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Rapid mutational declines of viability in *Drosophila*

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Summary

High rates of mildly deleterious mutation could cause the extinction of small populations, reduce neutral genetic variation and provide an evolutionary advantage for sex. In the first attempts to estimate the rate of mildly deleterious mutation, Mukai and Ohnishi allowed spontaneous mutations to accumulate on *D. melanogaster* second chromosomes shielded from recombination and selection. Viability of the shielded chromosomes appeared to decline rapidly, implying a deleterious mutation rate on the order of one per zygote per generation. These results have been challenged, however; at issue is whether Mukai and Ohnishi may have confounded viability declines caused by mutation with declines resulting from environmental changes or other extraneous factors. Here, using a method not sensitive to non-mutational viability changes, I reanalyse the previous mutation-accumulation (MA) experiments, and report the results of a new one. I show that in each of four experiments, including Mukai's two experiments, viability declines due to mildly deleterious mutations were rapid. The results give no support for the view that Mukai overestimated the declines. Although there is substantial variation in estimates of genomic mutation rates from the experiments, this variation is probably due to some combination of sampling error, strain differences and differences in assay conditions, rather than to failure to distinguish mutational and non-mutational viability changes.

1. Introduction

High rates of mildly deleterious mutation could provide an evolutionary advantage for sex (Kondrashov, 1988; B. Charlesworth, 1990; Peck, 1994), influence the evolution of mating systems (D. Charlesworth *et al*., 1990), reduce neutral molecular variation (B. Charlesworth *et al*., 1993) and cause the extinction of small populations (Gabriel & Bürger, 1994; Lande, 1995; Lynch *et al*., 1995). A thorough understanding of these effects would require precise estimates of U, the number of deleterious mutations per diploid genome per generation, as well as detailed characterization of the distribution of effects of the mutations on fitness. For practical reasons, such detailed information is essentially impossible to obtain. In contrast, it is often feasible to estimate ΔM, the per-generation rate by which deleterious mutations reduce fitness or one of its components when natural selection is minimized (Kondrashov, 1998). Minimization of selection can be accomplished by maintaining lines at very small population size (e.g. Mukai, 1964), and/or by artificially equalizing the reproductive success of individuals (Shabalina *et al*., 1997).

ΔM is useful for three reasons. First, it permits some assessment of the extent to which small populations may be endangered by deleterious mutations, by putting an upper bound on the rate of mutational fitness decline in small natural populations, in which selection is not minimized. The second use of ΔM is in predicting the additive genetic variance for fitness that will be maintained at equilibrium under mutation–selection balance (Burt, 1995); by Fisher's Fundamental Theorem, the variance for total fitness due to deleterious mutations should in fact equal ΔM for fitness. The most widespread use of ΔM, however,

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is in putting a lower bound on U, by the formula of Bateman (1959) and Mukai (1964):

$$U \geqslant c\frac{[\Delta M]^2}{\Delta V}. \quad (1)$$

Here, ΔV is the mutational variance, usually estimated from the rate of trait divergence among independent mutation-accumulation (MA) lines, and c scales the estimate to the entire genome.

The largest body of information on ΔM comes from studies of egg-to-adult viability of *Drosophila melanogaster*. In four separate experiments (Mukai, 1964; Mukai *et al*., 1972; Ohnishi, 1977; Fry *et al*., 1999), second chromosomes have been propagated through males with minimal selection and no recombination, and the viability of the resulting MA lines monitored. Reported rates of viability decline, after excluding lines with lethal and severely deleterious mutations, ranged from 0·17% (Ohnishi, 1977) to 0·48% per generation (Mukai *et al*., 1972). When extrapolated to the entire genome, these results suggest a surprisingly fast decline of 1–2% per generation. Lower bounds for U (by (1), with $c = 5$) varied from 0·10 (Fry *et al*., 1999) to 0·85 (Mukai *et al*., 1972), with about half this variation being caused by variation in the ΔM estimates (summarized in Fry *et al*., 1999).

