 to 10 percent of the normal viability. The data are consistent with a constant average effect per mutant at all concentrations, but this is about three times as high as that for spontaneous mutants. It is obvious that one can obtain only a lower limit for the mutation rate, since some mutants may have effects so near to zero that they cannot be detected. The possibility of measuring something other than the lower limit is discussed. The ratio of the load due to detrimental mutants to that caused by lethals, the D/L ratio, is about 0.2 to 0.3 for EMS-induced mutants, as compared to about 0.5 for spontaneous mutants. This is to be expected if EMS treatment produces a large fraction of small deletions and other chromosome rearrangements which are more likely to be lethal.

THE purpose of the present experiments was to study the nature of viability-affecting mutations induced in *Drosophila melanogaster* by relatively low concentrations of ethyl methanesulfonate (EMS) and to compare these with

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spontaneous mutations. This paper deals with homozygous effects; heterozygous effects are discussed in the following article (OHNISHI 1977b).

MUKAI and his colleagues (MUKAI 1964; MUKAI *et al.* 1972) have found a high rate of spontaneous polygenic mutation affecting viability. One of my objectives was to repeat these experiments with different chromosomes, but with the same test system. MUKAI (1970) also studied polygenic mutations induced by very high concentrations of EMS (2.5×10^{-2} M) and estimated the polygenic mutation rate as more than 0.59 per second chromosome. The average homozygous effect per mutant was larger than for spontaneous mutants. A second objective of my experiments was to extend these observations to lower concentrations likely to include a larger fraction of point mutations and more relevant to the study of human mutation risks.

In the preceding paper (OHNISHI 1977a) it was reported that EMS induces second chromosome recessive lethal mutations at a rate of about 0.01 per 10^{-4} M. Therefore, concentrations in the range of 0×10^{-3} M were used to study polygenic mutations.

The data are available in more extensive form in a thesis (OHNISHI 1974).

MATERIALS AND METHODS

The wild-type stock (+/+) and the marker stock (*Cy/Pm*) are those described in the preceding paper (OHNISHI 1977a). The procedures for treating with EMS are also given there. The following concentrations were used: $0, 5 \times 10^{-5}, 1 \times 10^{-4}, 5 \times 10^{-4}, 1 \times 10^{-3}, 2.5 \times 10^{-3}$, and 5×10^{-3} M. Mutations were allowed to accumulate over several generations of treatment by essentially the same technique as used by DOBZHANSKY, SPASSKY and SPASSKY (1952), PAXMAN (1957) and MUKAI (1964).

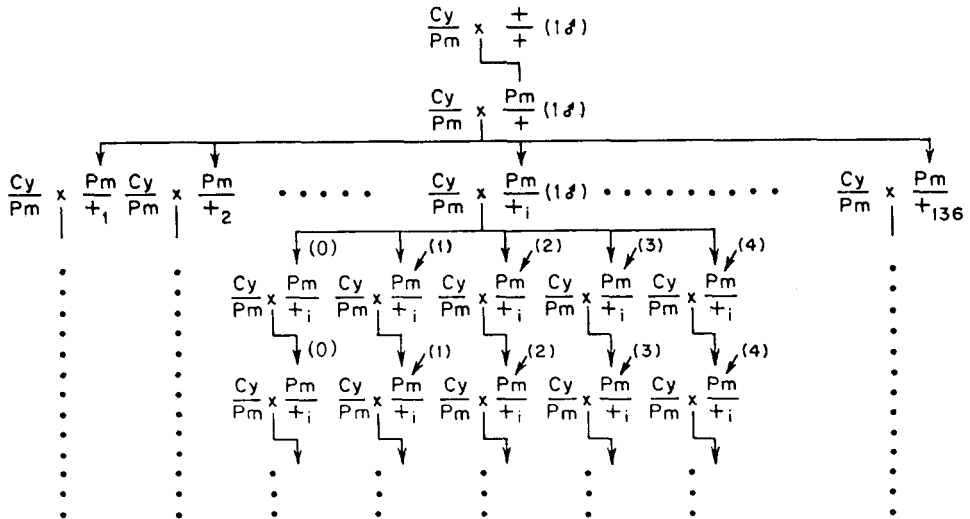


FIGURE 1.—Mating scheme for accumulation of spontaneous and EMS-induced mutations. 136 lines were each divided into 5 treatment groups. (1), (2), (3), and (4) indicate treatment of *Pm*/+ flies by EMS concentrations of $5 \times 10^{-5}, 1 \times 10^{-4}, 5 \times 10^{-4}, 2.5 \times 10^{-3}$ M. (0) indicates no treatment, to measure the spontaneous rate.

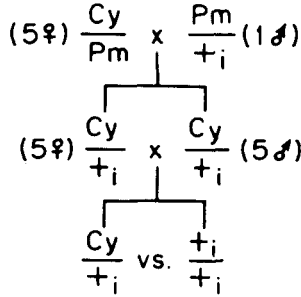


FIGURE 2.—Mating scheme for the test of homozygous viability.

The mating scheme is given in Figure 1. A single male from the inbred wild-type stock was mated to two *Cy/Pm* females. A single progeny male from this mating was mated to 10 *Cy/Pm* females in a half-pint bottle. From this mating, 136 *Pm/+* males were each mated with five *Cy/Pm* females to start 136 lines. Next generation, 10 males from each line were selected and divided into 5 groups of two each. The two males from the *j*th group of the *i*th line are designated *Pm/+_{ij}* (*i* = 1 to 136, *j* = 0 to 4). In each line, the males of group 0 were untreated, and groups 1, 2, 3, and 4 were treated with EMS in each generation with EMS at concentrations 5×10^{-5} , 1×10^{-4} , 5×10^{-4} , and 2.5×10^{-3} M. After treatment each male was mated with two *Cy/Pm* females. The procedure was repeated in successive generations for each group in each line. In each group only one mating was used, the other being insurance against sterility or error. Although the intention was to test all 136 lines at each concentration, some were unsuccessful in mating and some were lost accidentally, so that the actual numbers

