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Deleterious genomic mutation rate for viability in *Drosophila melanogaster* using concomitant sibling controls

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New deleterious mutations may reduce health and fitness and are involved in the evolution and maintenance of numerous biological processes. Hence, it is important to estimate the deleterious genomic mutation rate (*U*) in representative higher organisms. However, these estimated rates vary widely, mainly because of inadequate experimental controls. Here we describe an experimental design (the Binscy assay) with concomitant sibling controls and estimate *U* for viability in *Drosophila melanogaster* to be 0.31. This estimate, like most published studies, focuses on viability mutations and the overall deleterious genomic mutation rate would therefore be higher.

Keywords: mutation rate; mutation accumulation; concomitant sibling controls; *Drosophila melanogaster*

1. INTRODUCTION

Health and fitness may be reduced each generation by newly arising deleterious mutations (Drake *et al.* 1998; Lynch *et al.* 1999). These genetic changes are also involved in the evolution and maintenance of sexual reproduction and recombination, aging, diploidy, inbreeding avoidance, degeneration of Y chromosomes, genetic control of DNA-element transpositions, DNA repair, and the risk of extinction of endangered species (Charlesworth & Charlesworth 1998; Lynch *et al.* 1999). With relaxed selection, the increased accumulation of deleterious mutations is a particular threat to the genetic well-being of future humans (Crow 1997).

Hence, it is important to estimate the deleterious genomic mutation rate (*U*) in representative higher organisms. This rate seems to be very high in humans, with indirect estimations of up to three new deleterious mutations per individual (Eyre-Walker & Keightley 1999). However, estimated rates in other higher organisms vary widely. For example, *U* estimates in the model organism *Drosophila melanogaster* range from 0.01–1, with extensive ongoing debates on the correct value (Drake *et al.* 1998; Lynch *et al.* 1999; Fry 2001; Keightley & Eyre-Walker 2001; Kondrashov 2001; Garcia-Dorado & Caballero 2002; Rice 2002; Garcia-Dorado *et al.* 2004). One of the main reasons for difficulties in determining *U* in *D. melanogaster* is inadequate experimental controls (Kondrashov 1998; Houle & Nuzhdin 2004). In the standard methods of measuring the accumulation of deleterious mutations over time in *D. melanogaster*, the comparative controls have in many cases been unreliable. Examples of these controls include the original base stock, the most fit lines at the end of the experiment and base stocks frozen at the beginning of the experiment. Problems have arisen in controls because of contamination with other flies, low recovery of frozen stocks, and genetic changes in the base stock over time (Kondrashov 1998).

What is needed is an experimental design where deleterious mutations both do and do not accumulate in morphologically distinct siblings that have similar genetic backgrounds and are raised in identical environments (Kondrashov 1998). In this study, we will describe such an experimental design, the Binscy assay.

2. MATERIAL AND METHODS

(a) *The Binscy assay*

In the synthesis of the Binscy assay stocks (figure 1), we modified a mating scheme of Muller (Muller & Oster 1963). Binscy is a balancer X chromosome with the *B* (Bar eyes, dominant) and *y* (yellow body, recessive) mutations, plus multiple inversions that eliminate recombination of the X chromosome in females. We confirmed the balancing ability of the Binscy X chromosome, as described by Lindsley & Zimm (1992), by observing no recombinants among 622 progeny from *w m f*/Binscy females, whereas 206 recombinants were recovered among 584 progeny from *w m f*/+++ females. C(1;Y^S)oc ptg is a compound X and short arm of the Y chromosome, with the *oc* (ocelliless, missing ocelli, homozygous female sterile) and *ptg* (pentagon, thoracic trident dark) recessive mutations; and RY^L is the long arm of the Y chromosome in the shape of a ring (Lindsley & Zimm 1992). The Binscy/RY^L males are sterile because of the missing male fertility factors in the short arm of the Y chromosome, and C(1;Y^S)oc ptg/C(1;Y^S)oc ptg females are sterile because of the homozygous *oc* mutation (Lindsley & Zimm 1992). Each generation, one Binscy/C(1;Y^S)oc ptg female was mated with one C(1;Y^S)oc ptg/RY^L male per vial at 25 °C on standard cornmeal and agar medium. This mating scheme is designed to allow new deleterious mutations to accumulate over generations on the Binscy balancer X chromosome in Binscy/C(1;Y^S)oc ptg females. Since C(1;Y^S)oc ptg homozygous females and Binscy/RY^L males from this mating scheme are sterile, new deleterious mutations that occur on the X chromosome of C(1;Y^S)oc ptg/RY^L males are in a hemizygous state and are eliminated by selection. To the extent that deleterious mutations might temporarily be present on C(1;Y^S)oc ptg, our estimate of *U* will be reduced. Conversely, deleterious mutations that occur on the Binscy X chromosome in Binscy/C(1;Y^S)oc ptg females are buffered from selection because mutations are maintained as heterozygotes against wild-type alleles on the C(1;Y^S)oc ptg chromosome. Hence, with time deleterious mutations will accumulate on the Binscy chromosome, but are not expected to remain on the C(1;Y^S)oc ptg chromosome. We also tested for hybrid dysgenesis in our crosses (see methods in Boussy *et al.* 1998). None was observed.