A variety of causes, including some artefactual ones, may have contributed to the variation in ΔM estimates. Based on new statistical analyses, Keightley (1996) and García-Dorado (1997) have argued that Mukai and Ohnishi may have overestimated ΔM, and therefore U. These authors point out that both Mukai *et al*. (1972) and Ohnishi (1977) estimated ΔM by regressing the mean viability of their MA lines against generation number, a method that would overestimate ΔM if the viability declines had been caused by non-mutational events such as environmental changes (Keightley, 1996; García-Dorado, 1997). In contrast, Mukai (1964) estimated ΔM by the 'order method', which employs a simultaneous comparison of the viabilities of all MA lines with those of MA lines inferred to have few or no deleterious mutations, by virtue of their having had the highest viabilities in one or more later assays. While this method is not subject to bias caused by non-mutational changes, it appears that Mukai (1964) applied the method incorrectly (Keightley, 1996). Finally, Fry *et al*. (1999) estimated ΔM by comparing viabilities of the MA lines with that of relatively large homozygous 'control' populations. While such populations are expected to show less mutational change than small MA lines, their viability could still be altered by adaptive or deleterious mutations, resulting in over- or underestimation of ΔM. Thus all the ΔM estimates, as well as the resulting lower bounds for U, are subject to challenge (for a recent review see Keightley & Eyre-Walker, 1999).

The goal of this study was to re-examine the diverse estimates of ΔM for *D. melanogaster* viability. I used the order method, because of its advantage of being unaffected by non-mutational viability changes, to obtain new ΔM estimates from three of the previously published experiments (Mukai, 1964; Mukai *et al*., 1972; Fry *et al*., 1999), as well as from a new MA experiment (unfortunately, Ohnishi did not publish data from which it is possible to calculate order method estimates). The new ΔM estimates are high and reasonably consistent among studies. Most notably, the results strongly suggest that Mukai (1964) and Mukai *et al*. (1972) did not overestimate ΔM.

2. Materials and methods

The MA experiments considered here have made use of two special second chromosomes bearing dominant visible markers and recessive lethals; I will denote these by *Cy* and *Pm*. The *Cy* chromosome has the marker *Curly*, and also has multiple inversions that effectively suppress recombination between it and normal sequence homologues (i.e. it is a so-called balancer chromosome). The *Pm* chromosome has a dominant marker, informally called *Plum* (dark eyes). Each MA experiment was initiated by crossing a single $Cy/+$ or $Pm/+$ male to females from a Cy/Pm balanced lethal stock. $Cy/+$ and/or $Pm/+$ progeny of the original male were then crossed individually to Cy/Pm stock females. (The *Pm* chromosome is not a balancer but, since crossing-over is absent in *Drosophila* males, progeny of $Pm/+$ males that do not receive the *Plum* marker must have received the intact wild-type homologue.) Each of these crosses established one MA line; the lines were subsequently maintained by crossing one or a few $Cy/+$ and/or $Pm/+$ males to Cy/Pm females each generation. In this way, the + second chromosomes, all of which descended from a single progenitor chromosome, were propagated without recombination, and with minimal selection. Selection is minimal because population size is extremely small (1 or 2 males per MA line in most cases), and because mutations are never allowed to occur in homozygous condition.

All studies considered here have used the '*Curly*' method for measuring viability. In this method, $Cy/+_i$ females and males are intercrossed, where $+_i$ denotes a wild-type chromosome derived from the ith MA line. In the absence of differential viability, this cross is expected to produce $+_i/+_i$ and $Cy/+_i$ progeny in a 1:2 ratio. The departure of the ratio from 1:2 can be used as a measure of the relative viability of the mutant homozygotes to balancer heterozygotes:

$$RV = \frac{2 \times (\text{no. of wild-type progeny})}{(\text{no. of } \textit{Curly} \text{ progeny})}. \quad (2)$$

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A one is usually added to the denominator as a slight bias correction (Haldane, 1956). Two studies (Mukai, 1964; Ohnishi, 1977) have used an alternative viability measure, the percentage of wild-type flies. As Latter & Sved (1994) have pointed out, this measure underestimates the true fitness difference between genotypes. Therefore, when necessary, I have converted means to the relative viability scale, using the relationship

$$\overline{RV} = \frac{2\overline{\%WT}}{100 - \overline{\%WT}}. \quad (3)$$

Applying (3) to data from the two recent MA experiments described below shows that it is extremely accurate (J. D. F., unpublished results).