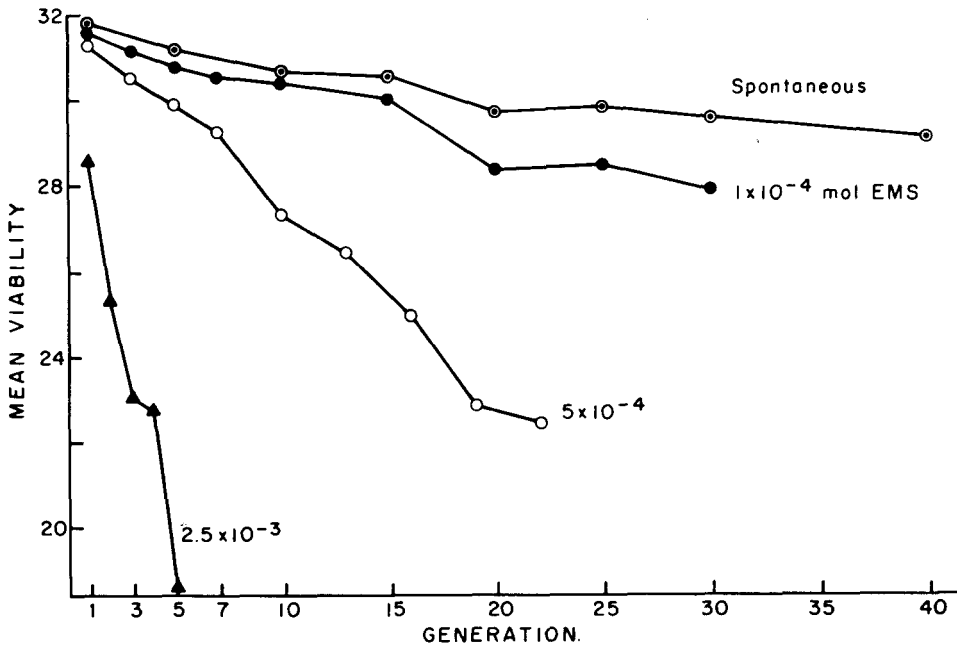


FIGURE 3.—Mean viability index plotted against the number of generations of mutation accumulation. The viability index is $100 \times (+/+ \text{ flies}) / [(Cy/+) + (+/+)]$. Data are for all nonlethal lines.

tested in groups 0, 1, 2, 3, and 4 were 136, 107, 120, 112, and 136, respectively. Males were always less than 24 hr old when treated and were mated immediately after treatment each generation.

At specified generations, the chromosomes were made homozygous and tested for viability. The number of generations treated and the generations at which homozygous tests were made can be seen in Figure 3. The mating scheme for homozygous tests is adapted from WALLACE (1956) and is almost the same as that used by MUKAI (1964, 1970). It is shown in Figure 2. A single $Pm/+$ male from each group in each line was mated to 5 Cy/Pm females. From the progeny of each of these, three replicate matings were made, each with 5 $Cy/+$ females and 5 $Cy/+$ males, in half-pint bottles. Females were allowed to deposit eggs for four days, and then they were discarded. Curly and wild-type progeny were counted five times at two-day intervals, starting with the 10th day after mating. The ratio $100 \times (\text{number of } +/+ \text{ flies} / \text{total number})$ was used as a viability index.

In addition to these long-run experiments, two single-generation treatments at higher concentrations were given. The procedure was the same and the tests were done at the same time as generations 30 and 40 of the long-run experiments. Each line was divided for treatment into three groups, a control and treatments of 1×10^{-3} and 5×10^{-3} M. In these experiments five flies from five different lines were treated in the same vial. The line identification is thus lost, but this does not matter in a single-generation experiment.

The tests done at the time of generation 40 were modified slightly to eliminate any effect of mosaic lethals. The test was delayed one generation by an additional mating of treated $Pm/+$ males to Cy/Pm females before the test mating. Although the viability indices for these single generation experiments were computed from their own controls (based on 68 chromosomes in the generation 30 experiment and on 35 chromosomes in the generation 40 experiment), the viability indices were adjusted to correspond to the mean viability of the control lines at the beginning of the long-run experiment.

METHOD OF ESTIMATION OF POLYGENIC MUTATION RATES AND AVERAGE EFFECTS OF AN INDIVIDUAL MUTANT GENE

The method of estimation is that of BATEMAN (1959) and MUKAI (1964). The mutants are assumed to have additive effects on viability and a Poisson distribution occurrence. Then, if the mutation rate parameter is u per chromosome and if the distribution of individual effects has mean \bar{s} and variance σ_s^2 , then the decrement of mean variability, $m(t)$, and the genetic variance, $\sigma_g^2(t)$, at generation t are given by

$$m(t) = u \bar{s} t \quad (1)$$

and

$$\sigma_g^2(t) = u \bar{s}^2 t + u \sigma_s^2 t . \quad (2)$$

Therefore, we expect the mean decrement and the genetic variance to change linearly with generation number.

Since there are three replications for the viability test of each chromosome, we can estimate the accumulated genetic variance $\sigma_g^2(t)$ by analysis of variance (MUKAI 1964; MUKAI *et al.* 1972). The decrement of mean viability, $m(t)$, was estimated by subtracting the mean viability at the beginning of the long-run experiment from the mean viability at time t . The decrement in mean viability and genetic variance per generation, m and σ_g^2 , were estimated by the slope of the unweighted least-square regression line.

The values for m and σ_g^2 per generation are

$$m = u \bar{s} \quad \text{and} \quad \sigma_g^2 = u \bar{s}^2 + u \sigma_s^2 . \quad (3)$$

Solving these for u and using the fact that $u > 0$, we have

$$0 \leq \sigma_s^2 \leq (\sigma_g^2)^2 / 4 m^2 , \quad (4)$$

$$u \geq m^2 / \sigma_g^2 , \quad (5)$$

and

$$\bar{s} \leq \sigma_g^2 / m . \quad (6)$$

Using these formulae, we can obtain the lower limit of the mutation rate u , and the upper limits of the mean viability effect \bar{s} and the variance of the individual effects σ_s^2 .

EXPERIMENTAL RESULTS

All chromosome lines with homozygous viability indices less than 1 were regarded as lethal, those with indices from 1–10 as semilethal, those from 10–20 as deleterious, and those above 20 as quasi-normal.

