

## Inferences on genome-wide deleterious mutation rates in inbred populations of *Drosophila* and mice

Armando Caballero<sup>1</sup> & Peter D. Keightley<sup>2</sup>

<sup>1</sup> Dep. Bioquímica, Genética e Inmunología, Facultad de Ciencias, Universidad de Vigo, 36200 Vigo, Spain (Phone: 34-86-812568; Fax: 34-86-812556; E-mail: armando@uvigo.es); <sup>2</sup> Institute of Cell, Animal and Population Biology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, Scotland

**Key words:** evolution, fertility, natural selection, population genetics, viability

### Abstract

A theoretical analysis was carried out on the mutation load observed in long-maintained inbred lines from two experiments with *Drosophila* and mice. The rate of decline in fitness and its sampling distribution were predicted for both experiments using Monte Carlo simulation with a range of mutational parameters and models. The predicted rates of change in fitness were compared to the empirical observed rates, which were close to zero. The classical hypothesis of many deleterious mutations (about one event per genome per generation) of small effect (1–2%) resulting in a mutation pressure for fitness of about 1% per generation is incompatible with the data. Recent estimates suggesting an overall mutation pressure for fitness traits of about 0.1% are, however, compatible with the observed load.

### Introduction

Knowledge of the rate of appearance of deleterious mutations and of the distribution of their effects is essential for several aspects of population genetics. Theoretical predictions on the maintenance of variation, the evolution of sex and recombination, and the genetic load all depend on mutational parameters (Kondrashov, 1988, 1995; Barton & Turelli, 1989; Charlesworth, 1990; Crow, 1993; Charlesworth & Hughes, 1998). Until recently, the only direct information on the genomic mutation rate and the mean effects of slightly deleterious mutations came exclusively from experiments on the accumulation of mutations in second chromosomes of *Drosophila melanogaster* protected from natural selection with balancer chromosomes (Mukai, 1964; Mukai et al., 1972; Ohnishi, 1977). The results of these experiments lead to the conclusion that the genomic mutation rate for viability alone is about one event per diploid genome per generation, with homozygous effects of the order of 1–2%. More recent indirect estimates from naturally self-fertilising plants, rely-

ing on the assumption of mutation-selection balance, indicate a similar figure for the genomic mutation rate (Charlesworth, Charlesworth & Morgan, 1990; Charlesworth, Lyons & Litchfield, 1994; Johnston & Schoen, 1995), although they do not give estimates of mutation effects. The above results (which we will refer to as 'Mukai's results' henceforth) imply that the rate of decline of viability in *Drosophila* can be about 1–2% per generation in populations of small effective size, say  $N_e \ll 100$ . For populations of this size, mutations with selection coefficient of the order of 1% behave as quasi-neutrals, i.e.,  $N_e s \ll 1$  (Crow & Kimura, 1970), and their selective elimination is largely overcome by genetic drift. Considering that viability in *Drosophila* may account only for about one-third of the total fitness effects (Sved, 1975; Mackay, 1985), Mukai's results imply that the rate of decline in total fitness due to newly arising deleterious mutations can be very substantial in short periods of time.

Two recent experiments seem to contradict Mukai's classical estimates. Results on viability and fecundity from mutation accumulation experiments in full-sib lines of *Drosophila melanogaster* (Fernández &

López-Fanjul, 1996; García-Dorado, 1997) and reproductive output and longevity in *Caenorhabditis elegans* (Keightley & Caballero, 1997) indicate that the genetic damage from deleterious mutations might be of much smaller magnitude than implied from Mukai's estimates. Moreover, a comparison of the classical spontaneous mutation experiments with effects of ethyl methanesulfonate (EMS)-induced mutation suggests that Mukai's estimates of the mutation rate and effects may not be a general feature for all species (Keightley, 1996). The parameters obtained from *C. elegans*, *Drosophila* full-sib lines, and EMS-induced mutation experiments indicate a much lower detectable mutation rate, about one hundred times smaller than Mukai's value, and a selection coefficient for detectable new mutations about ten times larger. They also coincide with a distribution of detectable mutant effects with little kurtosis or nearly equal effects. However, the experiments may not have detected mutations with very small effects having biological importance. Irrespective of the nature of the underlying distribution of effects and the genomic mutation rate, this new set of parameters implies a much lower rate of decline in fitness. For example, in the *C. elegans* experiment, the mean reproductive output after 60 generations of accumulation of mutations in lines of effective size one was about 2% less than that in the initial generation (Keightley & Caballero, 1997), whereas a set of parameters such as that from Mukai's data would imply a total decline of at least 60%.

There are also other experiments where initially highly inbred lines are allowed to accumulate mutations, and one or more fitness components are measured periodically. This allows an assessment of the decline in fitness due to mutation load. In the *Drosophila* selection experiment of Merchante, Caballero and López-Fanjul (1995), an initially isogenic control population was maintained for 47 generations. The mean egg-to-pupa viability was measured every five generations, and the observed decline was quite small. In a mouse selection experiment for body weight starting from a highly inbred line (Keightley & Hill, 1992; Caballero, Keightley & Hill, 1995), litter size was measured in each of 47 generations. In this case there was no observed decline in litter size.

