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The mutational rate of *Drosophila* viability decline: tinkering with old data

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Summary

In the first 25 generations of his classical mutation accumulation experiment, T. Mukai estimated a large rate of early linear decay for the relative viability of *Drosophila melanogaster* chromosome II ($\Delta M_{II} = 0{\cdot}004$). Mukai forced through zero the regression of viability decline on generation number, but it has recently been shown (Fry, 2001) that a similar decline ($\Delta M_{II} = 0{\cdot}006$) is obtained from unforced regression even if generation 32 instead of generation 25 (whose validity has been questioned) is included. We show that, from the perspective of the whole long-term experiment, it is hard to decide up to which generation viability can be considered to decline linearly. Depending on this decision, and on whether or not the regression is forced through the origin, very different estimates are obtained. Furthermore, the particular behaviour of the lines used as control suggests that they could have been different from the remaining lines at the beginning of the experiment, and casts doubts on the adequacy of a forced regression. Estimates from the linear unforced regression ($\Delta M_{II} = 0{\cdot}011$) or from the linear term in a quadratic unforced regression ($\Delta M_{II} = 0{\cdot}001$) are very different. The data fit both models very well, and the choice between them should be based on biological grounds.

1. Introduction

The finding of high rates of viability decline in early mutation accumulation (MA) experiments with Drosophila (Mukai, 1964; Mukai *et al.*, 1972) suggested the common occurrence of mildly deleterious mutations (those with an effect of a few per cent) in natural populations. This raised concern on how populations cope with the corresponding mutational load, as well as on the effect of mutation on the extinction risk of endangered species. The rate of occurrence of mildly deleterious mutations continues to be controversial, and the above results are at the centre of the debate (see reviews by Lynch *et al.*, 1999; Keightley & Eyre-Walker, 1999; García-Dorado *et al.*, 1999).

Recently, Fry (2001) reanalysed Mukai's experiments to test the relevance of some doubts about the generality and causes of the viability decline (Keightley, 1996; García-Dorado, 1997). By applying the rank-order control method originally used by Mukai (1964), he revised the estimates of viability decline in the experiments of Mukai (1964) and Mukai *et al.* (1972) as well as in his own experiments. His reanalysis suggests that the early conclusions of Mukai (1964) are consistent with the results from the later Mukai *et al.* (1972) MA experiment. In this paper, we re-examine the Mukai 1964–1969 long-term experiment and show that inferences are very unstable against different reasonable decisions that can be made in the analysis.

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2. Reanalysis of the Mukai 1964–1969 data

(i) *The basic results*

In his long-term MA experiment, Mukai and colleagues (1964–1969) measured the viability of each MA chromosome II when homozygous, as the percentage P of wild-type ($+/+$) flies in the offspring of crosses between $Cy/+$ individuals, where Cy is a

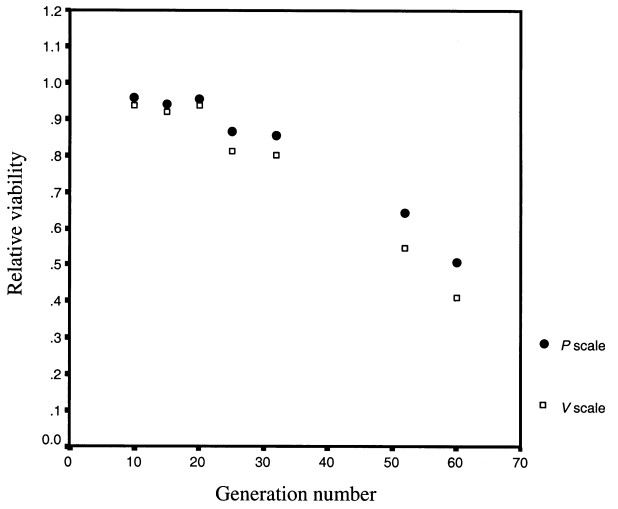

Fig. 1. Relative viability against generation number in Mukai's (1964–1969*b*) MA experiment. The *P* scale refers to the percentage of wild-type flies. The *V* scale refers to the transformed viability scale (see text).

recessive lethal balancer chromosome. The black circles in Fig. 1 represent the *P* values obtained by Mukai in the different assays of the experiment, relative to the corresponding rank-order control (see below). These points correspond to those in figure 1 of Mukai (1969*b*), except that Mukai 'dressed' them with a fitted curve, while here the bare points are presented, so that the reader is not chained to any particular interpretation.