The basic data are given graphically in Figures 3 to 6. The average number of flies counted per test culture was from 750 to 950; hence the average number of flies on which the viability of a chromosome at a particular generation was estimated was 2000–3000. The total number of flies in all experiments combined, including heterozygous tests reported in the following paper (OHNISHI 1977b), was over 6 million. The data are presented more fully in my thesis (OHNISHI 1974).

The decrease in mean viability is shown graphically for all nonlethal lines in Figure 3 and for quasi-normal lines in Figure 4. Corresponding graphs for the increase in genetic variance are given in Figures 5 and 6. (Since the number of generations in the concentration 5×10^{-5} M was limited, these values are omitted from the graphs and from the subsequent analysis.) It can be seen that the mean viability decreases approximately linearly over all treatment concentrations. The genetic variance also increases linearly in the early stages, but plateaus at about 50 in the nonlethal lines and at about 6 in the quasi-normals for the high concentrations. This phenomenon may be explained by the fact that some chromosome lines, which showed low viability and had a large contribution to genetic variance in the previous test generation, became lethal or exhibited variability less than the threshold of quasi-normals. As a result, a large amount of variance lost from the sample may be balanced by the increment of variance due to newly arisen mutations. On the other hand, the mean viability still can continue to decrease by reducing the relative number of chromosomes with higher viability. For this reason the estimates of m and σ_g^2 are based on only two generations for the concentration 2.5×10^{-3} and 13 for 5×10^{-4} M. For the concentration 1×10^{-4} M and the control, all generations were used.

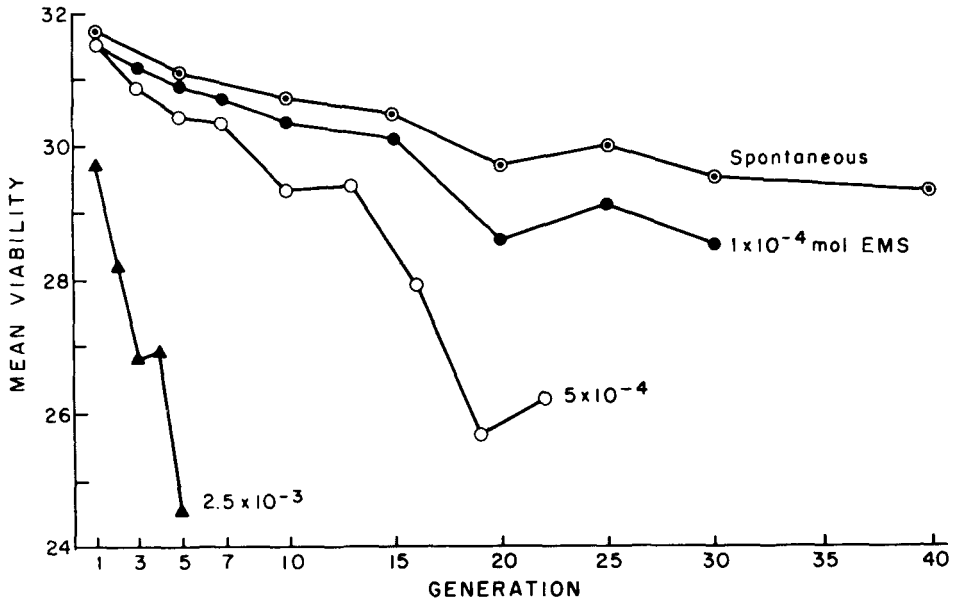


FIGURE 4.—Mean viability index for quasi-normal lines.

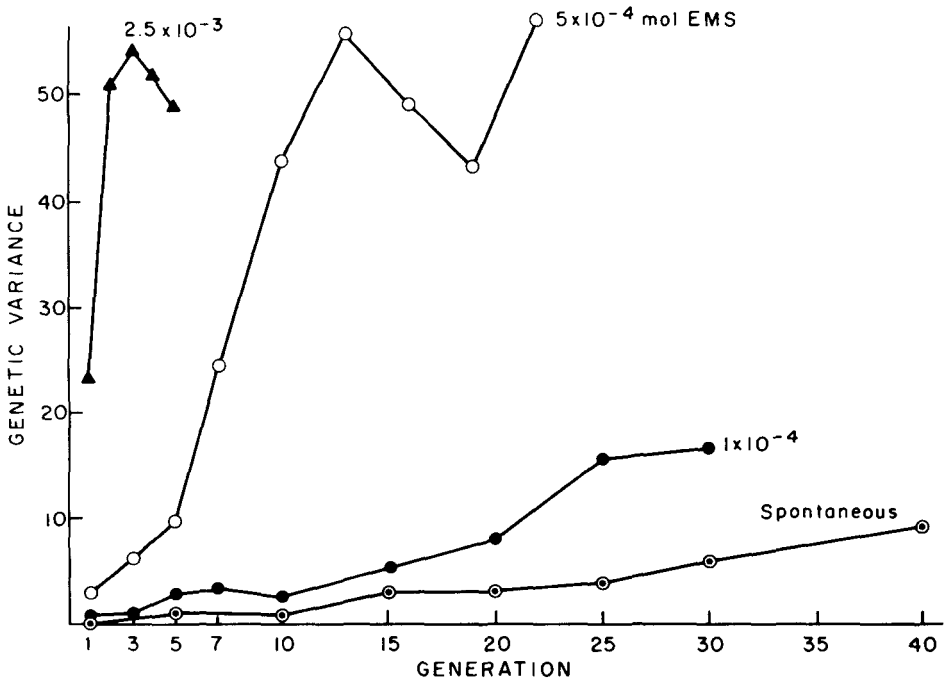


FIGURE 5.—Genetic variance of the viability index as a function of the number of generations of accumulated mutations. Data are for all nonlethal lines.