A total of 108, 225 and 225 lines were initiated in the three runs, and 80, 141 and 71 lines completed each run, respectively. Run A was for 16 generations, whereas Runs B and C were each for 25 generations. Viability was measured for each line and each generation by counting the ratio of Binscy/RY^L males to total males (Binscy/RY^L and C(1;Y^S)oc ptg/RY^L males). Any line that gave three generations or more with no Binscy/RY^L males was considered to contain a new Binscy lethal mutation and was discarded. Only those lines that completed all generations were included in the final determination of viability.

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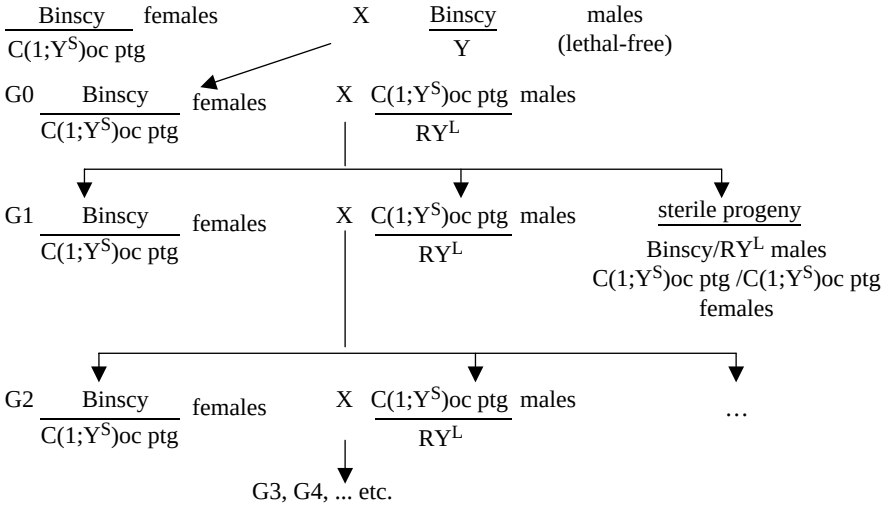

Figure 1. The mating scheme for the accumulation of deleterious mutations in the Binscy X chromosome of *Drosophila melanogaster*. In every generation (G0, G1, G2 … etc.), one single female and one single male are randomly selected to mate for each line.

(b) *Method to estimate deleterious genomic mutation rate*

The genomic mutation rate can be calculated using a method proposed by Bateman (1959) and Mukai (1964). The accumulation of deleterious mutations will lead to a predicted steady reduction in mean viability and an increase in variance between lines. The rate of decline in mean viability (ΔM) and the rate of increase in variance of viability between lines (ΔV) can be estimated by regression analysis.

If one assumes that spontaneous mutations are distributed on the X chromosome according to a Poisson distribution, ΔM and ΔV can be expressed as

$$\Delta M = M_s U_x, \tag{2.1}$$

$$\Delta V = (M_s^2 + V_s) U_x, \tag{2.2}$$

where M_s and V_s are the mean and the variance of s (the effect of a mutation on viability), respectively, and U_x is the mean number of deleterious mutations in the X chromosome in one generation.