The short-term rate of decline of relative viability reported for Mukai's first experiment (Mukai, 1964) was based on the comparison up to generation 25 (the first four points in Fig. 1) of the average *P* values of the MA lines with those of a 'rank-order method' (OM) control. The OM control average at any generation t is a synchronous estimate of the average viability of lines carrying no mutations. It is computed as the average viability at generation t of lines showing the top viability at some subsequent generation $t' > t$. Mukai (1964) computed the regression slope of the difference $M_C - M_Q$ on generation number, where M_C and M_Q are the mean viability of the OM control and that of the quasi-normal MA lines, respectively. The rate of relative viability decline for chromosome II was estimated as this regression slope relative to M_C averaged over generations, and gives $\Delta M_{II} = 0{\cdot}0038$ (see Mukai *et al.*, 1972).

(ii) *Fry's (2001) reanalysis of the short-term experiment*

The average percentage of wild-type flies *P* is not the best estimate of the wild-type viability, although it is roughly proportional to it for moderate ranges of viability values. Thus, Fry (2001) transformed average *P* to average viability *V* of wild-type chromosome II homozygotes (relative to *Cy* heterozygotes) as $V = 2P/(100-P)$, and applied the OM to the transformed means. (A representation of the *V* values of the whole long-term experiment is shown as white squares in Fig. 1). Fry (2001) also omitted the control viability given by Mukai (1964) for generation 25, which was computed from lines showing the top viability in an independent evaluation obtained in the same generation. Instead, he included data from generation 32 given by Mukai & Yamazaki (1968) (the fifth point in Fig. 1). Then, he obtained $\Delta M_{II} \approx 0{\cdot}0060$, irrespective of whether or not the set of OM control lines was the same as used by Mukai (1964), or whether or not the regression was forced through the origin. Fry's (2001) reanalysis supports the large short-term ΔM estimates obtained by Mukai (1964).

The question for this short-term analysis is: For how many generations can the decline be assumed to be roughly linear, so that it can be used to estimate the deleterious properties of individual mutations? If we force the regression line through the value of 1 on the ordinate axis, the linear period embraces up to generation 32, which was the choice made by Fry (2001). Once data from generations 25 or 32 have been included, the estimated rate of decline is large, even if the regression is not forced through the origin (unforced slopes: $0{\cdot}0072 \pm 0{\cdot}0042$, or $0{\cdot}0063 \pm 0{\cdot}0022$, respectively). However, if generations after $t = 20$ are not included, forcing the viability *V* line through an initial viability of 1 gives a large decline in viability ($0{\cdot}0042 \pm 0{\cdot}0009$), while not forcing the regression gives practically no decline ($0{\cdot}0001 \pm 0{\cdot}0022$).

An alternative approach is to analyse the data from the wider perspective of the whole experiment, in order to find an appropriate estimate of the early rate of decline. This will be attempted in the following three sections.

(iii) *Forcing the regression for the whole experiment through the origin*

The first point to be noted is that the viability decline could be non-linear on generation number. In fact, Mukai (1969*b*) concluded that synergistic epistasis was responsible for the accelerated viability decline. The synergistic hypothesis relied on forcing the regression of the decline on generation number through the origin, which gives

$$V = 1 - 0{\cdot}0084\,t \quad (p < 2{\cdot}3 \times 10^{-5})$$

$$V = 1 - 0{\cdot}0031\,t - 0{\cdot}0001\,t^2 \quad (p < 0{\cdot}4 \times 10^{-5}),$$

(the significance of the model is given in parentheses). Mukai (1969*b*) gave the regressions of the means on the estimated average number of deleterious mutations carried per line. This would be equivalent to the regressions given above after the independent variable

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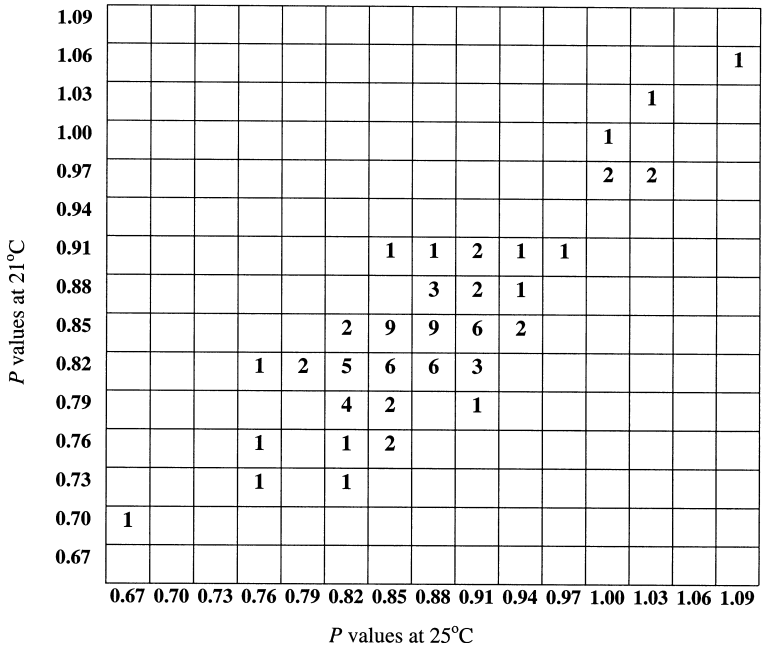