I report here new order method estimates of ΔM from four experiments: that of Mukai (1964), that of Mukai *et al.* (1972), that of Fry *et al.* (1999), and a new, previously unpublished experiment. In recalculating order method estimates from data in Mukai (1964), I corrected Mukai's misapplication of the method (see below), and converted the data to relative viabilities. Mukai *et al.* (1972) did not present order method estimates, but the paper contains data from which such estimates can be calculated. Order method estimates from the experiment of Fry *et al.* (1999) were calculated using previously unpublished data from that experiment.

The order method depends on assaying viability of the MA lines on two or more occasions (always at least five generations apart in the studies considered). The lines with the highest viability in the later assay(s) are assumed to carry few or no deleterious mutations; the mean viability of these lines in the earlier assay is therefore used to estimate non-mutant viability. In most cases, ΔM is estimated as

$$\Delta M = \frac{\bar{X}_C - \bar{X}_Q}{g\bar{X}_C}. \quad (4)$$

Here, g is the generation number of the earlier assay, and $\bar{X}_C$ and $\bar{X}_Q$ are mean viabilities of the order-method control lines and of all 'quasinormal' lines, respectively. Mukai (1964) and Mukai *et al.* (1972) used slightly different definitions of 'quasinormal', but to a good approximation their quasinormal lines were those with at least one-half the viability of the controls; this was the criterion used for the new experiment and that of Fry *et al.* (1999). As an alternative to (4), if data from several generations are available, ΔM can be estimated by regressing the differences $\bar{X}_C - \bar{X}_Q$ against generation number, and dividing the resulting slope by the grand mean of the controls. Details of the individual experiments and datasets will be presented along with the results.

The advantage of the order method is that it involves simultaneous comparison of 'control' lines and lines carrying deleterious mutations; therefore non-mutational changes over time do not confound estimates of ΔM. The order method may underestimate or overestimate ΔM if the controls contain significant numbers of deleterious or adaptive mutations, respectively; these possibilities are addressed below.

3. Results

(i) *Mukai (1964)*

Mukai (1964) conducted a 25-generation MA experiment, and used the order method to estimate non-mutant viability. If the data are converted to relative viabilities, Mukai's ΔM estimate becomes 0·60 %; his lower bound estimate of U was 0·70. Keightley (1996) called these estimates into question, because Mukai used an inappropriate method to identify control lines at generation 25; the lines were chosen based on their viability in that generation, rather than in a subsequent generation, possibly resulting in an overestimate of control viability. I have reanalysed Mukai's data by omitting the questionable generation 25 control data, and converting the means he presents from percentage wild-type to relative viability. I have also included data from generation 32 of the same experiment (Mukai & Yamazaki, 1968), for which five order method controls were identified based on their viability at generations 52 and 60.

The basic data are presented in Table 1. Two methods are used to identify order method controls. Method 1 is Mukai's (1964) method, which results in varying numbers of control lines per generation (for rationale see Mukai, 1964). In method 2, I have held the number of control lines per generation constant at five; for generations 10–20, these are the five lines with the highest viability in the subsequent assay. To estimate ΔM, Mukai (1964) regressed the decrement in viability against generation number, forcing the regression through the origin. Applying the same procedure to the data in Table 1 gives ΔM estimates of 0·56 % and 0·51 % for methods 1 and 2, respectively. If instead the regressions are not forced through the origin, the estimates are 0·69 % and 0·76 %. Allowing the y-intercepts to differ from zero, however, does not significantly improve the fit of the regressions ($P > 0{\cdot}6$ in both cases); therefore the former estimates make use of all available information and are to be preferred. These estimates are only slightly lower than Mukai's original estimate of 0·60 %. Therefore Mukai's estimates of ΔM and U do not appear to have been seriously affected by his inappropriate method for choosing order method controls at generation 25 (Keightley, 1996).

