

Mutation-selection balance accounting for genetic variation for viability in *Drosophila melanogaster* as deduced from an inbreeding and artificial selection experiment

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Abstract

We carried out an experiment of inbreeding and upward artificial selection for egg-to-adult viability in a recently captured population of *Drosophila melanogaster*, as well as computer simulations of the experimental design, in order to obtain information on the nature of genetic variation for this important fitness component. The inbreeding depression was linear with a rate of $0.70 \pm 0.11\%$ of the initial mean per 1% increase in inbreeding coefficient, and the realized heritability was 0.06 ± 0.07 . We compared the empirical observations of inbreeding depression and selection response with computer simulations assuming a balance between the occurrence of partially recessive deleterious mutations and their elimination by selection. Our results suggest that a model assuming mutation-selection balance with realistic mutational parameters can explain the genetic variation for viability in the natural population studied. Several mutational models are incompatible with some observations and can be discarded. Mutational models assuming a low rate of mutations of large average effect and highly recessive gene action, and others assuming a high rate of mutations of small average effect and close to additive gene action, are compatible with all the observations.

Introduction

Compilations of empirical data indicate that traits closely connected to fitness usually exhibit low heritabilities (Mousseau & Roff, 1987; Roff & Mousseau, 1987; Hoffmann, 2002), high levels of inbreeding depression (Charlesworth & Charlesworth, 1999; DeRose & Roff, 1999), substantial dominance variance (Crnokrak & Roff, 1995) and considerable asymmetry in the response to divergent artificial selection (Frankham, 1990). These observations have been explained by the action of natural selection eroding the additive variance of fitness (Fisher, 1930; Robertson, 1955) and by the fact that most

deleterious mutations segregating in natural populations are partially recessive (Charlesworth & Charlesworth, 1999). Thus, the simplest model to explain the genetic variation of fitness components is the balance between the input of partially recessive deleterious mutations and their elimination by natural selection (the mutation-selection balance). This is the null hypothesis against which other models of variation, such as antagonistic pleiotropy or selection in heterogeneous environments, can be tested (Charlesworth & Hughes, 1999).

A substantial amount of information is becoming available on the mutational properties of some fitness components, particularly viability in *Drosophila melanogaster*. The first estimates of the parameters describing the properties of minor deleterious mutations were obtained from mutation-accumulation experiments in the 1960s and 70s (Mukai, 1964, 1969b; Mukai *et al.*, 1972; Ohnishi, 1977a,b; for a review, see Lynch *et al.*, 1999). These classical studies suggested that deleterious mutations occur at a rate in the order of $\lambda \approx 0.25\text{--}0.5$ mutations per haploid genome and generation, with

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average homozygous effect of about $s \approx 0.02$. The average coefficient of dominance of minor deleterious mutations (h , where sh is the mutational heterozygous effect) estimated by Ohnishi (1977b) was 0.49. Estimates by Mukai and co-workers ranged from around zero, implying almost complete recessive gene action, to around 0.4, implying close to additive gene action, depending on the analyses (Simmons & Crow, 1977; García-Dorado *et al.*, 1999, 2004). The estimates close to zero were generally disregarded and an estimate of $h \approx 0.4$ has been generally cited for the average coefficient of dominance of newly arisen minor deleterious mutations.

More recent mutation-accumulation studies and reanalyses of previous results (García-Dorado *et al.*, 1999, 2004; García-Dorado & Caballero, 2000), suggest much smaller mutation rates ($\lambda \approx 0.015$), substantially larger homozygous effects ($s \approx 0.2$), and gene action closer to complete recessivity ($h \approx 0.15$). These parameters refer to mutations of minor or moderate effect. However, it is experimentally difficult to quantify the properties of mutations of extremely low effect ($s < 5 \times 10^{-4}$; García-Dorado *et al.*, 2004), and it is possible that many of those mutations have passed undetected and were not considered in the above estimates (Keightley & Eyre-Walker, 1999).

The two above models of spontaneous deleterious mutations provide quite different predictions for a number of evolutionary parameters (García-Dorado, 2003), which can be, at least in principle, empirically tested. Thus, the classical model involving many mutations of small effect implies a larger equilibrium additive variance than the more recent model of few mutations of large effect, as well as a larger inbreeding depression and a larger rate of fitness decline due to deleterious fixation in small populations. Several earlier studies have focused on the last of these predictions, showing that the observed fitness decline in small populations is incompatible with the classical model but compatible with the more recent model (Caballero & Keightley, 1998; Caballero *et al.*, 2002). Here, we focus on the first two predictions relative to the additive genetic variance and the inbreeding depression.

