

Nature of Deleterious Mutation Load in *Drosophila*

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ABSTRACT

Much population genetics and evolution theory depends on knowledge of genomic mutation rates and distributions of mutation effects for fitness, but most information comes from a few mutation accumulation experiments in *Drosophila* in which replicated chromosomes are sheltered from natural selection by a balancer chromosome. I show here that data from these experiments imply the existence of a large class of minor viability mutations with approximately equivalent effects. However, analysis of the distribution of viabilities of chromosomes exposed to EMS mutagenesis reveals a qualitatively different distribution of effects lacking such a minor effects class. A possible explanation for this difference is that transposable element insertions, a common class of spontaneous mutation event in *Drosophila*, frequently generate minor viability effects. This explanation would imply that current estimates of deleterious mutation rates are not generally applicable in evolutionary models, as transposition rates vary widely. Alternatively, much of the apparent decline in viability under spontaneous mutation accumulation could have been nonmutational, perhaps due to selective improvement of balancer chromosomes. This explanation accords well with the data and implies a spontaneous mutation rate for viability two orders of magnitude lower than previously assumed, with most mutation load attributable to major effects.

THE rate at which fitness declines due to the fixation of spontaneous mutations is of crucial importance for population and evolutionary theory concerning mutation load (SIMMONS and CROW 1977; CROW and SIMMONS 1983), the evolution of sex and recombination (KONDRASHOV 1988), the maintenance of variation (BARTON and TURELLI 1989; CABALLERO and KEIGHTLEY 1994; HUDSON and KAPLAN 1995; CHARLESWORTH *et al.* 1995), and conservation of small populations (LANDE 1995; LYNCH *et al.* 1995). Current knowledge of the extent of mutation load comes largely from a small number of experiments on the accumulation of spontaneous mutations in *Drosophila* (MUKAI 1964; MUKAI *et al.* 1972; OHNISHI 1977), which imply that the overall rate of genetic erosion of viability from mutations occurring throughout the genome is between 1 and 2% per generation. This loss of viability would have to be resisted by natural selection and has led to the hypothesis that populations may be vulnerable to extinction due to loss of fitness from fixation of deleterious mutations (LANDE 1995; LYNCH *et al.* 1995).

Classic mutation accumulation experiments in *Drosophila melanogaster* involve the maintenance of replicates of wild-type (+) second chromosomes sheltered against a balancer chromosome in conditions that minimize the opportunity for natural selection. The balancer contains multiple inversions and suppresses recombination, thereby keeping the wild-type chromosome intact. The competitive viability of a wild-type

chromosome relative to the *Cy* balancer is measured at different generations by mating *Cy/+* individuals *inter se* and measuring the ratio of the number of wild type (+/+) to *Cy* (*Cy/+*) progeny (WALLACE 1956) (*Cy/Cy* is lethal). In cases where such an experiment has been performed (MUKAI 1964; MUKAI *et al.* 1972; OHNISHI 1977), mean relative viability of sheltered chromosomes declined approximately linearly with time, (*i.e.*, the relative number of emerging wild-type progeny became smaller with increasing generation number). A small number of chromosomes accumulated lethal or severely deleterious mutations, but the majority of "quasi-normal" chromosomes also declined markedly in viability relative to the balancer (see Figure 1, a and b). This latter observation has been interpreted as demonstrating the occurrence of a large number of mutations with very small effects on viability (CROW and SIMMONS 1983), since a smaller number of mutations with major effects would lead to higher variance among the quasi-normals.

Previously, I developed a method to infer mutation rates and parameters of distributions of mutation effects using information in the distribution of phenotypic values of chromosomes or sublines obtainable from mutation accumulation experiments (KEIGHTLEY 1994) and showed that the distribution of mutation effects for viability in *Drosophila* is highly leptokurtic. Prompted by a suggestion by Dr. M. TURELLI, in this paper I investigate further the nature of deleterious mutation load by comparing the fit of continuous and discontinuous distributions of effects of mutations for viability to published data on spontaneous mutation

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accumulation and to data on the distribution of viabilities of EMS-treated and control chromosomes in *D. melanogaster*.