(ii) *Mukai et al. (1972)*

Mukai *et al.* (1972) conducted three 40-generation MA experiments, each starting with a different

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Table 1. *Results of the mutation-accumulation experiment of Mukai* (1964), *converted to relative viabilities*

Generation	Mean of order method controls (*n*)		Mean of 'quasinormal' lines (*n*)
	Method 1	Method 2	
10	0·982 (12)	1·003 (5)	0·924 (98)
15	0·845 (10)	0·770 (5)	0·774 (97)
20	0·951 (5)	0·951 (5)	0·896 (89)
25	–	–	0·792 (84)
32	0·978 (5)	0·978 (5)	0·779 (80)
ΔM (%) $\pm$ SE, forced	0·555 $\pm$ 0·087	0·507 $\pm$ 0·151	
ΔM (%) $\pm$ SE, unforced	0·691 $\pm$ 0·251	0·755 $\pm$ 0·436	

To estimate ΔM, the differences between MA and control viabilities were regressed against generation number, and the resulting slopes and standard errors were divided by the control mean. *Y*-intercepts were either assumed to be zero or estimated from the data ('forced' and 'unforced', respectively). The generation 32 data come from Mukai & Yamazaki (1968).

Table 2. *Reanalysis of the three mutation-accumulation experiments of Mukai* et al. (*1972*)

Group	Mean of order method controls	Mean of quasinormal lines	ΔM (%) from order method	ΔM (%) from regression
CH	1·012	0·973	0·383	0·362
PQ	0·968	0·963	0·054	0·439
RT	0·795	0·715	1·005	0·645
Mean	–	–	0·480	0·482

The viability means are from generation 10 and come from tables 1–3 and 8 of the paper; there were four order method control lines for each group of *c*. 50 MA lines.

progenitor chromosome. ΔM was estimated by regressing mean viability of the MA lines against generation number. Pooling data from the three experiments gave $\Delta M = 0{\cdot}48\,\%$ *and* $U \geqslant 0{\cdot}85$. One of Mukai *et al.*'s (1972) experiments, for which the viability distribution at generation 40 was given in a figure, has been the subject of reanalyses by Keightley (1996) and García-Dorado (1997). Both authors concluded that Mukai *et al.*'s estimate of ΔM, and therefore U, may have been inflated by a non-mutational viability change. Such a change would invalidate estimates of ΔM by the regression method, but should have no effect on estimates by the order method.

Mukai *et al.* (1972) present data from which it is possible to calculate order method estimates of ΔM at generation 10; these new estimates are presented in Table 2, along with the original regression method estimates. The mean order method estimate (0·48 %) is identical to the regression method mean. The estimates vary considerably among the three experiments, but this probably reflects the relatively small sample sizes on which they are based. Overall, this analysis gives no support for the view that Mukai *et al.* (1972) overestimated ΔM or U.

(iii) *Ohnishi* (*1977*)

Ohnishi (1974, 1977) performed a 40-generation MA experiment, and estimated ΔM by regressing mean viability against generation number. On the relative viability scale, his estimates are $\Delta M = 0{\cdot}25\,\%$ and $U \geqslant 0{\cdot}37$. Ohnishi's viability data from generation 40 have been reanalysed by Keightley (1996) and García-Dorado (1997), both of whom concluded that Ohnishi may have overestimated ΔM due to a non-mutational change. Unfortunately, neither Ohnishi's (1977) paper nor his thesis (1974) contain data from which order method estimates of ΔM can be calculated. Nonetheless, two points deserve comment. First, Ohnishi's ΔM estimate is only about one-half the order method estimates from Mukai (1964) and Mukai *et al.* (1972)

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Table 3. *Estimates of* ΔM *from the reanalysed experiment of Fry* et al. (*1999; experiment 1*), *and from a new experiment that used essentially identical methods* (*experiment 2*)