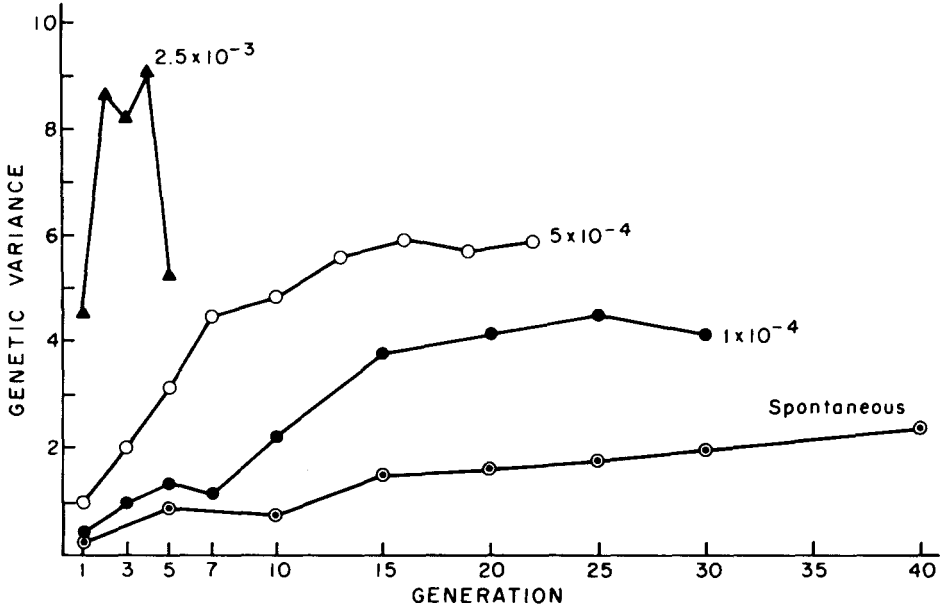


FIGURE 6.—Genetic variance of the viability index as a function of the number of generations of accumulated mutations. Data are for all quasi-normal lines.

TABLE 1

Estimated decrement of mean viability and increment of genetic variance per generation

Conc. of EMS (mol)	Non-lethal	Quasi-normal
Mean viability decrease		
0	0.066 + 0.019*	0.055 + 0.022
1 x 10 ⁻⁴	0.127 ± 0.027	0.100 ± 0.030
5 x 10 ⁻⁴	0.358 ± 0.086	0.181 ± 0.084
1 x 10 ⁻³	1.77 ± 0.78	0.86 ± 0.43
2.5 x 10 ⁻³	3.47 ± 1.27	1.57 ± 0.57
5 x 10 ⁻³	4.25 ± 1.49	2.29 ± 0.37
Genetic variance increase		
0	0.219 + 0.064	0.052 + 0.015
1 x 10 ⁻⁴	0.578 ± 0.149	0.185 ± 0.043
5 x 10 ⁻⁴	2.403 ± 1.366	0.387 ± 0.141
1 x 10 ⁻³	17.35 ± 5.49	2.64 ± 1.10
2.5 x 10 ⁻³	27.20 ± 9.53	4.10 ± 1.79
5 x 10 ⁻³	37.94 ± 12.74	6.77 ± 2.80

* ± indicates approximate 95% confidence limits.

The decrease in mean viability and increase of genetic variance per generation are given in Table 1. The figures for the single-generation treatment done at the time of generation 30 include a slight correction for the viability-reducing effect of undetected mosaic lethals (see OHNISHI 1974 for the correction). In the long-run experiments, the effect of mosaic mutants would be to cause a slight displacement in the regression line, but would not change the slope. Thus, there would be no effect on the calculations.

Table 2 gives the estimates for minimum mutation rate per chromosome, maximum mean effect per mutant, and maximum variance of individual mutant effects (from equation 5, 6, and 4). All values for treated chromosomes have been corrected by subtracting the effect of spontaneous mutants. The most reliable values for polygenic mutations are probably from the quasi-normals, since the variance is not inflated by a few chromosomes of very low viability. The values from nonlethals can be considered mostly for detrimental mutants.

The minimum spontaneous mutation rate is estimated as 0.058 per second chromosome per generation. The unweighted average of the treated groups is 0.017 per 10^{-4} M. The mean effect per mutant is approximately the same for

TABLE 2

Estimated EMS-induced polygenic mutation rate and the mean and variance of effect of an individual EMS-induced polygenic mutation on the viability index

Conc. of EMS (mol)	Non lethal	Quasi-normal
Mutation rate (minimum)		
0	0.020	0.058
1 x 10^{-4}	0.010	0.015
5 x 10^{-4}	0.039	0.047
1 x 10^{-3}	0.17	0.25
2.5 x 10^{-3}	0.43	0.57
5 x 10^{-3}	0.46	0.73
Mean (maximum)		
0	3.31	0.95
1 x 10^{-4}	5.89	2.96
5 x 10^{-4}	7.48	2.66
1 x 10^{-3}	10.05	3.21
2.5 x 10^{-3}	7.93	2.67
5 x 10^{-3}	9.02	3.01
Variance (maximum)		
0	2.75	0.23
1 x 10^{-4}	8.66	2.18
5 x 10^{-4}	13.99	1.77
1 x 10^{-3}	25.27	1.77
2.5 x 10^{-3}	15.71	1.78
5 x 10^{-3}	20.32	2.26