This study focuses on the rate of decline of fitness from directional mutation pressure. The objective is to find out the range of values for mutational parameters that are consistent with the linear rate of decline in fitness observed in the experiments of *Drosophila* and mice described above. Although the asymptotic rate

of decline in fitness can be predicted from the average probability of fixation of mutations (Kimura, 1962), this asymptotic rate may not be reached in a short term experiment with a population of moderate size. Moreover, the sampling distribution of expected rates cannot easily be obtained in this way. Thus, we used stochastic simulation to compare the expected decline in fitness with those experimentally observed. It is concluded that the genetic deterioration in the experiments is inconsistent with the parameter values inferred from Mukai's data, but they are generally consistent with the *C. elegans* and new *Drosophila* data.

## Materials and methods

### *Experimental lines*

#### *Drosophila isogenic control line*

The present analysis concerns an unselected control line in an experiment on divergent artificial selection carried out for 40 generations starting from an isogenic base population (Merchante, Caballero & López-Fanjul, 1995). The aim of the experiment was to compare the response to selection from new mutations under different mating systems. The line was maintained for 40 generations with 20 pairs of parents per generation, randomly mated in individual vials, and its effective population size was estimated to be 45 from pedigree analysis. After generation 40, the line was maintained for a further 7 generations in a bottle with about 100 individuals. Every five generations (from generation 5 to 40, and in generation 47), egg-to-pupa viability was scored as described by Merchante, Caballero and López-Fanjul (1995). Pupa-to-adult viability was also measured, but only in generations 35, 40 and 47, and data are not analysed here. Fecundity (number of eggs laid per female) was also evaluated, but it is not the main subject of analysis because Mukai's data refers to viability. Therefore, we have information on the egg-to-pupa viability for 47 generations in a randomly mated unselected line initially devoid of variation.

#### *Mouse inbred selection lines*

The maintenance of these lines has been described in detail previously (Keightley & Hill, 1992). Selection lines for high and low body weight at 6 weeks of age were derived from a long-established inbred strain (C3H/He). They were maintained by within-

family selection with 16 matings per line (12 up to generation 14), and are currently at generation 50. In this case, there was no unselected control line, and the selected lines are the object of the present analysis. Data on litter size and viability from birth to the weaning age of 21 days are available throughout the experiment. To promote local inbreeding, from generation 21 half of the matings were between full sibs and the rest were at random (but not between full sibs). Before this point, a form of circular mating was used, but it was discontinued on theoretical grounds (Caballero, Keightley & Hill, 1991). At generation 36, the lines were moved by embryo-transfer to a new mouse house, where pathogen levels were lower and the environmental conditions more constant. Food and temperature were, as far as possible, kept constant throughout the experiment.

*Prediction of rate of change of fitness and its sampling distribution*

*Drosophila viability analysis*

Effects of selection on viability were modelled by Monte Carlo simulation with the aim of computing the expected rate of change of viability and the sampling distribution of this rate of change as a function of expected mutant numbers per genome and their effects on viability. Because the unselected *Drosophila* control population was randomly mated, selection models of fertility and viability are basically equivalent, and the presence of sexes is irrelevant. Thus, for simplicity, the simulated population was assumed to be an idealised random mating population of constant size 45 (the effective size of the control line) initially inbred, and a model of differential fertility of parents was assumed. Every generation, an average of  $U$  (a Poisson variable) mutations arose per haploid genome. Mutations were assumed to be unlinked and to have additive effects between loci on the fitness of the individual. A multiplicative model of fitness effects was also simulated, but it gave very similar results and it is not shown. Mutation effects were assumed to be equal, or sampled from a gamma distribution with average selection coefficient,  $s$ , against mutant homozygotes (see Keightley, 1994, for details about this distribution of mutant effects). The initial viability of the simulated population was 0.8, the estimated egg-to-pupa viability observed in the base population (Fernández & López-Fanjul, 1996). The genotypic viability of an individual was calculated as  $0.8(1 - v)$ ,

where  $v = \sum_{(i=1,n)} s_i + \sum_{(i=1,m)} s_i h_i$ . Here,  $n$  ( $m$ ) is the number of homozygous (heterozygous) mutations carried by the individual, and  $s_i$  and  $h_i$  are the selection coefficient and coefficient of dominance of mutation  $i$ . The mean genotypic viability of the line ( $V$ ) was the average of viabilities of individuals. Gene action between alleles was generally assumed to be additive ( $h = 0.5$ ), but dominance was also considered in some runs. The model of Deng and Lynch (1996) was used in this case. Assuming a given distribution of mutant effects, the coefficient of dominance is obtained by a function,  $h = \exp(-ks) / 2$ , where  $k$  was chosen so that the average dominance coefficient of mutations is 0.36, a consensus value (Lynch, Conery & Burger, 1995). With this type of function, mutants of small effect (small  $s$ ) tend to be additive ( $h = 0.5$ ), whereas mutants of large effect tend to be recessive ( $h = 0$ ), as empirically deduced (see Caballero & Keightley, 1994).