Rearranging equations (2.1) and (2.2) gives

$$U_x = (1+k)\frac{\Delta M^2}{\Delta V}, \tag{2.3}$$

$$M_s = \frac{1}{1+k}\frac{\Delta V}{\Delta M}, \tag{2.4}$$

$$V_s = \left[1 - \frac{(1-k)^2}{(1+k)^2}\right]\left(\frac{\Delta V}{2\Delta M}\right)^2, \tag{2.5}$$

where $k = V_s/M_s^2$. Let

$$U_{xl} = \frac{U_x}{1+k} = \frac{\Delta M^2}{\Delta V}, \tag{2.6}$$

$$M_{su} = M_s(1+k) = \frac{\Delta V}{\Delta M}, \tag{2.7}$$

$$V_{su} = \frac{V_s}{1 - \frac{(1-k)^2}{(1+k)^2}} = \left(\frac{\Delta V}{2\Delta M}\right)^2, \tag{2.8}$$

then U_{xl} is a lower bound of U_x, M_{su} is an upper bound of M_s and V_{su} is an upper bound of V_s.

The Binscy assay estimates the haploid genomic mutation rate using the X chromosome. Since an X chromosome contains around 15.97% of the genes in the haploid genome in *D. melanogaster*[1], the lower bound of the diploid genomic mutation rate that we report here (U_l) is about two times U_{xl} divided by 15.97%.

(c) *Statistical analysis*

A bootstrap resampling method (Charlesworth *et al.* 2004) was employed to get interval estimations of U_l, M_{su} and V_{su}, using the MINITAB software package. From the observed viability data of each of the three experimental runs, the same number of lines as in that run were sampled with replacement 300 times. For each bootstrap sample, ΔM, ΔV, U_l, M_{su} and V_{su} were calculated by the method mentioned above. Confidence intervals of these parameters then can be obtained from those bootstrap samples.

Table 1. Lethal mutation rates (with standard errors) in three runs of mutation accumulation experiments.

	lethal chromosomes	chromosomes screened	mutation rate ±s.e.
Run A	6	1302	0.0046±0.0019
Run B	17	4068	0.0042±0.0010
Run C	24	3076	0.0078±0.0016
total	47	8446	0.0056±0.0008

3. RESULTS AND DISCUSSION

The numbers of Binscy X chromosomes with new lethal mutations and the total numbers of Binscy X chromosomes assayed in three experimental runs are recorded in table 1. If any line had no Binscy/RY^L males for three generations or more, a new lethal mutation in a Binscy X chromosome was counted. The total number of Binscy X chromosomes assayed is the summation of the number of lines screened in every generation. There were 47 lethal mutations among 8446 X chromosomes screened in all three runs. Therefore, the estimated spontaneous lethal mutation rate for the Binscy X chromosome in females is 0.0056. This rate is similar to some previous estimations (Woodruff *et al.* 1983, 1984).

As predicted, the mean viability of Binscy/RY^L males decreased and the variance increased for each of the three experimental runs (figure 2). The rate of decline in the mean viability (ΔM) and the rate of increase in the variance of viability between lines (ΔV) were determined from the slopes of the curves, which can be estimated by linear regression. Using the method described above, our estimations of mutational parameters are given in table 2. Weighting each run by the inverse of its bootstrap variance (Charlesworth *et al.* 2004), the average genomic mutation rate for viability (U_l) is 0.31 and the average effect of a deleterious mutation (M_{su}) is 0.14.

The value of U_l (U_l=0.31) is a lower bound of the deleterious genomic mutation rate (U). U equals to U_l only when the mutational effect (s) is