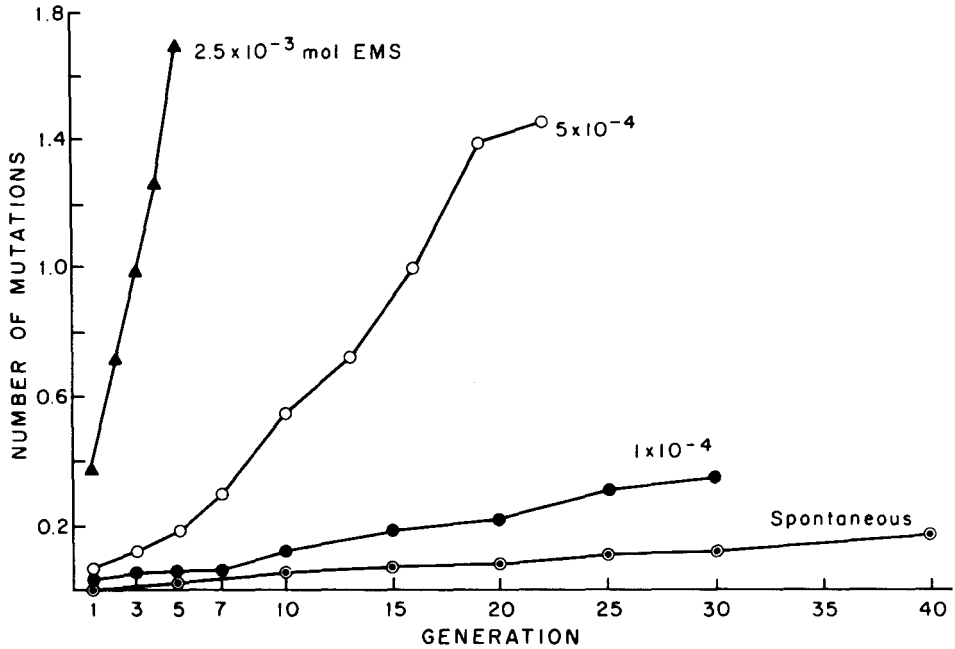


FIGURE 7.—Frequency of lethal mutations, assuming a Poisson distribution, as a function of the number of generations of accumulation.

all doses, both in the nonlethal group and the quasi-normal group. It would seem that the average effect per mutant is substantially greater for the mutants produced by EMS than for the spontaneous mutants.

In the course of the long-run experiment, many recessive lethal mutations accumulated. The frequency of lethal mutations per chromosome, assuming a Poisson distribution, is shown graphically in Figure 7. The estimated mutation rates, with standard errors, are given in Table 3. The rate increases approximately linearly with concentration, and the unweighted average is 0.013 per

TABLE 3

Recessive lethal mutation rate and its standard error

Conc. of EMS (M)	Mutation rate	Standard error	Number of generation on which the estimation is based	Mutation rate per 10^{-4} M
0	0.0041	0.0002	40	
5×10^{-5}	0.0075	0.0064	5	0.015
1×10^{-4}	0.0118	0.0005	30	0.012
5×10^{-4}	0.0556	0.0094	13	0.011
1×10^{-3}	0.153	0.042	1	0.015
2.5×10^{-3}	0.326	0.026	5	0.013
5×10^{-3}	0.541	0.053	1	0.011
Unweighted mean				0.013

10^{-4} M. This is in good agreement with the results of the single-generation treatment reported in the preceding paper (OHNISHI 1977a), when the complete and mosaic lethals are added, which is the appropriate comparison.

Table 4 gives the decrease in mean viability caused by the various classes of mutations. It is clear that the largest contribution is made from lethals. The last column shows the D/L ratio, the ratio of the genetic load due to nonlethal mutations to that due to lethals. The value is fairly consistent for all EMS concentrations (considering the uncertainty of the measurements), but is clearly lower for EMS-induced than for spontaneous mutations. Despite the fact that individual spontaneous nonlethal mutants are smaller in effect than EMS-induced mutants, their greater relative number makes their contribution to the load greater than that of the EMS-induced mutants. The figures in parentheses in the last column give the loads calculated on a multiplicative model (see GREENBERG and CROW 1960) and with the classification that they used for lethals (less than 10 percent of the expected viability). The values are not greatly different from the values based on an additive model.

The maximum reduction in viability per mutant in Table 2 is given in units of the viability index, whose control value is 31.71. To convert these numbers into percent decrease, divide by 0.3171. Thus, the maximum estimate for the percent viability decrease per EMS-induced mutant among quasi-normals is about 9 percent, compared to 3 percent for spontaneous mutations. The mean viability decrease shown in Table 4 is given as the proportionate reduction from the control value.

It is in some respects more desirable to use as a viability index the ratio $(+ / + \text{ flies}) / (Cy / + \text{ flies})$. This has the advantage of making the decrease in viability linear with the mutation rate. If s is the fraction by which the viability is reduced, this index is proportional to $(1-s)/2$ rather than to $(1-s)/(2+1-s)$, the value with the index I have used. Using the other index, the mutation rate

TABLE 4

Decrease of mean viability caused by various classes of mutations and D/L ratio

Conc. of EMS (mol)	Quasi-normal	Deleterious & Semilethal	Subtotal	Lethal	Total	D/L
0	0.0017	0.0004	0.0021	0.0041	0.0062	0.51 (0.51)*
1×10^{-4}	0.0031	0.0009	0.0040	0.0117	0.0157	0.34 (0.34)
5×10^{-4}	0.0054	0.0052	0.0106	0.0541	0.0647	0.20 (0.20)
1×10^{-3}	0.0233	0.0246	0.0479	0.1421	0.1900	0.34 (0.37)
2.5×10^{-3}	0.0358	0.0432	0.0790	0.2779	0.3569	0.28 (0.36)
5×10^{-3}	0.0420	0.0360	0.0780	0.4177	0.4957	0.19 (0.27)

* (D/L) is estimated under the assumption of multiplicative effect of mutations.

estimates are higher. For example, the newly estimated spontaneous mutation rate is 0.073 as compared to the corresponding value of 0.058. The calculations may be found in OHNISHI (1974).

DISCUSSION

Polygenic mutations can ordinarily be studied only by making mathematical and statistical assumptions and by statistical observations. Therefore, we cannot discuss the nature of the mutations at the same level as is possible with the more usual qualitative mutations. It is proper to investigate the validity of the underlying assumptions.

In this study the following assumptions are made: (1) Mutations affecting viability occur in the same manner as the usual qualitative mutations. Furthermore, the mutation rate per generation remains constant under a fixed treatment condition. (2) The effect of polygenic mutations on viability as measured by the reduction of survival to the adult stage is linear. The assumptions appear to be satisfied, within the accuracy of the experiments, by the linearity of the mean viability and the accumulated genetic variance with the number of generations of accumulated exposure. I found no indication of the quadratic component in mean viability effect reported by MUKAI (1969) in comparable experiments, although this might have appeared after a large number of generations. As mentioned earlier, the choice of viability index makes no substantial difference in the estimate of mutation rate.