To compare the observed rate of decline in viability in the *Drosophila* experiment with predictions, the simulations used an analysis similar to the experimental one. The regression of viability on generation number ( $b$ ) was calculated using data from every five generations, starting at generation 5. Sampling and environmental variation was included as follows. In the experiment, egg-to-pupa viability was estimated from the progeny (an average of about 30 eggs laid per female) from 20 females. The variance of individual viability (a binomial trait) is  $V_0(1 - V_0)$ , where  $V_0$  is the observed average viability of the offspring of a female. Thus, the sampling variation for the observed estimates in each generation is  $V_0(1 - V_0) / (30 \times 20)$ . In the simulations, the mean phenotypic viability for a particular generation was then obtained as a random normal deviate with mean the average genotypic viability ( $V$ , calculated as explained above) and variance  $V(1 - V) / (30 \times 20)$ . For example, at generation 0, the mean viability is 0.8 for all simulations. The sampling variance is thus 0.0003, and the standard deviation is about 2%. This is of the same order as the standard error of mean viabilities obtained with a similar design in the control estimates of Fernández and López-Fanjul (1996, Table 5), which includes environmental and sampling variation. Therefore, the simulated decline in viability accounted for genetic variation (occurrence of different mutations and their chance) and sampling variation. Possible environmental trends and environmental effects common to generations, however, were ignored.

The effective size of the control population was 45 up to generation 40, although possibly larger (about 100 or so) between generations 40 and 47. However no change was made in the simulations, for simplicity, because the expected rate of decline in fitness with  $N_e = 100$  is nearly the same as with  $N_e = 45$  (data not shown). One thousand simulated replicates were run for each set of parameters. From these replicates, the expected rate of change in fitness was calculated, and 95% confidence limits were obtained by ranking the regression coefficients and determining the upper and lower 2.5% percentiles.

#### Mouse litter size analysis

Effects of selection on litter size were also modelled by Monte Carlo simulation in a similar way to the *Drosophila* analysis. Possible correlated effects of selection on body weight were ignored. Only additive gene action was assumed in this case. Thus, the genotypic value,  $g$ , of a female was one-half of the product of the number of mutations it carried and the mutant effect. In the simulation, the initial average litter size was set to 5.6, the average of the observed mean litter sizes in the high and low lines. The number of progeny per litter was assumed to be Poisson distributed, which was in good agreement with the observations from the experiment because mean and variance of litter size were very similar. The actual litter size of a female,  $n$ , was a Poisson variable with parameter  $5.6(1 - g)$ . Thus, as in the *Drosophila* experiment, genetic and other sampling effects were accounted for in the simulations but, again, environmental trends and generation effects were ignored. Because mating was nonrandom in these lines, and to allow for the possibility of negative pleiotropic effects of mutations on viability, a pleiotropic model was also investigated in addition to the above pure fertility model. In the pleiotropic model, mutations had the above fertility effect on the mother, with litter size modelled as above, but individual male and female progeny were rejected with probability  $1 - g_p$ , where  $g_p$  was the genotypic value of the progeny, one-half of the product of number of mutations it contained and the mutant effect. As far as was possible, the simulation modelled the mating scheme used in the experiment. Thus, there were 12 pair matings up to generation 14, and 16 thereafter, and circular and half-full-sib matings were carried out in appropriate generations. Where a mating failed to produce any offspring, or all the offspring were the same sex, a substitute was used from a mating nearby in the mating schedule, as

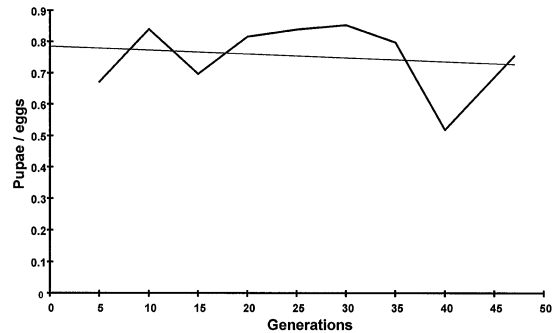


Figure 1. Egg-to-pupa viability in an initially isogenic unselected control population of *Drosophila melanogaster* (number of pupae per egg) plotted against generation number, and its fitted regression line.

was done in the experiment. The result of one replicate from the simulation,  $b$ , was the average rate of change of litter size, estimated by linear regression from two independent simulation runs over 47 generations. The average of two runs was taken, because the experiment involved two independent lines. The expected change in litter size for a given set of parameter values and 95% confidence limits were obtained as for the *Drosophila* analysis.