We carried out upward artificial selection and inbreeding in a recently captured population of *D. melanogaster*, obtaining estimates of the selection response and inbreeding depression for egg-to-adult viability. The simultaneous use of selection and inbreeding is a useful tool in this context, as it may involve a quite effective purging of deleterious mutations, and the mean realized inbreeding coefficient evolves with selection (Wright & Cockerham, 1985). The results of these experiments were used in combination with the mutational information in order to infer the properties of the genetic architecture of viability. Using a range of mutational parameters and assuming a simple deleterious mutation-selection balance model of variation, we simulated the experimental conditions and compared simulated data and

empirical observations. The objectives of the study were: (i) to ascertain whether the genetic variation for viability in a natural population can be explained by a model of mutation-selection balance; (ii) to investigate the set of mutational parameters that better explain the empirical data. We conclude that a model assuming mutation-selection balance can explain the frequency of genes affecting viability in the natural population, but the two main contrasting models of mutation debated in the literature produced similar outcomes.

Methods

Base population, culture conditions and trait scored

We collected 306 inseminated females in a wine cellar close to Vigo (north-west Spain). Flies were reared in a standard medium formula composed of 1 L water, 200 g brewer's yeast, 50 g sucrose, 12 g agar, 2.5 g NaCl and 5 mL propionic acid. All cultures were incubated at 25 ± 1 °C and maintained under continuous lighting. Flies were handled at room temperature under CO₂ anaesthesia. The trait considered was egg-to-adult viability, which was evaluated as follows. Four-day-old virgin females were individually mated to males of the same age in glass vials (20 mm diameter, 100 mm height) with 10 mL medium added. After 2 days, both parents were transferred to a new vial with fresh medium to which food colouring was added to make the eggs visible. Oviposition was allowed for 24 h. The number of eggs laid (with an average of 54.85 ± 0.39 eggs across lines, replicates and generations) has been analysed elsewhere (Fernández *et al.*, 2003). From these layings, 30 eggs were transferred to a fresh vial and allowed to develop into adults. The trait measured was the proportion of adults emerged from the 30 eggs up to the eleventh day after the transfer, when emergence had been completed, except for rare exceptions. When the number of eggs laid was smaller than 30 (14% of the cases), all eggs were transferred. The untransformed data were analysed.

Previous to the start of the selection and inbreeding experiments, flies were maintained by single pair matings in vials for three generations (denoted with a minus sign; see Fig. 1a) as follows. From those wild females producing at least four offspring of each sex (232 of 306), 120 were randomly taken as ancestors of the experimental lines (generation -3). These females were randomly divided into three blocks of 40, to form the three replicates of the experiment. In each block (replicate), four male and four virgin female offspring were taken from each vial and mated in pairs following a circular scheme (Fig. 1a), to initiate four lines of 40 matings each (selected inbred, selected non-inbred, control inbred and control non-inbred lines were eventually derived from these lines; see below). Thus, each replicate of the experiment was derived from a group of 40 wild females sampled from the base population, whereas the four treatments in each