Comparison of the maximum average effect of an individual mutant in the treated and control groups reveals two important results: (1) the maximum average effect is about 3 times as large in the EMS treated as in the control group, and (2) the average effect within the EMS treated group does not depend on the concentration at the concentrations tested here. Furthermore, the minimum mutation rate increases linearly with the concentration at these dose levels. Finally, not unexpectedly, EMS treatment produces a larger increase in lethals than polygenic mutations, in comparison with spontaneous mutants.

Note that for each concentration the estimated mutation rate in Table 2 is higher in the quasi-normal class than in the nonlethal class. This obviously makes no sense, since the latter class includes the former. It is not unexpected, however, because of the implicit assumption in these estimates that $\sigma_s^2 = 0$. This may be approximately correct for the quasi-normal group, but clearly cannot be true for the nonlethal group, which includes some mutants with large effects. A few mutants with large effect contribute greatly to the mean and even more to the variance. A large estimated value of the effect of an individual EMS-induced mutation implies the occurrence of some mutants with large individual effects, but does not deny the possibility of many with very minor effects. For these reasons the estimates for the quasi-normal group are likely to be more nearly correct.

The estimates of mutation rate reported here appear to be only one-half of those reported by MUKAI (1964) and by MUKAI *et al.* (1972). Since homozygotes with mutations show lower viability in high density test conditions, such

as the five-pair matings in vials used by MUKAI, than in less dense conditions as in the present experiments (OHNISHI 1974), the discrepancy between two reports with respect to the estimated genetic variance and decrement of mean viability is most likely due to the different culture conditions.

Can we do more than set upper limits for the individual effects and lower limits for the mutation rates? Let us return to the estimating formulae. We know σ_g^2 and m for each concentration from the experimental data. Eliminating \bar{s} from the equations for σ_g^2 and m , we obtain

$$\sigma_s^2 u^2 - \sigma_g^2 u + m^2 = 0 \quad (7)$$

Then, from the relation $\bar{s} = m/u$, we also have

$$\sigma_s^2 m - \sigma_g^2 s + m \bar{s}^2 = 0 \quad (8)$$

Solutions for \bar{s} and u for the observed values of σ_g^2 and m are plotted against σ_s^2 in Figures 8–11. Note from Figures 8 and 9 that the estimated value of \bar{s} , when σ_s^2 is maximum, is one-half its maximum value, as is clear from algebraic considerations. Note also that, because of the inverse relationship between u and \bar{s} , the upper values of \bar{s} in the parabola-like curves in Figure 8 and 9 correspond to the lower values for u in Figures 10 and 11. If there is no *a priori* relationship

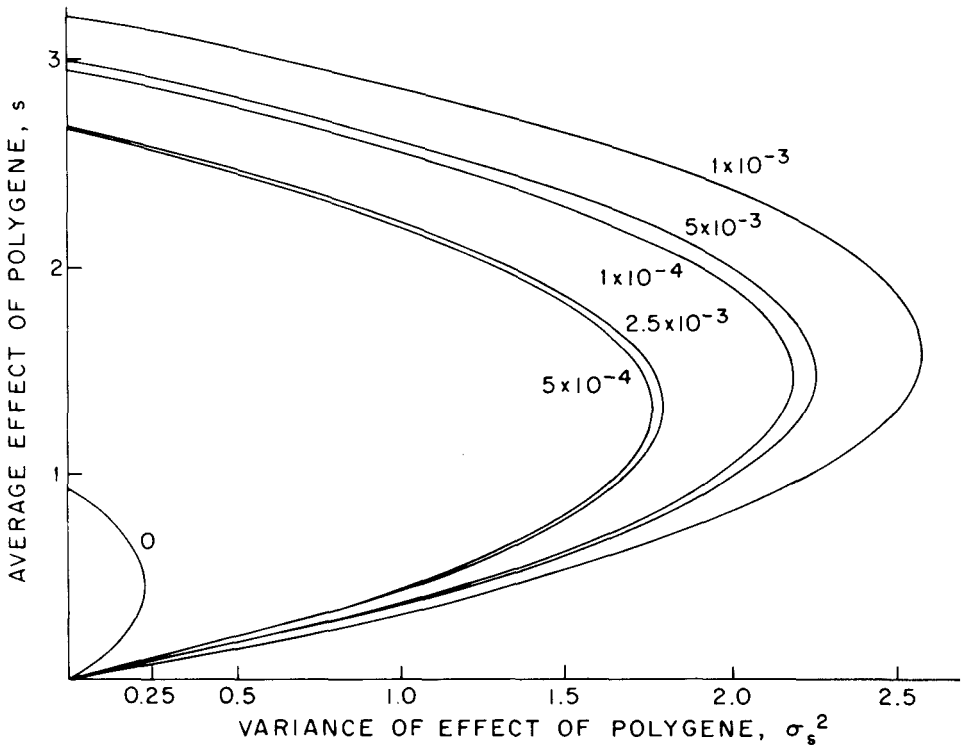


FIGURE 8.—Relation between average of viability effects of individual mutants and their variance. Quasi-normal lines.