## Results

### Observed rates of change of fitness

#### *Drosophila* data

The observed egg-to-pupa viability in the unselected control line and the fitted regression line are presented in Figure 1. The regression coefficient was  $-0.0013 \pm 0.0035$ ; i.e., viability declined an average of 0.13% per generation. There are no data available for generation 0, but a non-contemporaneous estimate of the same trait in the same base population was 0.82 (average of control estimates from Fernández & López-Fanjul, 1996), in close agreement with the intercept of the regression line. As observed in the figure, the viability remained practically invariable for 47 generations, except for a lower value in generation 40. If this generation were not considered, the regression coefficient would become positive. As mentioned above, fecundity (number of eggs laid per female) was also scored during the experiment. The regression of the control fecundity on generation number was positive, but with a large sampling variation

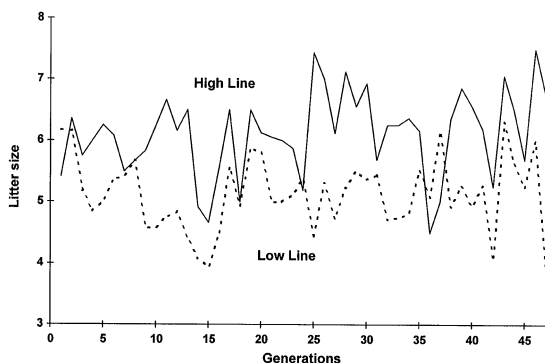


Figure 2. Mean litter size in the high and low mouse inbred selection lines plotted against generation number.

(i.e.,  $0.26 \pm 0.50$  eggs per generation; cf., Merchante, Caballero & López-Fanjul, 1996).

#### Mouse data

Mean litter size for the high and low lines is plotted against generation number in Figure 2. There is a clear difference of about one pup in litter size between the high and low lines, presumably because there is a negative environmental covariance between body weight (the target of artificial selection) and litter size. A linear regression model was also used in this case to estimate the rates of change of litter size per generation, with an effect included to account for the change in environmental conditions that occurred at generation 36. Rates are positive, but very small and not significantly different from zero ( $0.0065 \pm 0.0091$  for the high line and  $0.0027 \pm 0.0091$  for the low line). The combined regression coefficient was  $0.0006 \pm 0.0059$ . Viability from birth to the weaning age of 21 days was also scored every generation. The linear regression of viability on generation number was  $0.00036 \pm 0.00090$  for the high line and  $-0.00051 \pm 0.00120$  for the low line. Therefore, the observed rates of change of viability were also very close to zero. We focus the following analysis, however, on litter size data alone.

#### Comparison of observed and simulated rates of change in fitness

##### *Drosophila* viability

Simulations were used to illustrate the rate of decline expected in a 50 generation experiment. Figures 3 and 4 illustrate the simulated fitness decline for different values of haploid mutation rate ( $U$ ), selection coefficient against mutations ( $s$ ), distribution of mutant effects

(gamma distribution with parameter  $\beta$ ), and degree of dominance ( $h$ ).

Figure 3 assumes additivity and equal effects of mutations for simplicity. The thick lines show the decline in fitness expected if all mutations were randomly fixed, i.e., a rate of decline of 1% per generation for  $Us = 0.01$ , and 0.2% per generation for  $Us = 0.002$ . For a population of effective size 45 (thin continuous lines), there is substantial selection for mutations of  $s = 0.1$ , but mutants with effect of 0.01 are randomly fixed and substantially reduce the fitness of the population. The observed rate of decline in viability in the unselected control population (thick broken line) was 0.13% per generation (a 6% reduction in 50 generations).

A mutation pressure,  $Us$ , of 0.002 (three uppermost lines), or a mutation pressure of 0.01 but with few mutations of large effect ( $U = 0.1$ ,  $s = 0.1$ ), would be compatible with the observed decline in viability. However, a mutation pressure of  $Us = 0.01$  due to many mutations ( $U = 0.5 - 1$ ) of small effect ( $s = 0.01 - 0.02$ ) seems inconsistent with the observed decline.

In Figure 4, the effects of a highly leptokurtic distribution of mutant effects – gamma distribution with parameter  $\beta = 0.5$  or  $\beta = 1$  (exponential) – as well as the effect of partial dominance of mutations are illustrated. Although both factors reduce the rate of decline in fitness, a mutation pressure caused by many mutations of small effect is still inconsistent with the observed decline.

The above figures are only illustrations of the expected rate of decline. A more formal analysis is to compare the observed linear rate of change in viability (0.13%) with confidence limits on estimated rates of change of fitness from the simulation. If, for example, the upper/lower 95% confidence limits for the linear rate of decline in viability for  $U = 1$ ,  $s = 0.01$  from the simulation are more/less than 0.13%, it could be concluded that this combination of mutation rate and mutant effect are incompatible with the data. Values of the compound parameter  $Us$  above or below which, by the above criterion, produce rates of change of viability greater (or smaller) than the observed rate, are plotted against  $U$  in Figure 5. The analysis refers to an additive model of mutations with equal effects (broken line) or a gamma distribution of effects with  $\beta = 0.5$  (continuous line). The area between the upper and lower lines determines the set of parameters compatible with the observed decline in viability. For mutation rates smaller than 0.4 or so, a large range of values of  $s$  are compatible with the data. For larger values of