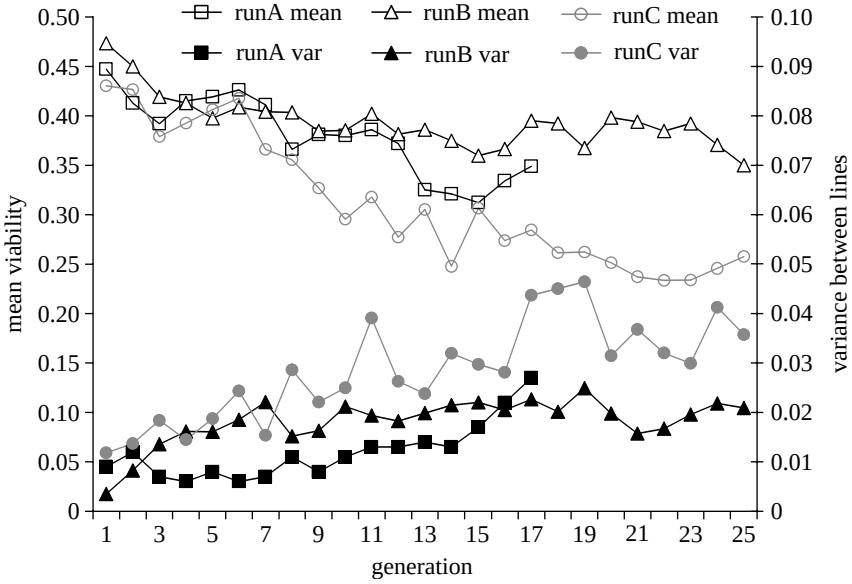

Figure 2. Changes in the mean viability and variance over time for deleterious mutations in three runs (Run A has 80 lines, Run B has 141 lines and Run C has 71 lines) of the Binscy assay of *Drosophila melanogaster*.

Table 2. Deleterious mutation rate for the X chromosome (U_{xl}) and the diploid genome (U_l) in three runs.
(M_{su} and V_{su} are upper bounds of mean and variance of s (effect of a single mutation on viability). ΔM is the rate of decline in mean viability and ΔV is the rate of increase in variance of viability between lines. Values in parentheses are 95% bootstrap confidence intervals.)

	ΔM	ΔV	U_{xl}	U_l	M_{su}	V_{su}
Run A	0.007	0.0009	0.054	0.68	0.13	0.0041
	(0.0053, 0.0089)	(0.0006, 0.0012)	(0.033, 0.088)	(0.41, 1.10)	(0.08, 0.18)	(0.0018, 0.0081)
Run B	0.0026	0.0004	0.017	0.21	0.15	0.0059
	(0.0019, 0.0034)	(0.0002, 0.0005)	(0.010, 0.030)	(0.13, 0.38)	(0.10, 0.22)	(0.0023, 0.0118)
Run C	0.0083	0.0011	0.063	0.79	0.13	0.0044
	(0.0071, 0.0096)	(0.0008, 0.0014)	(0.042, 0.105)	(0.53, 1.31)	(0.09, 0.18)	(0.0019, 0.0078)

constant. The relation between U_l and U depends on the coefficient of variance of s (i.e. $\sqrt{k}$), which is determined by the distribution of the parameter s. For example, if s follows an exponential distribution, $k=1$, $U=2U_l$ and $M_s=0.5M_{su}$ (Drake *et al.* 1998). Hence, our estimation of the deleterious genomic mutation rate is at least 0.31 and could be higher.

This estimation of the deleterious genomic mutation rate in *D. melanogaster* is lower than the estimations of Mukai (0.7–0.8) (Mukai 1964; Mukai *et al.* 1974), but higher than some recent estimations (0.04–0.12) (Fry *et al.* 1999; Garcia-Dorado *et al.* 1999; Charlesworth *et al.* 2004). The average effect of a mutation (M_{su}) of 0.14 is substantially higher than reported by Mukai (0.02–0.03) (Mukai 1964; Mukai *et al.* 1974), but is more in accordance with other recent reports (0.08–0.25, Garcia-Dorado *et al.* 2004; 0.10, Charlesworth *et al.* 2004). In addition, the rate of decline in mean viability of about 4% (M_{su} of $0.14\times U_l$ of 0.31) is larger than previously reported. Although the reason for this large value is unknown, it may be related to the deleterious mutations recovered in this study being on the X chromosome.

The observed deleterious mutation rate of 0.31 is below 1, which is predicted to be the minimum level at which sex may have evolved to eliminate deleterious mutations through recombination leading to epistatic selection (Kondrashov 1988, 1998). The Binscy assay allows us to avoid one key criticism of other estimation techniques by providing concomitant sibling controls, but there are other complications that affect estimates in general, such as the occurrence of epistatic interactions and mutations affecting processes other than viability, that will make our rate an underestimate of the overall deleterious genomic mutation rate.

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ENDNOTE

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