Fig. 2. Numbers of homozygous lines for different classes of relative viabilities (*P* scale) at generation 25 at two temperatures (values are scaled to the average control viability: 33·06 at 21 °C and 32·92 at 25 °C; Mukai, 1964).

t had been adjusted by a factor of 0·141 (the deleterious mutation rate per generation for chromosome II estimated by Mukai, 1964), except that Mukai used *P* instead of *V* values.

The higher significance of the quadratic model suggests that the decline is not linear. Note that the rate of early decline for *V* estimated from the quadratic regression ($\Delta M_{II} = 0{\cdot}0031$) is about half that obtained from Mukai's (1964) paper ($\Delta M_{II} \approx 0{\cdot}0060$; Fry, 2001). This is even more conspicuous when regression is computed for *P* (results not shown), which gives an early rate of decline (0·0014) about one-third that obtained for Mukai's (1964) data (0·0038; reported by Mukai *et al*., 1972).

(iv) *Is it safe to force the regression through the origin?*

Forcing through the origin is a sound procedure if the control has our complete trust, i.e. if the OM control lines are identical to the remaining MA lines except that they carry no new deleterious mutations. Then, MA viability at $t = 0$ relative to such control should be 1, and the corresponding decline should be 0. However, below we give arguments suggesting that the difference in viability between the OM controls used by Mukai (1964) and Fry (2001) and the remaining MA lines may not be due just to the accumulation of deleterious mutations by the latter.

Figure 2 reproduces Mukai's (1964) figure 4, representing the numbers of lines (given in the grid) showing the joint *P* values of the MA lines assayed at two temperatures at generation 25. A group of 7 lines with outlying high viability (numbered 15, 16, 37, 58, 72, 91 and 92) can be clearly identified. Fig. 3 reproduces figure 6 of Mukai & Yamazaki (1968), representing the viability at generation 32 of heterozygotes for pairs of chromosomes II sampled from the MA lines against the average of the two homozygous chromosome II viabilities in the parentals. Again, an outlying set of 14 crosses can be distinguished whose constituents were all the seven lines of the outlying 25-generation group, plus line 44. Interestingly, for these 14 crosses from generation 32 the correlation between heterozygous and homozygous viabilities was negative, while for the remaining crosses the correlation was positive. In fact, Mukai & Yamazaki (1968) treated the outlying lines separately from the rest. Furthermore, at generation 52, the same eight lines as in generation 32 were the components of the heterozygous crosses which showed a negative homozygous–heterozygous correlation, while the remaining lines showed, again, a positive correlation (Mukai & Yamazaki, 1968).

Mukai (1969*a*) also indicated that the three top lines at generation 32 were lines 91, 44 and 58, and the three top lines at generation 78 were lines 15, 92 and 191 (a replicate of line 91). The absolute *P* means for the outlying group were $0{\cdot}329 \pm 0{\cdot}002$ at generation 25 (lines 16, 37, 58, 72, 91, 92), and 0·326 and 0·324 at generations 32 and 52 (lines 15, 16, 37, 44, 58, 72, 91; standard error not given), both close to the OM control mean of generation 10 ($0{\cdot}330 \pm 0{\cdot}005$). Thus, the outlying group identified at generation 25 did not suffer any appreciable decline up to generation 52. In contrast, the viability of the remaining quasinormal