MATERIALS AND METHODS

Data on spontaneous mutation accumulation: Relative viabilities of second chromosomes after 40 generations of maintenance against a balancer chromosome were obtained from MUKAI *et al.* (1972) and OHNISHI (1974) consisting of data on 43 and 106 sublines, respectively, and these were used in the analysis, but lines with lethal mutations were excluded. OHNISHI (1974) also provided data on viabilities of chromosomes at the start of the experiment, and this information was used in the analysis to estimate the base population mean and sampling variance simultaneously with the mutation parameters. In the case of the data set of MUKAI *et al.* (1972) the mean and sampling variance were estimated separately by regression (KEIGHTLEY 1994).

EMS mutagenesis. Data on competitive viabilities of 202 second chromosomes exposed for one generation to 2.5 mM EMS, along with estimated viabilities of 75 control chromosomes were kindly provided by Dr. O. OHNISHI (personal communication; see also CROW and SIMMONS 1983). Young adult males of an inbred stock were exposed to EMS (or not, in the case of the controls) according to the procedure of LEWIS and BACHER (1968), and replicates were backcrossed on to a *Cy/Pm* stock with otherwise the same genetic background, to extract mutagenized (or nonmutagenized) second chromosomes. Relative viability was measured as the ratio of emerging wild type to *Cy* flies in an intercross of 5 *Cy/+* females with 5 *Cy/+* males. The viability test was carried out in 180-ml milk bottles at 25°. The viability for a line was the average for three replicates. In the analysis, control data were included to allowed the simultaneous estimation of the population mean and sampling variance. A major difference between the design of this experiment and the above spontaneous mutation accumulation experiments was that the control and EMS-treated chromosomes were assayed contemporaneously.

Models: The fit of the data from the above three experiments was investigated under models of continuous or discontinuous distributions of mutation effects, with the number of mutation events per chromosome per generation assumed to be Poisson distributed with parameter U and mutation effects assumed to be unconditionally deleterious.

Gamma distribution. This continuous distribution has two parameters specifying scale (α) and shape (β), variation in which can generate distributions with arbitrary mean and a wide variety of shapes (see Figure 2a).

Gamma + equal distribution. This mixed distribution of mutation effects was investigated in which a proportion $1 - p$ of mutations have effects sampled from a standard gamma distribution, and a proportion p of mutations have a fixed effect ϵ (see Figure 2b). In addition to the gamma shape and scale parameters, p and ϵ required to be estimated.

Gamma + environmental change. A model was investigated in which the relative viability of each chromosome was assumed to have shifted downwards for some nonmutational reason by a constant amount k , a parameter that was estimated in the model. Mutation was also included, the effects of which were assumed to be from a gamma distribution.

Likelihood analysis: The fit of different distributions of mutation effects for viability was compared by evaluating the likelihood of the data sets under the different models by Monte Carlo integration using the method described in detail by KEIGHTLEY (1994). The parameters to be estimated were the mutation rate, gamma shape and scale parameters, the

equal effects proportion and value where appropriate, the nonmutational effect where appropriate, and the population mean and variance where data on nonmutagenized chromosomes were available. The likelihood of an observation was the average of likelihoods from at least 10^7 sampled genotypic values (Equation 3 in KEIGHTLEY 1994). Each genotypic value was simulated by sampling the sum of n gamma deviates, or gamma deviates plus equal effects (ϵ), where n is a random variable from the Poisson distribution parameter U . The overall log likelihood of the complete data set was the sum of log likelihoods for the individual independent observations. The Simplex algorithm (NELDER and MEAD 1965) was used to maximize the likelihood of the data. Convergence to the maximum likelihood (ML) was checked by increasing the number of replicates by factors of 10 and restarting the procedure until no significant increase in likelihood was observed. Satisfactory convergence was achieved after 10^6 replicates. The parameter space was explored by starting the procedure with several different initial values for the parameters.