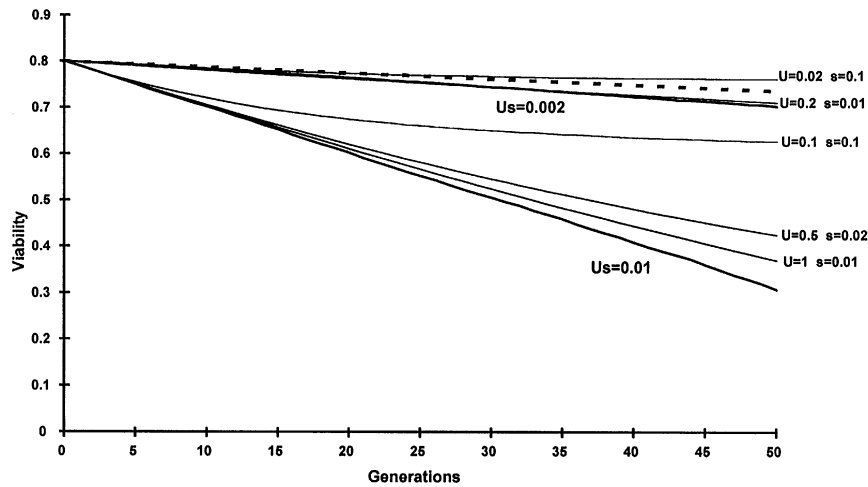


Figure 3. Simulated (average from 1000 replicates) viabilities in a population of effective size 45 for different values of  $U$  and  $s$ . Thick broken line: observed regression line for viability on generation number. Thick continuous lines: viability plots if all mutations were randomly fixed. Additive and equal effects of mutations are assumed.

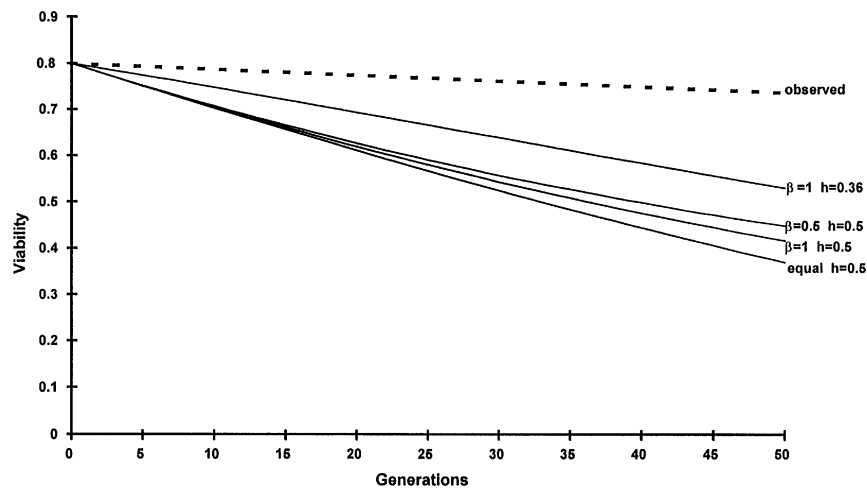


Figure 4. As Figure 3 ( $U = 1$ ,  $s = 0.01$ ) for mutations with equal or variable effects (gamma distributed with  $\beta = 0.5$  or 1), and additive ( $h = 0.5$ ) or variable coefficients of dominance ( $h = \exp[-ks]/2$ ), where  $k = 40$  so that the average  $h$  equals 0.36.

$U$ , however, the experimental data is only compatible with values of  $Us$  between about 0.0005 and 0.0025, which correspond to selection coefficients of between about 0.0003 and 0.0050.

#### Mouse litter size

Simulation of the pure fertility model showed that accumulation of deleterious mutations leads to an essentially linear decline of litter size, at a rate approximately equal to the product  $Us$  on the population per generation (Figure 6), where  $s$  is the effect of the mutation on litter size (equal effects assumed for all

mutations). For example, with  $U = 1$  and  $s = 0.01$  (a mutation pressure of 1% per generation), the mean litter size at generation 47 was 3.0, equivalent to a 46% reduction from generation 0, which is very close to a reduction of 47%, if all mutations were randomly fixed. With higher fertility effects, e.g.,  $s = 0.1$ , the rate of decline is only slightly lower than the product  $Us$ . Thus, in this case the effectiveness of selection in eliminating deleterious mutations was weaker than in the *Drosophila* experiment (cf., Figures 3, 6), presumably because in the mouse experiment, selection was only within families. Because families contribute

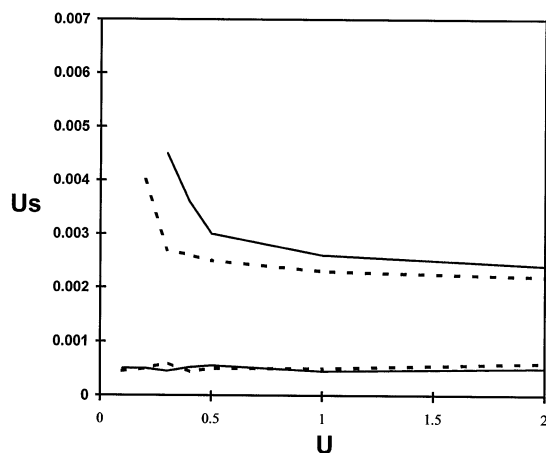


Figure 5. Values of the mutation pressure compound parameter,  $Us$ , above/below which the simulated rate of change in viability in the control population of *Drosophila* is incompatible with the experimental data, plotted against the mutation rate,  $U$ . Broken lines: equal effects of mutations. Continuous lines: mutant effects sampled from a gamma distribution with parameter  $\beta = 0.5$ . Additive gene action.

equally, there is little opportunity for fertility selection to eliminate deleterious mutations, some of which can drift to high frequencies or to fixation. The predicted rates of decline in litter size for mutation pressures of  $Us = 0.002$  and  $Us = 0.01$  both fall below the rate seen in the experiment (thick broken line).