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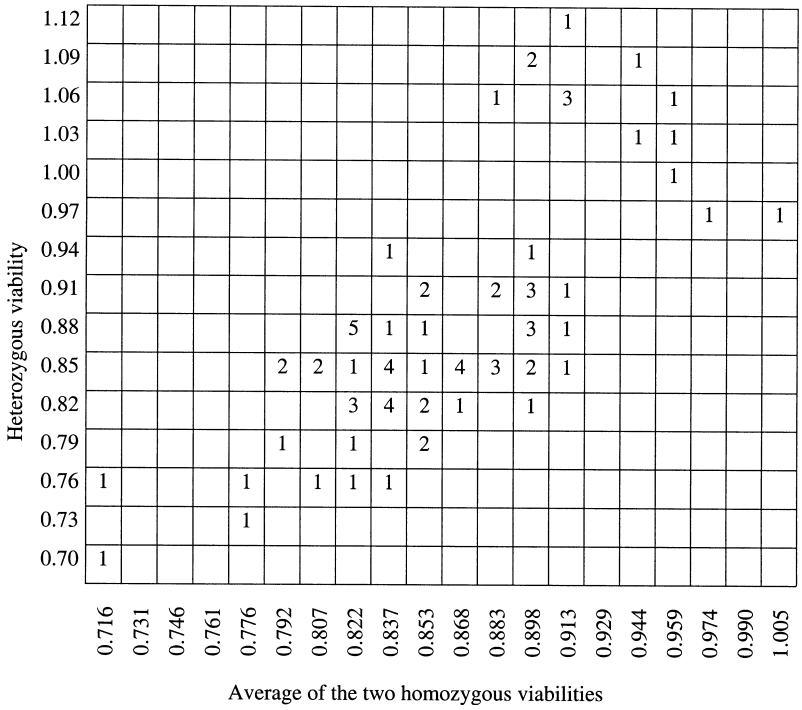

Fig. 3. Numbers of lines for different classes of relative viabilities (P scale) for heterozygous lines against the sum of the corresponding homozygous viabilities at generation 32 (values are scaled to the average control viability, 32·84; Mukai & Yamazaki, 1968).

MA lines declined continuously ($0{\cdot}316 \pm 0{\cdot}002$, $0{\cdot}283 \pm 0{\cdot}001$, $0{\cdot}280 \pm 0{\cdot}003$ and $0{\cdot}213 \pm 0{\cdot}007$ at generations 10, 25, 32 and 52, respectively). All this suggests that the seven top lines identified at generation 25 were genetically different from the remaining ones, and this difference could go back to the origin of the MA experiment. In any case, the existence and behaviour of the outlying viability group is unexpected on the basis of a high rate of accumulation of common mildly detrimental mutations.

In the analysis by both Mukai and Fry, most of the MA lines making up the OM controls belonged to the outlying group. Only Fry's OM control for generation 15 (his method 2) included no lines of the outlying group. Interestingly, no viability decline was observed for this generation using that control ($\Delta M_{II} = -3{\cdot}5 \times 10^{-4}$). Thus, at least some of the lines of the OM controls could have been different from the remaining MA lines at the beginning of the MA experiment. We might speculate about the different mechanisms that could be responsible for an original difference, as well as for the surprising estimates of the degree of dominance for this set of lines (Mukai & Yamazaki, 1968; see also García-Dorado & Caballero, 2000). These speculations would refer to events that occurred at some preliminary cross (contamination with an external mate, movement of an active transposable element in some lines, etc.), but none of them can now be tested. It is possible that the viability of OM control lines had not suffered any relevant change from generation 10 onwards, thus providing an adequate control for subsequent mutation accumulation. However, since they may have been different initially from the remaining MA lines, we believe there is a considerable risk in forcing the regression of the viability decline on generation number through the origin.

(v) *Results from the whole experiment using unforced regression*

The linear simple and quadratic regression equations of relative viability V on generation number t, without forcing through the origin, are given by

$$V = 1{\cdot}0984 - 0{\cdot}0108t \quad (p < 1{\cdot}1 \times 10^{-4})$$

$$V = 0{\cdot}9719 - 0{\cdot}0012t - 0{\cdot}0001t^2 \quad (p < 2{\cdot}3 \times 10^{-4}).$$

The significance of the above regressions is not comparable with that of the regressions forced through the origin because, in the forced regression ANOVA, the regression deviation includes the deviation from the average to the origin (i.e. the significance of the fraction of quadratic deviations explained by the model refers to deviations from the origin, instead of deviations from the mean), increasing the proportion of dispersion explained by the regression model. Since the significance is not improved under the quadratic model, the linear fitting seems statistically more parsimonious, suggesting a dramatically high rate of viability decline ($\Delta M_{II} = 0{\cdot}0108$). This value would

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imply a genomic decline of 2·7% per generation just for viability and, therefore, an even larger rate of decline for fitness. The figure is exceedingly high in the light of subsequent evidence, including the average estimates of the later MA experiment of Mukai *et al.* (1972). The significance under both the linear and the quadratic models is very similar, and the synergistic non-linear hypothesis may be preferred on biological grounds. In this case it is assumed that the mutational viability decline accelerates as the experiment goes on. Thus, the mutational properties of the original natural chromosomes are harder to estimate, and should be inferred from the early rate of decline, estimated as the linear regression term ($\Delta M_{II} = 0{\cdot}0012$).