RESULTS

Spontaneous mutation accumulation: Distributions of viabilities of spontaneous mutation accumulation chromosomes after 40 generations from the two published experiments are shown in Figure 1, a and b. Previous analysis showed that a model in which all mutations have equal effects fits both data sets very poorly, but a gamma distribution (Figure 2a) of effects fits the experimental data sets significantly better (KEIGHTLEY 1994). With a gamma distribution, the parameters giving the highest likelihood is a highly leptokurtic distribution ($\beta \rightarrow 0$) with very small mean mutant effect [$E(a) = \beta/\alpha \rightarrow 0$], and a very high mutation rate ($U \rightarrow \infty$) (KEIGHTLEY 1994). However, the gamma + equal distribution (Figure 2b), was found to give a significantly better fit (Table 1). The expected distribution of viabilities of chromosomes assuming ML estimates of the parameter values is compared to the data in Figure 3. The improved fit to the observations for the discontinuous distribution is obvious from visual inspection. The likelihood of OHNISHI's (1974) dataset is flat for p values in the range $0 < p < 1$. For MUKAI *et al.*'s (1972) dataset the likelihood is flat for most of this range, but increases for p values very close to 1, and the best fit is two classes of equal effect explaining the two modes in the data. Previously, I thought this model gave a poorer fit than the gamma distribution (KEIGHTLEY 1994), but this was an error due to a failure to find the global maximum likelihood. The proportion of the observed change of mean viability explained by the equal effects class varies little for p values in the range $0 < p < 1$, their effects account for ~80% and ~60% of the observed reduction of mean relative viability, and minimum estimates of U are 0.2 and 0.04 for MUKAI *et al.*'s and OHNISHI's data, respectively. To check the robustness of the discontinuous model, a distribution in which there could be a large class of approximately equivalent effects generated by displacing the gamma distribution from zero by an amount δ (an additional

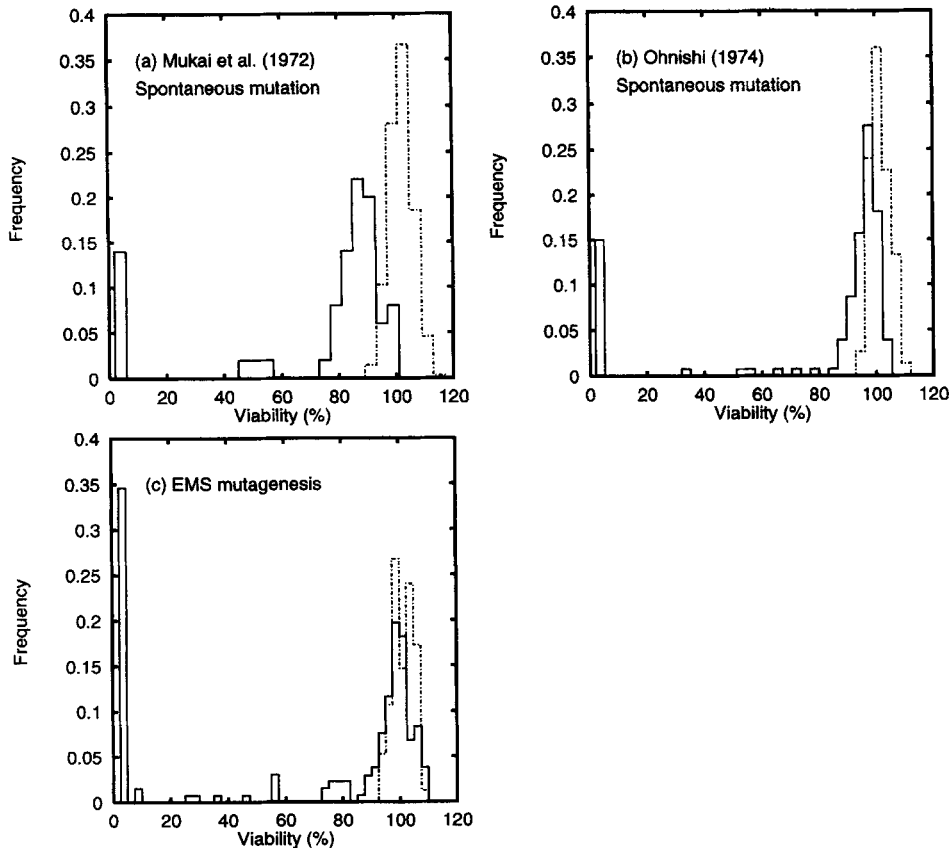


FIGURE 1.—Frequency distributions of competitive viabilities of chromosomes after 40 generations of spontaneous mutation accumulation or one generation of EMS mutagenesis (solid lines) along with distributions of viabilities of non-mutagenized chromosomes (dotted lines). In the case of MUKAI *et al.* (1972), the distribution for generation 0 was inferred by linear regression of the observed mean and variance of viability on generation number from data from generations 10, 20, 30, and 40, and was assumed to be normal.

parameter in the model) was also investigated and found to give significantly higher likelihoods than the unmodified gamma distribution (data not shown). An interpretation of these results is that there is a real discontinuity in the distribution of viability mutation effects such that there is a deficit of minor effects close to zero. A distribution with this shape does not accord with the nearly neutral model (OHTA 1992), which assumes a frequency distribution increasing monotonically as it approaches zero.