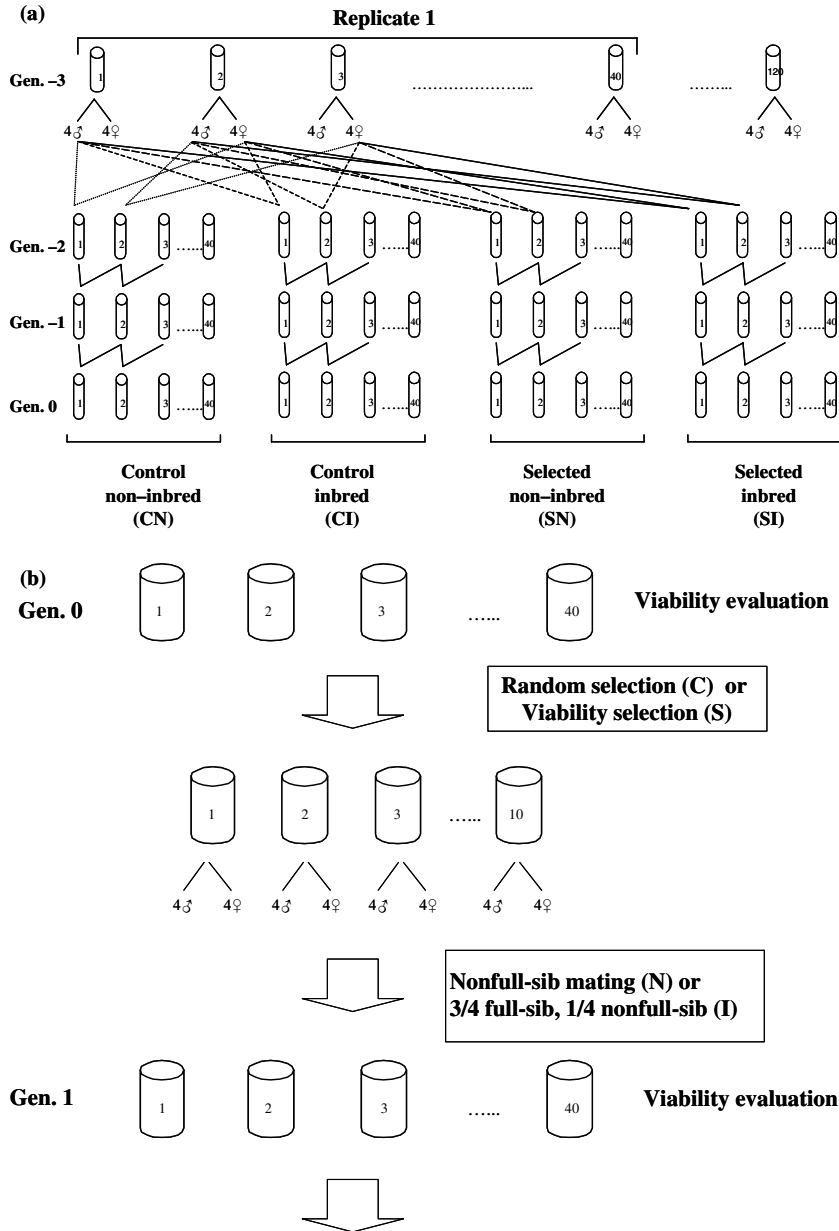


Fig. 1 Experimental design: initial generations (a); selection and inbreeding experiments (b) (see text for further explanation).

replicate (selected and control, inbred and non-inbred) were derived from the same set of 40 wild females. Each replicate line was subsequently maintained with circular mating for two further generations before the start of the inbreeding and selection experiments (generation 0). The circular scheme used guaranteed virtually no inbreeding at generation 0 (Fig. 2a).

Selection and inbreeding procedure

At generation 0, the 40 pairs of virgin individuals from each of the replicates were mated and the viability was

evaluated (Fig. 1b). From these 40 pairs, 10 pairs were either chosen at random (in the control, C lines) or selected because of their larger egg-to-adult viability (in the selected, S lines). Each of these 10 pairs contributed four male and four virgin female offspring in all cases, for a total of 40 male and 40 female progeny. These individuals were then mated again in 40 pairs, either randomly but avoiding full-sib matings (in the non-inbred, N lines), or making three-fourths of the crosses between full sibs and one-fourth between unrelated individuals (in the inbred, I lines). For the latter in particular, three male and three female offspring from

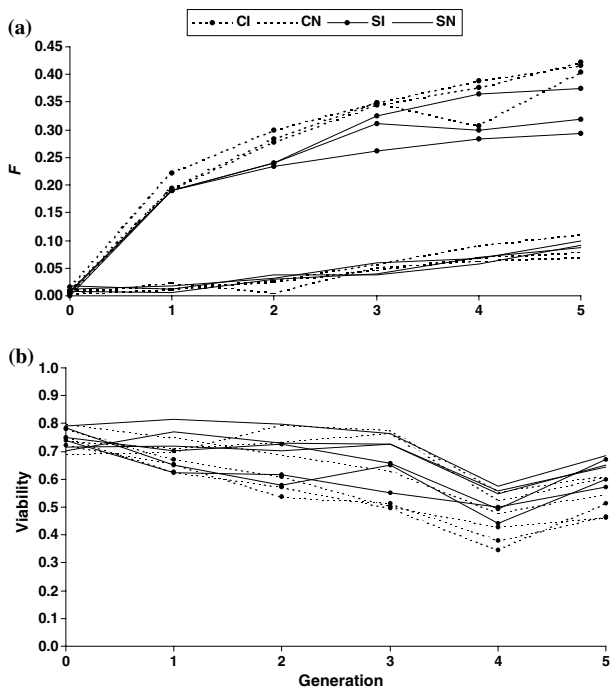


Fig. 2 Average inbreeding coefficient (a) and egg-to-adult viability (b) per generation for the different experimental lines. CI, control inbred lines; CN, control non-inbred lines; SI, selected inbred lines; SN, selected non-inbred lines.

each vial were mated in pairs and the remaining male and female were mated to individuals from other randomly chosen vials. The viability of these 40 matings was evaluated (generation 1). Note that all parents in generations 0 and 1 were non-inbred, and that the progeny used to evaluate generation 1 was the first inbred (in the case of inbred lines). The procedure followed between generations 0 and 1 was repeated for five consecutive generations. In all, there were four groups of lines: selected inbred (SI), selected non-inbred (SN), control inbred (CI) and control non-inbred (CN), with three replicates each. Pooling replicates and generations, the proportion of sterile matings (zero eggs laid) was 6.6% in the inbred lines and 4.1% in the non-inbred lines. In parallel, the overall proportion of vials with zero viability (no adults emerged from any of the eggs laid) was 5.2% in the inbred lines and 2.1% in the non-inbred lines. These vials were excluded from the analysis (as they could correspond to non-fertilized eggs), but their inclusion did not change the results substantially (data not shown).