3. Discussion

Table 1 shows a summary of the alternative estimates from the analysis of Mukai's 1964–1969 experiment (rates adjusted by a factor of 2·5 to apply to the whole haploid genome). Note first that, for reasons stated above, the significance of forced and unforced models can not be compared, so that the choice between these two alternative analyses must be based on a biological rationale. In our view, in order to obtain estimates whose validity does not rely on the assumption of the original identity between the OM controls and the remaining MA lines, the safer choice is not to force through the origin. After choosing between forced or unforced regression, the choice is between the linear and the quadratic approach. If this decision is to be based on statistical grounds, the model chosen must be the one providing more significant fitting to the data (i.e. a smaller p value), irrespective of the magnitude of the standard error for any specific regression coefficient. For unforced regression, the linear coefficient is about 1 order of magnitude larger in the linear than in the quadratic model, and it has a 4 times smaller standard error. However, both the linear and the quadratic models provide similarly good statistical fitting (p values on the order of 10^{-6}). Thus, results can be satisfactorily accounted for either by a model where viability decay is large and linear ($0{\cdot}0108 \pm 0{\cdot}0010$ per generation for chromosome II), or by a model where viability decay is initially non-significant but accelerates later (quadratic regression coefficient on generation number amounting to $0{\cdot}00014 \pm 0{\cdot}00005$ for chromosome II). The fortunate, and very valuable, availability of frequent viability assays through this MA experiment provides an appealing suggestion for accelerated viability decline. The quadratic model, proposed by Mukai, is in agreement with the observed accelerated rate of increase in between-line variance (Mukai, 1969*b*). It is also supported by the accelerated rate of lethal mutation, and by the observation that the mutation rate from deleterious to lethal chromosomes is of surprisingly large magnitude (Mukai, 1964). Unfortunately, the number of evaluations (the seven points in Fig. 1) is not large enough to allow precise estimation of both the quadratic and the linear genetic coefficients. Thus, results are compatible with a wide range of initial rates of viability decline.

Evidence from *Drosophila* MA experiments has continued accumulating since Mukai's seminal long-term experiment, and Table 1 shows a summary of the ΔM estimates obtained so far. The patterns of observed declines are far from homogeneous.

Table 1. *Haploid genomic rates of mean decline* (ΔM) *for the viability scale of quasi-normal lines observed in different* Drosophila melanogaster *MA experiments*

Experiment	Statistical procedure	$\Delta M \pm$ SE[a]
Mukai (1964–1969*b*)	Forced linear regression[b]	$0{\cdot}0210 \pm 0{\cdot}0002$ ($p < 0{\cdot}2 \times 10^{-6}$)
	Forced quadratic regression[b]	$0{\cdot}0077 \pm 0{\cdot}0027$ ($p < 0{\cdot}0 \times 10^{-6}$)
	Unforced linear regression[b]	$0{\cdot}0271 \pm 0{\cdot}0024$ ($p < 1{\cdot}1 \times 10^{-6}$)
	Unforced quadratic regression[b]	$0{\cdot}0031 \pm 0{\cdot}0098$ ($p < 2{\cdot}4 \times 10^{-6}$)
Mukai *et al.* (1972)	Linear regression	$0{\cdot}0101 \pm 0{\cdot}0004$
Ohnishi (1977)	Linear regression	$0{\cdot}0060 \pm 0{\cdot}0024$
Fernández & López-Fanjul (1996)	Comparison with large control	$0{\cdot}0016 \pm 0{\cdot}0003$
Chavarras *et al.* (2001)	Comparison with large control	$0{\cdot}0022 \pm 0{\cdot}0005$
Fry *et al.* (1999)	Comparison with large control	$0{\cdot}0060 \pm 0{\cdot}0004$
Average for all above experiments (García-Dorado *et al.*, 1999)	Minimum distance	$0{\cdot}0020 \pm 0{\cdot}0004$
Fry (2001)	Comparison with OM control	$0{\cdot}0080 \pm 0{\cdot}0025$

[a] ΔM is given with its standard error (SE), in some cases roughly inferred from the corresponding source. For Mukai (1964–1969*b*) data, the p values for the whole-model fitting are also given for the different analysis.
[b] For viability relative to that of an OM control.