A similar comparison between the observed linear rate of change in fertility (which was actually very close to zero, 0.0006) with confidence limits on estimated rates of change of fertility from the simulation is made in Figure 7. In this case, as the observed linear rate of decline was very close to zero and positive, there is no lower limit for  $Us$ . The results refer to pure fertility selection and the model incorporating both viability and fertility selection, as well as equal effects of mutations or gamma distributed effects ( $\beta = 0.5$ ). Although the results are less clear-cut than for the *Drosophila* data, a combination of mutation rates larger than about 0.25 is incompatible with effects on fertility of about 0.01. For example, for  $U = 0.5$ , the largest value of  $Us$  that is compatible with the observed data is  $Us = 0.00125$  (i.e.,  $s = 0.0025$ ) for equal effects, and  $Us = 0.004$  (i.e.,  $s = 0.008$ ) for highly leptokurtic mutant effects.

The mouse experiment involved half of the matings between full sibs and the remaining matings at random (but not between full sibs) between generations 22 and 47. It is possible, therefore, to look for differences in fertility between the two types of mating (Table 1).

Table 1. Average litter sizes in the high and low lines of mice for matings involving random individuals or full sibs, or matings where the mother came from parents mated at random or full sibs

Mating type	Mean litter size (SE)	
	High line	Low line
Between random individuals	6.16 (0.17)	4.68 (0.16)
Between full-sib individuals	6.14 (0.14)	4.70 (0.16)
Mother from random mating	6.07 (0.17)	5.08 (0.16)
Mother from full-sib mating	6.22 (0.15)	4.35 (0.16)

Matings in which the parents were full sibs produced almost identical mean numbers of progeny compared to matings where the parents were chosen at random, indicating that inbreeding had no effect on viability of pups. A detectable effect of inbreeding is seen, however, in matings where the mother came from a full-sib mating. In the low line, such matings produced 4.35 pups, on average, compared to 5.08 pups for mothers derived from random matings (the standard error of the difference is 0.22). No significant inbreeding effect is seen in the high line.

## Discussion

Direct estimates of mutational parameters, such as rates of appearance of deleterious mutations and their distribution of effects and dominance, are still scarce. Most direct information comes from *D. melanogaster* mutation accumulation experiments involving replicated second chromosomes protected from natural selection (Mukai, 1964; Mukai et al., 1972; Ohnishi, 1977). These experiments lead to the conclusion that many slightly deleterious mutations (of the order of one mutant per zygote per generation with average effect of 1-2%) occur that affect viability. Indirect estimates from relative fitnesses of selfed and outbred progeny, assuming mutation-selection balance in the populations agree with the above estimate of genomic mutation rate (see Lynch, Conery & Burger, 1995, for a review). These results have led to the conclusion that populations of effective sizes smaller than 100 (or even greater) are likely to develop a substantial load of deleterious mutations in a few dozen generations, making the population highly vulnerable to extinction in a short period of time (Lande, 1994, 1995; Lynch, Conery & Burger, 1995).

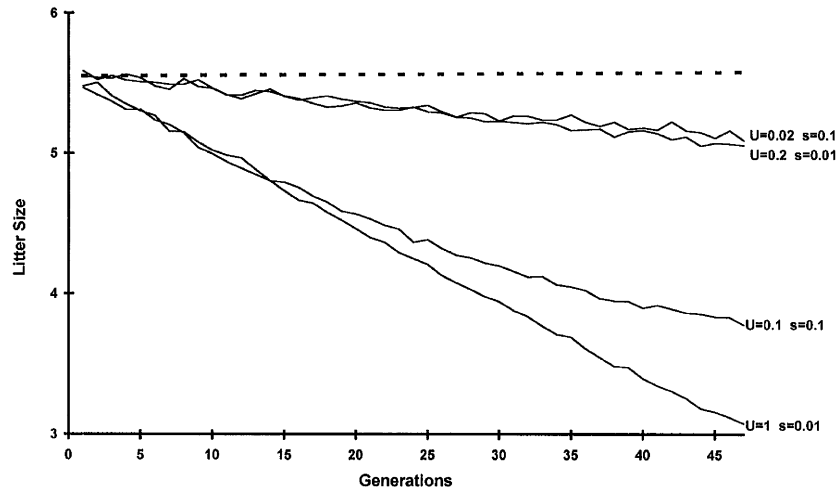


Figure 6. Simulated (average from 1000 replicates) mouse litter sizes for different values of  $U$  and  $s$ . Thick broken line: observed regression line for litter size on generation number (combined data from high and low lines). Additive and equal effects of mutations on fertility are assumed.