Experiment	Generation	No. of QN[a] lines	Mean viability, controls (SD)	Mean viability, QN lines (SD)	ΔM (%)
1	18	21	0·889 (0·014)	0·791 (0·101)	0·608
	19	22	0·861 (0·068)	0·844 (0·067)	0·105
	20	17	0·780 (0·023)	0·771 (0·133)	0·054
	21	22	0·985 (0·000)	0·870 (0·101)	0·558
	Mean				0·331
	(95% CI)				(0·052–0·520)
2	22	27	0·928 (0·024)	0·844 (0·083)	0·408
	24[b]	23	0·855 (0·045)	0·842 (0·126)	0·067
	24[b]	25	0·924 (0·028)	0·814 (0·127)	0·495
	Mean				0·323
	(95% CI)				(0·105–0·504)

There were two order method controls for each set of lines; see text for details.
[a] Quasinormal.
[b] The generation 24 assays were performed in two blocks.

described above, suggesting that it is not a serious overestimate. Second, although Ohnishi (1977) stated that the reduction in survival in his MA lines was linear, the rate of viability decline in fact slowed over time. A quadratic term significantly ($P < 0{\cdot}02$) improves the fit of the regression of viability against time (data from table 2a of Ohnishi, 1974); this is true whether data are analysed on the original percentage wild-type scale, or converted to relative viabilities. In terms of relative viability, the regression equation is $\overline{RV} = 0{\cdot}930 - (4{\cdot}81 \times 10^{-3})\,g + (6{\cdot}35 + 10^{-5})\,g^2$, where g is generation number. The predicted initial rate of viability decline is therefore 0·00481/0·930, yielding $\Delta M = 0{\cdot}52\%$. Thus it is possible that a mutational decline in viability similar to that in the Mukai studies ($\Delta M \approx 0{\cdot}5\%$) occurred, but was masked in later generations by an environmental change. An alternative possibility is that the rapid initial decline was itself due to an environmental change. Unfortunately, it is not possible to distinguish between these possibilities.

The slowing of the decline in viability in the later generations of Ohnishi's experiment contrasts with the famous result of Mukai (1969) showing an accelerating decline. The latter has been widely cited as evidence for synergism of deleterious mutations; Ohnishi's experiment effectively contradicts that evidence.

(iv) *Fry* et al. (*1999*), *and a new experiment*

On the basis of a MA experiment lasting 33 generations, Fry *et al.* (1999) estimated $\Delta M = 0{\cdot}24\%$ and $U \geqslant 0{\cdot}10$. Their ΔM estimate was obtained by comparing viability of the MA lines with that of three homozygous 'control' populations of *c.* 300 flies each. Confidence intervals for ΔM did not overlap the point estimates from Mukai (1964) and Mukai *et al.* (1972), leading Fry *et al.* (1999) to speculate that the latter authors had overestimated ΔM (and therefore U). On the other hand, the difference from Mukai's estimates could have been the result of the different methods for estimating non-mutant viability. For example, it is possible that some deleterious mutations accumulated in Fry *et al.*'s (1999) control populations, which would have caused underestimation of ΔM. To permit a comparison of the studies when the same method is used for analysis, I present here an order method estimate of ΔM from Fry *et al.*'s (1999) experiment. I also present a ΔM estimate from a new experiment that used a different progenitor chromosome; this experiment followed the same methods as that of Fry *et al.* (1999), except where noted below.

In both experiments, each MA line was assayed for viability on two occasions. In the experiment of Fry *et al.* (1999; hereafter, experiment one), each line was first assayed in one of four sets between G18 and G21 (Table 3), and was later retested at either generation 27 or 33. In the new experiment (hereafter experiment 2), lines were assayed in three sets between generations 22 and 24 (Table 3), with each line being retested at either generation 31 or 35. Within a set, lines were ranked based on their viability in the later assays, with the top two lines being chosen as order method controls; two order method controls per set results in roughly the same proportion of lines used as controls as in the earlier studies (Mukai, 1964; Mukai *et al.*, 1972). ΔM was then calculated for each set and averaged. Although lines were tested in several treatments in the later assays, only results in the 'standard' treatment (six $Cy/+$ pairs per vial on cornmeal-molasses medium at 25 °C; see Fry *et al.*,

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1999) were used for the ranking; this was the only treatment used for the earlier assays. (It is also the treatment that most closely resembles the assay conditions used by Mukai.) The fact that the later assays took place on two occasions adds a slight complication, but any errors in identifying lines with the fewest mutations that this might cause would tend to bias ΔM downward. Bootstrap 95% confidence intervals for ΔM were calculated by randomly sampling lines from each set with replacement 20000 times, repeating the calculations for each sample, and finding the 2·5th and 97·5th percentiles of the resulting distributions (Mooney & Duval, 1993).