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Mukai *et al*. (1972) used a design similar to that of the early experiment to study the rate of viability decline (V scale) in three sets of lines. This was fairly linear, giving an average $\Delta M_{II} = 0{\cdot}0040$, in qualitative agreement with the value previously reported by Mukai (1964). Since there was no control available, the conclusions relied on the assumption that the viability of the $Cy/+$ genotypes (used as the reference in the viability assays) remained constant over the experiment. Mukai *et al*. (1972) obtained estimates of the OM control using the means at generation 10 of lines with top viability in an independent assay. The average of these estimates was close to the viability of generation 0 inferred from the regression of V on t. This supported the linearity of the decline, and suggested that its magnitude was not affected by changes in the viability of the Cy chromosome, at least up to generation 10. Fry (2001) used this OM control to obtain an average $\Delta M_{II} = 0{\cdot}0048 \pm 0{\cdot}0028$ using generation 10 data, a value very close to the estimate obtained by Mukai *et al*. (1972) from the regression between generations 10 and 40 ($\Delta M_{II} = 0{\cdot}0048 \pm 0{\cdot}0008$). However, the separate OM estimates for the three sets of lines were extremely dispersed ($\Delta M_{II} = 0{\cdot}0038$, $0{\cdot}0005$ and $0{\cdot}0100$), and the fact that their average was so close to the regression estimate might be considered a coincidence.

In addition to the large linear decline reported by Mukai *et al*. (1972), and the initially slow but later accelerated decline found in the early Mukai experiment (1964, 1969*b*), Ohnishi (1977) reported an initially fast but later decelerated decline. The accelerated late decline observed by Mukai (1964, 1969*b*) could be due to unknown processes, such as accelerated transposition rates due to the crossing maintenance scheme (Keightley, 1996), although the strongly accelerated parallel increase in between-line variance suggests synergistic epistasis, as proposed by Mukai (1969*b*). The decelerated decline found by Ohnishi (1977), however, is hard to interpret in genetic terms because, as the mutational variance remained constant, this would imply both decelerated mutation rate and increasing deleterious effects (see García-Dorado & Caballero, 2000).

The long-term experiment of Fernández & López-Fanjul (1996) showed a linear but slow viability decline (Chavarrías *et al*., 2001). In this case, viability was not assayed over the first 100 generations, but the approximately linear decline observed over generations ~ 100, ~ 200 and ~ 250 was so small that it does not leave room for too much acceleration or deceleration during the first 100 generations. Minimum Distance reanalysis of all the above experiments (obtained ignoring the observed change in mean; see García-Dorado *et al*., 1999) gives a quite consistent picture suggesting small rates of mutational viability decline. Finally, Fry *et al*. (1999) and Fry (2001) reported a moderate rate of decline after about 30 generations.

The extent to which these discrepancies are due to different mutational properties of different genetic backgrounds depends to a large extent on the reliability of the corresponding controls. We have shown above that there could be problems with the OM control in Mukai's early experiment, at least regarding its original identity to the remaining MA lines. The validity of the decline observed in the experiment of Mukai *et al*. (1972) depends upon the genetic constancy of the Cy reference chromosome used to assay viability, which is partially supported by the good behaviour of the OM control up to generation 10. The Cy reference chromosome remained stable for ~ 30 generations in the experiment of Fry *et al*. (1999) relative to a large control population, but this does not necessarily imply a similar constancy for the Cy chromosome used in the Mukai or Ohnishi experiments. The decline observed in the experiment of Fernández & López-Fanjul (1996) relies on the genetic constancy of a large control population. This could have accumulated mutations to some extent, although computer simulations suggest that common mildly deleterious mutations causing substantial viability decline in the large control would cause a parallel decline in the MA lines much larger than experimentally observed (Caballero *et al*., 2002). Thus, controls have been the Achilles' heel of MA experiments.

The results and analysis of experiments on mutation accumulation by Mukai and colleagues are extremely valuable for understanding the properties of deleterious mutations. However, from the perspective of almost four decades, the rates of viability decline observed in different *Drosophila* MA experiments are far from consistent. It is likely that some keys for interpreting such inconsistency will remain unknown, and that only further experiments will improve our knowledge of the properties of deleterious mutations.

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