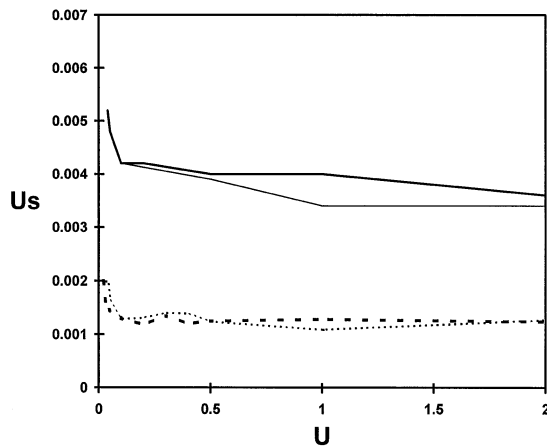


Figure 7. Values of the mutation pressure compound parameter,  $Us$ , above which the simulated rate of change in fertility is incompatible with the mouse experimental data, plotted against the mutation rate,  $U$ . Broken lines: equal effects of mutations. Continuous lines: mutant effects sampled from a gamma distribution with parameter  $\beta = 0.5$ . Thick lines: fertility model. Thin lines: fertility + viability model. Additive gene action.

Other recent estimates, however, clearly disagree with the above results. First, a mutation accumulation experiment in *C. elegans* (Keightley & Caballero, 1997) showed a nearly negligible decline in fitness after 60 generations. The estimated mutation rate for productivity, assuming a gamma distribution of mutation effects, was  $U = 0.0026$ , with 95% confidence intervals of 0.001 and 0.01. After correcting this estimate by the difference in genome size and number of germ line

divisions between *C. elegans* and *D. melanogaster*, the interval becomes  $U = 0.005 - 0.05$  (Keightley & Caballero, 1997), but it can be argued that gene number is a more appropriate way of scaling, and estimates of gene numbers in these two species are of similar order (see references in Keightley & Caballero, 1997). The estimated average effect of mutations was  $s = 0.2$  in this experiment (confidence intervals of 0.05 and 0.3), with equal effects or a slightly leptokurtic distribution of effects. Second, an experiment of accumulation of mutations in *E. coli* (Kibota & Lynch, 1996) showed a rate of decline in fitness of 0.0002% per generation, giving a minimum estimate of  $U = 0.0002$ , but this became closer to the *Drosophila* minimum estimate (0.3) after correction as above (Kibota & Lynch, 1996). Third, indirect calculations from effects of EMS on viability in *D. melanogaster* gave a minimum estimate of  $U = 0.002$  and a maximum estimate of  $s = 0.3$  (Keightley, 1996), in agreement with the *C. elegans* results. Finally, analysis of the mutation accumulation full-sib lines of Fernández and López-Fanjul (1996) gave estimates for viability of  $U = 0.015$  and  $s = 0.06$  (García-Dorado, 1997).

This set of experiments and analyses seem to indicate a much lower mutation pressure than implied by Mukai's data. In this paper, we have analysed the mutation load observed in inbred lines of *Drosophila* and mice. Our results indicate that observed rates of change in viability and litter size disagree with Mukai's estimates and are more consistent with the near absence of decline observed both for productivity and life span

in *C. elegans* (Keightley & Caballero, 1997) and for viability and fecundity in *Drosophila* (Fernández & López-Fanjul, 1996). For example, the summary of parameters reviewed by Lynch, Conery and Burger (1995), including Mukai's estimates and other indirect estimates, assumes  $U = 0.75$ ,  $s = 0.03$ , an average coefficient of dominance  $h = 0.36$ , and an exponential distribution of effects (gamma distribution with  $\beta = 1$ ). With these parameters, the expected mean rate of decline in viability for the *Drosophila* experiment is  $-0.74\%$  per generation, with 95% confidence limits  $-0.56\%$  and  $-0.93\%$ . This is a decline in viability similar to the lowest line in Figure 4, and much larger than the observed rate of decline ( $-0.13\%$ ).

In the analysis of the mouse fertility data, the observed rate of decline in fertility was close to zero, in agreement with the *Drosophila* control line. Selection on body weight was ignored in the mouse lines, but would be expected to lead to an even greater decline in fertility due to pleiotropy. This was not observed, however, because selection on the trait was weak, as within-family selection was practised with relatively low family sizes.