However, there is no obvious biochemical or genetic explanation for such a strongly discontinuous distribution, so the fit of the model with a nonmutational change was tested. Nonmutational change could occur, *e.g.*, because of environmental or background genetic change during the course of the experiments. Inclusion of the environmental effect gave a significant improvement in the likelihood (Table 1), and the nonmutational change (k) accounts for $\sim 70\%$ and $\sim 55\%$ of the decline of mean viability for MUKAI *et al.* (1972) and OHNISHI (1974), respectively, or a still higher proportion of the change in mean viability of the quasinormal chromosomes.

EMS mutagenesis: The distribution of viabilities of EMS-treated and control chromosomes is shown in Figure 1c. The fit of different models of the distribution of mutation effects to these data was tested (Figure 3c), but was not significantly improved by the inclusion of a class of mutations with equal effects or by a nonmuta-

tional change (Table 1). In contrast to the spontaneous mutation accumulation experiments, with EMS mutagenesis the quasinormal chromosomes show only a slight decline in viability relative to the controls, despite the very much higher proportion of lethal and severely detrimental chromosomes.

DISCUSSION

The likelihood analysis shows a clear difference between effects of spontaneous and EMS mutagenesis on the distribution of viabilities of chromosomes. Visual inspection of the data (Figure 1) shows a large drop in the viability of quasinormal spontaneous mutation accumulation chromosomes without a substantial increase in their variance, while the quasinormal chromosomes under EMS mutagenesis do not show such a drop. This difference could be explained in at least three different ways.

1. Characteristics of spontaneous and EMS-induced mutations differ such that spontaneous mutations include a large class with approximately equivalent effects. EMS generates primarily GC to AT transitions (DRAKE 1970). A significant frequency of chromosome aberrations is also produced in conditions where sperm storage occurs in females (VOGEL and NATARAJAN 1979), a phenomenon that would not have been important in the experiment described here. Based on the pattern of nucleotide variation

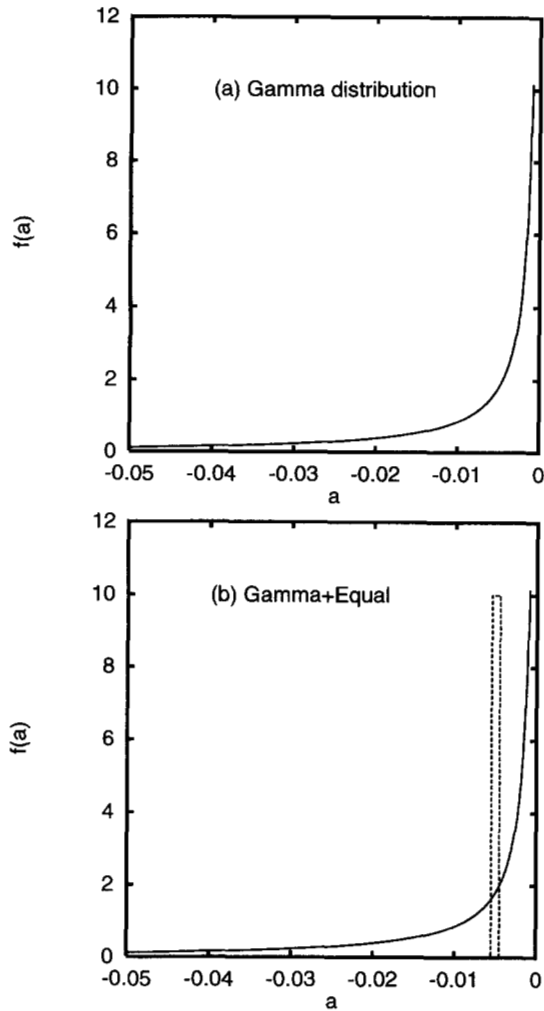


FIGURE 2.—Frequency distributions of mutation effects, a , used in a likelihood analysis to test for goodness of fit to the observed distributions of viabilities of chromosomes. (a) A gamma distribution reflected about zero such that effects are always negative. The particular distribution shown is highly leptokurtic with the majority of effects close to zero, but has a long tail of increasingly large effects which occur with decreasing probability. (b) A mixed “gamma + equal” distribution in which a proportion of mutations come from a standard gamma distribution and a proportion has an equal effect.