The two experiments give ΔM estimates of 0·33% and 0·32% (Table 3). The confidence limits overlap or approach the point estimates (0·48–0·56%) recalculated from Mukai (1964) and Mukai *et al.* (1972). Confidence intervals from the latter two studies cannot be calculated, unfortunately. Nonetheless the results indicate that order method estimates have large sampling errors; therefore the possibility that the difference in ΔM estimates between the old and new studies is due to sampling error should be entertained.

The ΔM estimates from the two recent experiments can be combined with ΔV estimates to produce lower bound estimates of U. I estimated ΔV by a modification of the covariance method (Fry *et al.*, 1999):

$$\Delta V = \frac{\mathrm{COV}(X_1, X_2)}{g\bar{X}_C^2}; \quad (5)$$

here, X_1 and X_2 are the mean viabilities of an MA line in the first and second assays, g is the generation number of the first assay, and $\bar{X}_C$ is the mean of the order method controls in that assay. Equation (5) was applied to the three or four sets of lines in each experiment separately and then averaged to produce a single estimate; as with the ΔM estimates, only quasinormal lines were used. This procedure gives $\Delta V = 3{\cdot}35$ (bootstrap 95% CI: 1·21–5·23) $\times 10^{-4}$ and 3·81 (1·82–5·55) $\times 10^{-4}$ for experiments 1 and 2, respectively. (The former estimate differs slightly from the one reported in Fry *et al.* (1999) because it is based on data from the standard treatment only, after a reanalysis showed that ΔV differed significantly among treatments; J.D.F., unpublished data.) Combining these estimates with the ΔM estimates gives $U \geqslant 0{\cdot}164$ (0·0076–0·454) and $U \geqslant 0{\cdot}137$ (0·017–0·372).

4. Discussion

In four MA experiments involving the *D. melanogaster* second chromosome, viability declined 0·3–0·6% per generation, even after excluding lethals and severely deleterious mutations. Extrapolated to the entire genome, the average decline was over 1% per generation. Notably, the revised ΔM estimates based on Mukai's studies (Mukai, 1964; Mukai *et al.*, 1972) are similar to those reported in the original publications. Because order method estimates of ΔM are unaffected by between-generation changes in viability, the ΔM estimates reported here do not support the conclusion that Mukai overestimated ΔM due to environmental changes (Keightley, 1996), evolution of the balancer stock (Keightley, 1996; García-Dorado, 1997) or changes in scoring accuracy of *Curly* and wild-type flies (Fry *et al.*, 1999).

The order method would consistently overestimate ΔM only if the control lines contained adaptive mutations. Even then, it would not be sufficient for one or two adaptive mutations to have occurred; the net effect of adaptive mutations in the approximately 10% of lines used as controls would have had to outweigh the effects of deleterious ones. This seems unlikely, given the probable rarity of adaptive mutations. In addition, in their reanalyses of one of the experiments of Mukai *et al.* (1972) and that of Ohnishi (1977), García-Dorado *et al.* (1998) estimated an extremely low rate of adaptive mutations.

In contrast to the experiments considered here, two whole-genome MA experiments on nematodes (Keightley & Caballero, 1997; Vassilieva & Lynch, 1999) and one on *Drosophila* (Fernández & López-Fanjul, 1996) reported rates of decline of fitness-related traits of only 0·3% per generation or less. In all three studies, however, fitness assays were performed under non-competitive conditions, in contrast to the competitive conditions used in the second chromosome MA experiments. Kondrashov & Houle (1994) and Shabalina *et al.* (1997) report evidence that in *Drosophila*, fitness declines caused by deleterious mutations are substantially greater when measured under competitive conditions. The latter study employed the 'middle-class neighbourhood' design, in which mutations are accumulated in an outbred population; productivity under competitive conditions declined an estimated 2% per generation when measured under competive conditions, but only approximately 0·2% per generation when measured under non-competitive conditions.