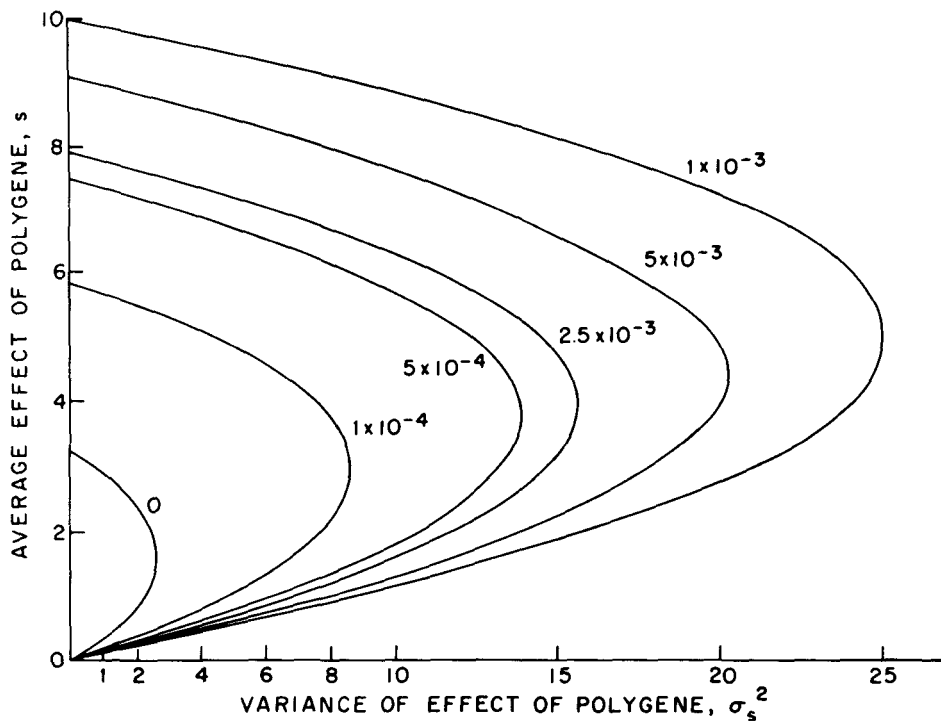


FIGURE 9.—Relation between average of viability effects of individual mutants and their variance. Nonlethal lines.

between σ_s^2 and \bar{s} or u , then any point on a line is possible. However, it is not reasonable to find a large \bar{s} with a small σ_s^2 .

Consider first the quasi-normal data in Figure 8. If we assume that there is no qualitative difference in the spontaneous and EMS-induced mutants, so that differences in σ_s^2 and m in the two groups depend entirely on differences in mutation rates, then the only possible combinations that are consistent with the data are in the lower left corner. These lead to mutation rates that seem unreasonably high; for example if we take $\sigma_s^2 = 0.5$ and correspondingly $\bar{s} = 0.1$, we have a mutation rate of more than 100 per chromosome for the higher concentrations. Furthermore, the consistency of the maximum estimates of \bar{s} within the EMS-treated group and the difference between these and the spontaneous group argue that the two groups are qualitatively different.

If we make the justifiable assumption that the distribution of s values is the same for all EMS doses, then the estimated mutation rates at different concentrations should be linear with the concentration. Then, there are only two possible models consistent with the observed data. The first one is that EMS-induced mutants have a small average effect, about 0.5 to 1.0, which is not much different from spontaneous, and a variance of about 1 to 2. For example, if we take σ_s^2 as 1.5, corresponding to $\bar{s} = 0.5 - 0.8$, then the mutation rates for the five increasing doses are 0.07, 0.16, 1.42, 1.88 and 3.54, as can be seen from Figure 10.

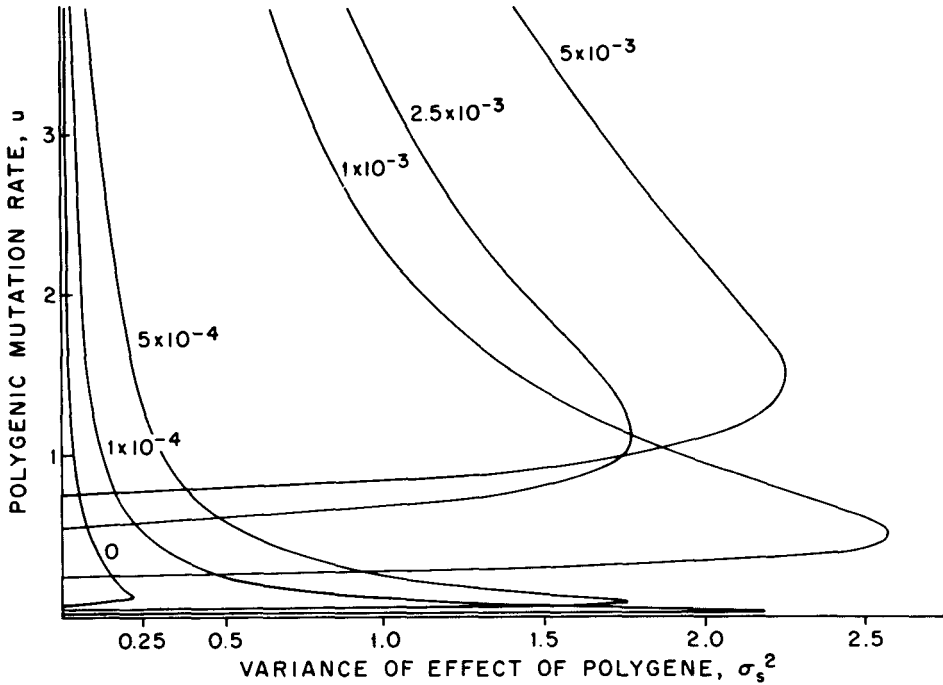


FIGURE 10.—Relation between mutation rate per chromosome, u , and variance of the individual mutant effects, σ_s^2 , corresponding to observed values of the per generation change in mean and genetic variance of the viability index. Quasi-normal lines.

An alternative interpretation for the observed values is that EMS-induced mutations have much larger individual effects, but a small variance. This places them in the upper middle and left portion of Figure 8. These give estimates of \bar{s} that are close to the maximum estimates, and correspondingly estimates of mutation rate near the minimum.

For the nonlethal case, it appears more difficult to interpret the observed results by a simple model of induced mutations. As a first approximation, it is reasonable to assume that mutations with severe effect on viability occur very rarely. This assumption is supported by the rarity of chromosomes with viability below 20 in the early generations of the experiments. In this case, σ_s^2 for the whole distribution of EMS-induced mutations can be very large in comparison with that for quasi-normals, but the mean \bar{s} remains relatively small and the mutation rate almost the same as for the quasi-normal case. Under these assumptions, we arrive at the conclusion from Figures 9 and 11 that the possible σ_s^2 values are either about 4–10 with \bar{s} values around 0.5 to 1.5, corresponding to the first explanation of the quasi-normal lines, or variances about the same but \bar{s} values ranging from 4 to 10, corresponding to the second model. The mutation rates are essentially the same as the minimum estimates for the quasi-normal chromosomes.