In general, for large values of  $U$ , the observed decline in viability is only compatible with very small average selection coefficients and a low overall mutation pressure (Figures 3–7). A variable distribution of mutant effects and partial dominance of mutations does not seem to change this conclusion drastically. With a leptokurtic distribution of mutant effects, the asymptotic fitness of a population is smaller than that with constant  $s$  if  $N_e s > 0.1$  or so (Lynch, Conery & Burger, 1995). However, in a short-term experiment (such as those analysed here), the decline in fitness is larger for constant  $s$  (see Figure 4), because the decline in the initial generations differs from the asymptotic decline. A variable distribution of mutant effects also implies an increase in the sampling variation. As a result of these two effects, the upper limits of  $Us$  compatible with the experiments are larger for variable effects than for equal effects (Figures 5 and 7). For the mouse data, the effect of the variable distribution was much more marked than for the *Drosophila* data (cf., Figures 5, 7). This is because the selected mouse lines produced estimates with much more noise, which was augmented by the variability in mutant effects. For example, for  $U = 2$  and  $s = 0.001$ , i.e.,  $Us = 0.002$ , the mean expected rate of decline in *Drosophila* viability and mouse fertility (this latter expressed as a percentage from the initial mean) was  $-0.19\%$  both with equal or variable effects. However, the confi-

dence limits for the *Drosophila* data were ( $-0.12\%$ ,  $-0.27\%$ ) for equal effects, and ( $-0.11\%$ ,  $-0.28\%$ ) for variable effects, both compatible with the observed decline ( $-0.13\%$ ). The confidence limits for the mouse data were ( $-0.05\%$ ,  $-0.32\%$ ) for equal effects and ( $+0.05\%$ ,  $-0.52\%$ ) for variable effects; only the last one is compatible with the observed decline ( $+0.01\%$ ). The reason for this much larger noise in the mouse experiment is likely to be due to the fact that mating was nonrandom (partial full-sib mating from generation 21), and this is prone to cause a large reduction in effective size when combined with weak selection (Caballero & Santiago, 1995).

Partial dominance obviously reduces the expected rate of fitness decline (Figure 4). The effect, however, is not very drastic with the realistic assumption that coefficients of dominance are negatively correlated with mutant effects. Thus, data suggest that although the average dominance coefficient may be 0.3–0.4, mutants of small effect have a tendency to be additive on average, or nearly so (see Caballero & Keightley, 1994, for a review on direct estimates of  $h$  on *P*-element-induced and spontaneous mutations). In the mouse experiment, it is expected that recessive mutation effects were expressed because half of matings were between full sibs in a substantial part of the experiment.

In this analysis it has been assumed that mutant effects act additively between loci. A multiplicative model of gene action was also considered but gave similar results. There is experimental evidence pointing to the existence of synergistic epistasis between minor viability mutations (Mukai, 1969; Crow, 1993). However, because the life span of the experiments analysed here was very short (less than 50 generations), it is very unlikely that synergistic epistasis would affect the conclusions (see simulation results by Charlesworth, Morgan & Charlesworth, 1993).

The discrepancy between estimates of  $U$  and  $s$  in the earlier *Drosophila* experiments (Mukai's data) and those from *C. elegans* and *Drosophila* full-sib lines can be ascribed to several possible causes. First, it has been proposed that the large apparent changes of mean viability observed in the *Drosophila* experiments involving balancer chromosomes were artifacts (see Keightley, 1996; Keightley & Caballero, 1997; and García-Dorado, 1997). Another possibility is that selection eliminated many mutations in the *C. elegans* and *Drosophila* full-sib experiments, compared to Mukai's experiment, because they did not use balancer chromosomes to protect from selective elimina-

tion. This is, however, a highly unlikely explanation, because the population size of the mutation accumulation lines ( $N = 1$  in *C. elegans* and  $N = 2$  in *Drosophila*) was small enough to make drift overcome selection for mutants of small or intermediate effects (see Figure 1 from Keightley & Caballero, 1997).

Finally, there may have been an absence of competition in the *C. elegans* and *Drosophila* full-sib experiments compared to that in Mukai's experiments, where viability was measured in high-density conditions with potential for larval competition. It is possible that a harsh environment increases the detrimental nature of mutations (Kondrashov & Houle, 1994), but other experimental evidence seems to disagree with this conclusion (Fernández & López-Fanjul, 1997). Finally, the inbreeding depression detected when viability was analysed by Mukai's technique (Mackay, 1985) is of the same order (about 0.7% per 1% increase in coefficient of inbreeding) as that detected when viability was measured with a non-competitive technique (López-Fanjul & Villaverde, 1989; García, López-Fanjul & García-Dorado, 1994), also pointing against this explanation.

The results of mutation accumulation experiments give almost no information on rates of mutations with very small effects (say  $s < 10^{-3}$ ), because these are simply undetectable with any reasonable experimental design, but such mutations may be crucial for our understanding of evolutionary processes. A high genomic mutation rate would therefore be compatible with the *Drosophila* and *C. elegans* experiments showing small rates of decline in fitness traits, but would imply that most mutation effects are in this undetectable range, and imply highly leptokurtic distributions of mutation effects.

The estimates of mutational parameters are of great relevance for conservation issues, given the impact of a large mutation pressure (particularly of a large input of mutations of small effect) on the fitness of small populations (see Lande, 1995). It is clear that the current estimates on mutational parameters are in question (Peck & Eyre-Walker, 1997), and more experimental data in different species, different environments, and different fitness related traits are urgently needed.

### Acknowledgements

This work was supported by grant 64102C605 from Universidad de Vigo (A.C), the Royal Society (P.K.), and an Acciones Integradas collaborative grant

HB1996-0158. We are grateful to A. García-Dorado, W. G. Hill, and C. López-Fanjul for useful comments on the manuscript.

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