Another possibility is that mutation rates in the second chromosome MA experiments were inflated over natural levels due to possible mutagenic effects of balancer chromosomes. Chromosomes extracted with balancers sometimes have multiple new TE insertions relative to the original chromosomes (Pasyukova *et al.*, 1988; García Guerreiro & Biémont, 1995; Kozhemiakina & Furman, 1995). Increased TE activity does not always result from using balancers, however. Maside *et al.* (2000) have recently estimated rates of movement of 11 families of TEs in a second chromosome MA experiment that used the balancer *SM1*; the overall transposition rate was similar between their experiment and an unpublished ex-

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periment involving sib-mated MA lines (B. Charlesworth, personal communication). In addition, *copia* transposed at a three-fold higher rate in the 10-pair MA lines of Nuzhdin and Mackay (1995) compared with Maside *et al*.'s (2000) experiment.

In conclusion, Mukai's estimates of ΔM and U (Mukai, 1964; Mukai *et al*., 1972) do not appear to have been inflated by failure to distinguish mutational and non-mutational viability changes. Point estimates of these parameters from a new experiment and that of Fry *et al*. (1999) are lower than those reported by Mukai – several-fold lower in the case of U. The differences persist when the same method is used to estimate the mean decline. Bootstrap confidence intervals for order method ΔM, when available, are wide (Table 3); therefore sampling error may be sufficient to explain the variation in the ΔM estimates. There is some evidence that other factors, possibly strain differences or differences in assay conditions, may be necessary to fully account for the variation in ΔV and U estimates (J. D. Fry, unpublished results). Regardless of the explanation, the variation in the estimates suggests that it may be difficult to generalize about the magnitude of mutational parameters for a given trait, even within a single species.

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References

Bateman, A. J. (1959). The viability of near-normal irradiated chromosomes. *International Journal of Radiation Biology* **1**, 170–180.

Burt, A. (1995). Perspective: the evolution of fitness. *Evolution* **49**, 1–8.

Charlesworth, B. (1990). Mutation–selection balance and the evolutionary advantage of sex and recombination. *Genetical Research* **55**, 199–221.

Charlesworth, B., Morgan, M. T. & Charlesworth, D. (1993). The effect of deleterious mutations on neutral molecular variation. *Genetics* **134**, 1289–1303.

Charlesworth, D., Morgan, M. T. & Charlesworth, B. (1990). Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multi-locus system with no linkage. *Evolution* **44**, 1469–1489.

Fernández, J. & López-Fanjul, C. (1996). Spontaneous mutational variances and covariances for fitness-related traits in *Drosophila melanogaster*. *Genetics* **143**, 829–837.

Fry, J. D., Keightley, P. D., Heinsohn, S. L. & Nuzhdin, S. V. (1999). New estimates of the rates and effects of mildly deleterious mutation in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the USA* **96**, 574–579.

Gabriel, W. & Bürger, R. (1994). Extinction risk by mutational meltdown: synergistic effects between population regulation and genetic drift. In *Conservation Genetics* (ed. V. Loeschke, J. Tomiuk & S. K. Jain), pp. 69–84. Basel: Birkhauser.

Garcia Guerreiro, M. P. & Biémont, C. (1995). Changes in the chromosomal insertion pattern of the copia element during the process of making chromosomes homozygous in *Drosophila melanogaster*. *Molecular and General Genetics* **246**, 206–211.

García-Dorado, A. (1997). The rate and effects distribution of viability mutation in *Drosophila*: minimum distance estimation. *Evolution* **51**, 1130–1139.

García-Dorado, A., Monedero, J. L. & López-Fanjul, C. (1998). The mutation rate and the distribution of mutational effects of viability and fitness in *Drosophila melanogaster*. *Genetica* **102/103**, 255–265.

Haldane, J. B. S. (1956). The estimation of viabilities. *Journal of Genetics* **54**, 294–296.

Keightley, P. D. (1996). Nature of deleterious mutation load in *Drosophila*. *Genetics* **144**, 1993–1999.