at silent sites in *Drosophila* (MORIYAMA and POWELL 1996), it can be inferred that spontaneous mutations involve transversions and transitions at approximately equal frequencies, and, due to the structure of the genetic code, that spontaneous nucleotide substitutions more often generate amino acid substitutions than the transitions produced by EMS. This is one difference between the mutational spectra for spontaneous and EMS mutagenesis. A large fraction of spontaneous mutations from classical *Drosophila* genetics are now known to be transposable element (TE) insertions (GREEN 1988). Furthermore, since about half the spontaneous mutation events in *Drosophila* are attributable to TE movements (NUZHIDIN and MACKAY 1995), the movement of TEs is likely to

TABLE 1

Natural log likelihoods, relative to the gamma distribution, for different models of distributions of mutation effects

Model ^a	Experimental data set		
	MUKAI <i>et al.</i> (1972)	OHNISHI (1974)	EMS
Gamma (2)	0	0	0
Gamma + equal (4)	9.3	11	0.4
Gamma + k (3)	4.0	11	0

^a Number of distribution parameters in parentheses.

be the most important difference between spontaneous and EMS mutagenesis. The decline in viability of the quasinormal chromosomes without a large increase in their variance could therefore be due to TEs causing small and rather nonvariable mutagenic effects, possibly by insertion in noncoding regions, a hypothesis originally proposed by MUKAI and YUKUHIRO (1983). Intuitively, insertions will tend to have stronger deleterious effects than single base pair changes, and this is born out by experimental evidence on effects of insertional mutagenesis in *Drosophila*, which shows that homozygous *P*-element inserts reduce viability by about 12%, on average, and greatly inflate the variance of the trait (MACKAY *et al.* 1992). It seems unlikely that the experimental crosses of MUKAI and OHNISHI led to mobilization of *P* elements, because lethal mutations accumulated at a “normal” rate (CROW 1993), but it is possible that movement of other families of elements specifically into noncoding sites generated mildly deleterious mutations at a sufficiently high rate to produce the observed distribution of viabilities. This explanation would imply that general information on the mutation load is difficult to obtain, because rates of TE insertion vary greatly between strains and species (PASYUKOVA and NUZHIDIN 1993; CHARLESWORTH *et al.* 1994). For example, most spontaneous mutations in *Drosophila* are caused by TE insertions (GREEN 1988), but only a small proportion of spontaneous mutations in mice can be linked with the insertion of foreign DNA (FAVOR 1994).

- An environmental change over the 40 generations of spontaneous mutation accumulation could have led to the change of relative viability of the wild-type chromosomes, but this is unlikely because the observed rates of change in the different experiments were approximately linear.
- A change in relative viability between the wild type and *Cy* tester chromosomes without an increase in the variance among the wild-type chromosomes would occur if an improvement in the performance of the *Cy* balancer chromosome had occurred. *Cy* contains inversions relative to *Pm*, which lead to a dramatic suppression of crossovers, but gene conver-