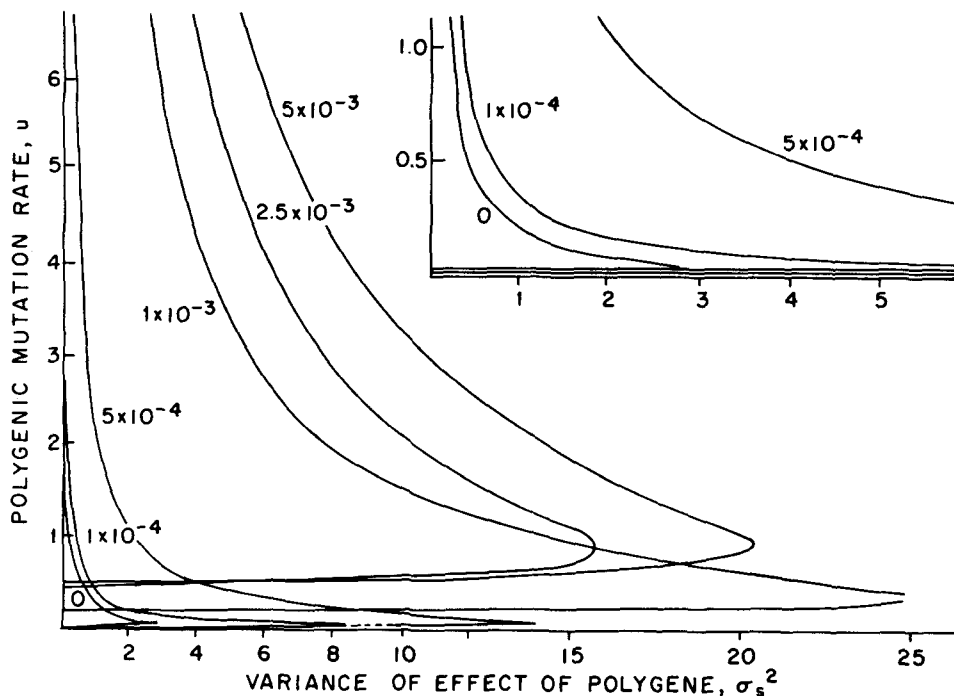


FIGURE 11.—Relation between mutation rate per chromosome, u , and variance of the individual mutant effects, σ_s^2 , corresponding to observed values of the per generation change in mean and genetic variance of the viability index. Nonlethal lines.

There is another way of looking at the problem. Equations (3) may be rewritten (MUKAI *et al.* 1972).

$$u = k m^2 / \sigma_g^2 \quad (9)$$

and

$$\bar{s} = \sigma_g^2 / k m, \quad (10)$$

where

$$k = \overline{s^2} / \bar{s}^2 = 1 + \sigma_s^2 / \bar{s}^2. \quad (11)$$

I have noted before (see Table 2) that, within the exposed group, m^2/σ_g^2 is proportional to the concentration, as it should be, for it is proportional to the mutation rate. On the other hand σ_g^2/m is independent of the concentration, as it should be, for this is proportional to the average effect per mutant.

Since σ_g^2/m is about 3 times as large in the EMS-induced group as in the spontaneous group, then $k \bar{s}$ must be 3 times as large. Thus, if k is the same for both groups, \bar{s} is three times as high for the EMS group. Constancy of k would be expected if the two distributions of viability effect have the same shape, which is perhaps reasonable, but certainly not established. On the other hand, it could

be true that the two distributions have the same mean, but that the EMS group has a greater mean square (or variance).

The only rigorous conclusion is that the distribution of viabilities of EMS-induced viabilities is different from that of the spontaneous mutants, but whether the EMS group has a greater mean, or a greater variance, or some mixture cannot be established from these data.

Assuming no significant production of viability-increasing mutants, the greater value of $k\bar{s}$ implies that the distribution of EMS mutants has a greater density of larger values of s . This might be expected as an extension of the observation that there is a greater proportion of lethals among the EMS-induced group. But whether EMS also induces a large number of very mild mutants cannot be determined.

It is known that EMS induces base pair substitutions in microorganisms. It is also known that EMS can induce translocations (YOST, IVES and HALL 1967; ABRAHAMSON, KIRIAZIS and SOBOL 1969) and deletions (BISHOP and LEE 1969; LIFSCHYTS and FALK 1969). It is therefore a reasonable conclusion that at high doses, and possibly at low doses as well, EMS induces some chromosome aberrations, as well as point mutations. Hence, a large average effect and a very large variance of individual effects may be expected, caused by a mixture of point mutations, small deletions, and possibly other types of rearrangements.

MUKAI (1970) arrived at the same conclusion for mutations induced by very high concentrations of EMS. It should be pointed out, however, that at high concentrations, many detrimental mutations may be lost from the sample, as is suspected for the high doses where the variance plateaued in my data. In fact, the minimum mutation rate and maximum average effect estimated from the genetic variance and decrement of mean viability at the plateau, $u = 0.7$ and $\bar{s} = 8$ (or about 0.25 when normalized), are close to MUKAI's $u = 0.59$ and $\bar{s} = 0.36$. Some of my unpublished data show that the increase in variance is not linear at concentrations above 10^{-2} M. Therefore, it is suggested that the polygenic mutation rate and the average effect should be estimated from relatively low doses, say less than 5×10^{-3} M.

Because we can be certain only about the minimum estimate of the mutation rate, and the maximum for the average effect of a mutant, it is for some purposes more revealing to look at the genetic loads and the ratio of the load due to detrimentals to that due to lethals (the D/L ratio). It is clear, as pointed out earlier, that the ratio is lower for EMS-induced than for spontaneous mutations. Although the data for X-ray-induced mutations are quite unreliable, the available data are consistent with the idea that the D/L ratio for EMS treatment is between that for X-ray-induced and spontaneous mutations. This is expected if EMS produces relatively more deletions and other chromosome changes than are found among spontaneous mutants, and if radiation produces still more.

Perhaps the most surprising result is the relatively low minimum frequency of detrimental mutations relative to lethals in the EMS-induced group, as compared to spontaneous mutations. For spontaneous mutations, the mildly detrimental group is at least 12 times as frequent as lethals. For the EMS-induced

mutations, the minimum estimate for mildly deleterious mutations is less than twice that of lethals.

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