Keightley, P. D. & Caballero, A. (1997). Genomic mutation rate for lifetime reproductive output and lifespan in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the USA* **94**, 3823–3827.

Keightley, P. D. & Eyre-Walker, A. (1999). Terumi Mukai and the riddle of deleterious mutation rates. *Genetics* **153**, 515–523.

Kondrashov, A. S. (1988). Deleterious mutations and the evolution of sexual reproduction. *Nature* **336**, 435–441.

Kondrashov, A. S. (1998). Measuring spontaneous deleterious mutation process. *Genetica* **102/103**, 183–197.

Kondrashov, A. S. & Houle, D. (1994). Genotype–environment interactions and the estimation of the genomic mutation rate in *Drosophila melanogaster*. *Proceedings of the Royal Society of London, Series B* **258**, 221–227.

Kozhemiakina, T. A. & Furman, D. P. (1995). Increased transposition rates of *copia*-like TEs while deriving isogenic lines of *Drosophila melanogaster*. *Drosophila Information Service* **76**, 100–103.

Lande, R. (1995). Mutation and conservation. *Conservation Biology* **9**, 782–791.

Latter, B. D. H. & Sved, J. A. (1994). A reevaluation of data from competitive tests shows high levels of heterosis in *Drosophila melanogaster*. *Genetics* **137**, 509–511.

Lynch, M., Conery, J. & Burger, R. (1995). Mutation accumulation and the extinction of small populations. *American Naturalist* **146**, 489–518.

Maside, X., Assimacopoulos, S. & Charlesworth, B. (2000). Rates of movement of transposable elements on the second chromosome of *Drosophila melanogaster*. *Genetical Research* **75**, 275–284.

Mooney, C. Z. & Duval, R. D. (1993). *Bootstrapping: A Nonparametric Approach to Statistical Inference*. Newbury Park, CA: Sage Publications.

Mukai, T. (1964). The genetic structure of natural populations of *Drosophila melanogaster*. I. Spontaneous mutation rate of polygenes controlling viability. *Genetics* **50**, 1–19.

Mukai, T. (1969). The genetic structure of natural populations of *Drosophila melanogaster*. VII. Synergistic interaction of spontaneous mutant polygenes controlling viability. *Genetics* **61**, 749–761.

Mukai, T. & Yamazaki, T. (1968). The genetic structure of natural populations of *Drosophila melanogaster*. V. Coupling-repulsion effect of spontaneous mutant polygenes controlling viability. *Genetics* **59**, 513–535.

Mukai, T., Chigusa, S. I., Mettler, L. E. & Crow, J. F. (1972). Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*. *Genetics* **72**, 335–355.

Nuzhdin, S. V. & Mackay, T. F. C. (1995). The genomic rate of transposable element movement in *Drosophila melanogaster*. *Molecular Biology and Evolution* **12**, 180–181.

Ohnishi, O. (1974). Spontaneous and ethyl methanesulfonate induced polygenic mutations controlling viability in *Drosophila melanogaster*. Ph.D. thesis. University of Wisconsin.

Ohnishi, O. (1977). Spontaneous and ethyl methanesulfonate-induced mutations controlling viability in *Drosophila melanogaster*. II. Homozygous effect of polygenic mutations. *Genetics* **87**, 529–545.

Pasyukova, E. G., Belyaeva, E. S., Ilyinskaya, L. E. & Gvozdev, V. A. (1988). Outcross-dependent transpositions of *copia*-like mobile elements in chromosomes of an inbred *Drosophila melanogaster* stock. *Molecular and General Genetics* **212**, 281–286.

Peck, J. R. (1994). A ruby in the rubbish: beneficial mutations, deleterious mutations and the evolution of sex. *Genetics* **137**, 597–606.

Shabalina, S. A., Yampolsky, L. Y. & Kondrashov, A. S. (1997). Rapid decline of fitness in panmictic populations of *Drosophila melanogaster* maintained under relaxed natural selection. *Proceedings of the National Academy of Sciences of the USA* **94**, 13034–13039.

Vassilieva, L. L. & Lynch, M. (1999). The rate of spontaneous mutation for life-history traits in *Caenorhabditis elegans*. *Genetics* **151**, 119–129.