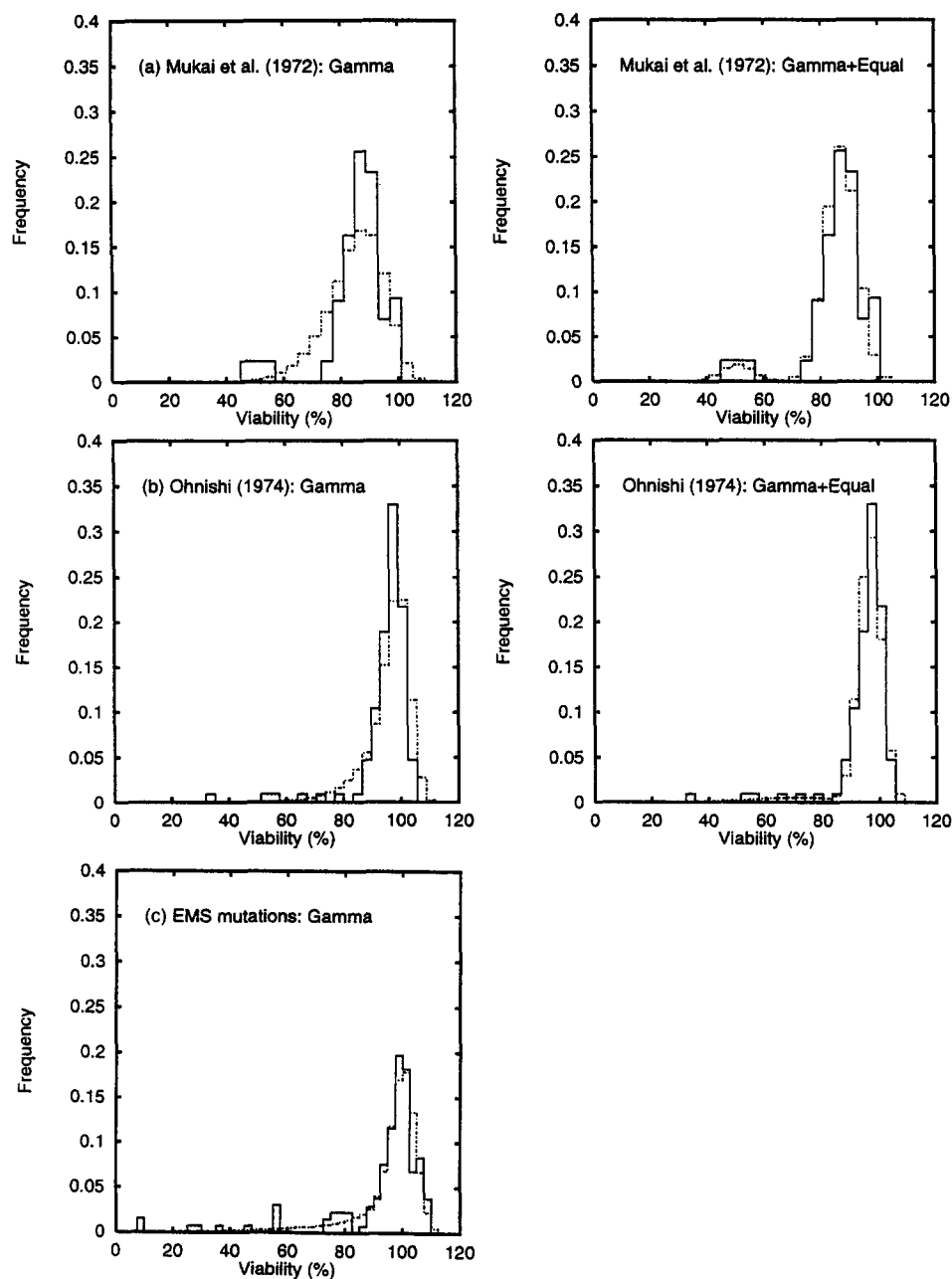


FIGURE 3.—The fit of models of distributions of mutation effects to the observed distribution of viabilities for spontaneous and EMS mutagenesis. The likelihood analysis with Monte Carlo integration was used to infer parameters of the distributions of mutation effects which give the highest likelihood for the different data sets. In addition to the distribution parameters (Figure 2), the genomic mutation rate, U , and the population mean and sampling variance were estimated. The solid lines represent the observations from the experiments, and the dotted lines the expected frequency distribution of relative viabilities assuming ML estimates of the model parameters.

sion between inverted segments has been observed to occur at the same rate as in standard chromosome arrangements (CHOVNICK 1973; ASHBURNER 1989, Chapt. 13). Although the rate of exchange between complex inversions is unknown, genetic exchanges between the *Cy* and *Pm* chromosomes in the tester stock would generate genetic variability among the population of *Cy* chromosomes, upon which natural selection would have the opportunity to act. The rate of exchange in *Drosophila* is of the order of 10^{-5} per locus per generation (CHOVNICK 1973; HILLIKER *et al.* 1994), and lengths of exchange tracts are about 350 base pairs (HILLIKER *et al.* 1994). Variation from gene conversion in the balancer stock could therefore be at least as important as mutation. Selection

on gene conversion-induced variability could explain the distribution of relative viabilities of wild-type chromosomes in spontaneous mutation accumulation experiments, along with the finding of a peculiar distribution of spontaneous mutation effects. This explanation requires that the *Cy* chromosome had not reached an equilibrium with *Pm*, but it is likely that *Cy* would have experienced a switch from a maintenance environment to carefully controlled experimental conditions.

In MUKAI'S (1964) first mutation accumulation experiment, an "order method" was used to obtain estimates for control viabilities at three different generations (10, 20 and 25). At generation 10, *e.g.*, the control viability

was the mean viability of a subset of lines at generation 10 showing the highest viabilities at generations 20 and 25. The implicit assumption was that the highest ranking lines had not accumulated deleterious mutations. On the basis of this method the overall deviation in viability from the control was negligible up to generation 20. However, there was a severe reduction in viability between generations 20 and 25 (see MUKAI 1964, Figures 2 and 5). The explanation is that MUKAI was unable to use the order method to infer a control viability for generation 25 (because there was no later generation on which to base the calculation). Instead, a spurious "control" value was estimated from the mean viability of six high ranking lines *within generation 25*. The rate of change in unscaled relative viability was similar to rates seen later (MUKAI *et al.* 1972; OHNISHI 1977). MUKAI's (1964) experiment was subsequently continued up to generation 60 (MUKAI and YAMAZAKI 1968; MUKAI 1969). The rate of change of viability increased during later generation and was accompanied by a huge increase in the between-line variance. This result was interpreted as evidence for synergistic epistasis between minor viability mutations (MUKAI 1969). An alternative explanation is instability of transposable element copy number leading to a nonlinear increase in the mutation rate (NUZHIDIN *et al.* 1996).

One other mutation accumulation experiment utilized the *Cy/Pm* method to accumulate mutations on *Drosophila* second chromosomes (HOULE *et al.* 1994), but comparison of the change of mean viability with the original mutation accumulation experiments reanalyzed here is not possible because a control line became genetically contaminated. More recently a mutation accumulation experiment in *Drosophila* with brother-sister mating for 105 generations has been carried out (FERNANDEZ and LOPEZ-FANJUL 1996). With full-sib mating, natural selection against mildly deleterious mutations would be ineffective. This experiment cannot suffer from any confounding effect due to the evolution of a balancer system. Viability traits showed only small changes downward relative to a control assayed noncontemporaneously. The drop in viability predicted from results of MUKAI and OHNISHI, taking into account the fact that FERNANDEZ and LOPEZ-FANJUL accumulated mutations for 105 generations in the whole genome, would be close to 100%.

In *Escherichia coli*, KIBOTA and LYNCH (1996) observed a fitness decline of about 0.0002% per generation during about 7500 generations of mutation accumulation and inferred a minimum estimate for the genomic mutation rate of 0.0002. KIBOTA and LYNCH argue that this figure is comparable with a genomic mutation rate of 0.3 in *Drosophila* if account is taken of differences in genome size and number of cell divisions. It can equally be argued, however, that such a low rate of decline in fitness agrees with the hypothesis that deleterious mutation load is much lower than previously thought.

We have recently performed a 60-generation mutation accumulation experiment in *Caenorhabditis elegans* and employed a cryopreserved control (P. D. KEIGHTLEY and A. CABALLERO, unpublished results). The mutational heritability and mutational coefficient of variation for fitness were typical values (HOULE *et al.* 1996), but mean fitness showed only a negligible decline, and the genomic mutation rate was estimated to be 0.0026. This result implies, at least, that the estimates of mutation load from *Drosophila* experiments involving balancers cannot be general.

Under the assumption that the distributions of effects of spontaneous and EMS-induced mutations are the same, and that they include the same proportions of lethals, the EMS data can be used to infer a minimum estimate for the spontaneous mutation rate per generation. The number of EMS-induced lethals is about 2.5 times greater than seen after 40 generations of spontaneous mutation accumulation (Figure 3). The likelihood analysis gives a minimum estimate for the mean number of EMS-induced chromosome 2 viability mutations (excluding lethals) of 0.2 per chromosome, so the minimum predicted spontaneous rate per generation is $0.2 / (40 \times 2.5) = 0.002$. A maximum estimate for the mean mutant effect is $\sim 30\%$. The estimated mutation rate is nearly two orders of magnitude smaller than previously thought (CROW and SIMMONS 1983) and may resolve the paradox (KONDRASHOV 1995) of how small populations are able to persist in the laboratory and in nature.

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