Analysis

Inbreeding depression

Genealogies were kept from the start of the experiment (generation -3) allowing the calculation of the expected

inbreeding coefficient of every individual. The inbreeding depression rate was estimated for the control inbred (CI) lines as the regression of viability on the mean inbreeding coefficient, correcting for environmental trends as indicated by Lynch & Walsh (1998). Basically, the method is a partial regression of the mean of CI lines at generation t , $\bar{Z}_I(t)$, on the mean of CN lines, $\bar{Z}_N(t)$, and the mean inbreeding coefficient, $F(t)$:

$$\bar{Z}_I(t) = a + b\bar{Z}_N(t) + IF(t) + e(t),$$

where I is the estimated inbreeding depression corrected for environmental trends and $e(t)$ is the deviation of the mean at generation t from the regression line. The mean values of the inbred lines corrected for environmental trends are obtained as:

$$\bar{Z}_I^*(t) = \bar{Z}_I(t) - b[\bar{Z}_N(t) - \bar{Z}_N],$$

where \bar{Z}_N is the average of CN mean values over generations. Note that I can also be estimated as the regression of $\bar{Z}_I^*(t)$ on $F(t)$. The calculations were made without transformations as the drop was approximately linear over generations in real scale.

Egg-to-adult viability has been previously considered as a trait fully dependent on the maternal genotype (López-Fanjul & Villaverde, 1989; García *et al.*, 1994), as the maternal component may account for up to 74% of the variation (Chapco, 1979). However, our results indicate that inbreeding depression has maternal and offspring components of similar magnitudes (see Results section below). Thus, in the estimation of the inbreeding depression rate, we used the geometric average of the mean heterozygosities, $F = 1 - \sqrt{(1 - F_m) \times (1 - F_o)}$, where F_m and F_o are the inbreeding coefficients from maternal and offspring generations, respectively.

Response to selection and heritability

The response to upward artificial selection was calculated for each replicate selected line (SN and SI) as the regression of the mean values of the selected lines, deviated from the corresponding control mean values (CN and CI) to correct for environmental effects, on generation number. The selection differential applied was calculated as the difference between the average egg-to-adult viability of the 10 selected vials and that for all vials scored. Note that if the trait is assumed to be fully dependent on the mother genotype (as in López-Fanjul & Villaverde, 1989; García *et al.*, 1994), the procedure involves individual selection, and the regression of the mean values of the selected lines on the cumulative selection differential directly estimates the trait heritability. However, if the trait is assumed to be dependent both on maternal and offspring genotypes, individual selection is carried out on the maternal component whereas family selection is carried out on the offspring component, complicating the interpretation of the estimate. Thus, heritabilities were obtained just for the purpose of comparison with those of previous experiments, which

assume fully maternal effects. Accordingly, the additive variance was estimated by multiplying the phenotypic variance by the realized heritability. All results were averaged over replicates.

Computer simulations

Model and mutational parameters

To investigate whether the experimental results could be explained by a mutation-selection balance model of genetic variation, we carried out simulations resembling the experimental conditions and using different mutational parameters. We assumed a model of variable deleterious mutations in which the genotypic fitnesses at a given locus are 1, $1 - sh$ and $1 - s$ for the wild homozygote, the heterozygote and the mutant homozygote, respectively, and effects are multiplicative among loci. Because we considered egg-to-adult viability as a trait dependent on the genetic constitutions of both the mother and the offspring in similar proportions (see Results section below), the probability of survival of a simulated individual was calculated as the geometric

mean of the genotypic fitnesses of the mother and that of the individual itself. Individuals survived if a random number between 0 and 1 was smaller than its probability of survival, and died otherwise.

Mutations were assumed to occur at a rate λ per haploid genome and generation and effects were sampled from a gamma distribution with shape parameter β and mean effect \bar{s} . A range of five models was used in the simulations as shown in the left-hand columns of Table 1. The first models ($\lambda = 0.25-0.5$, $\bar{s} = 0.02-0.03$) assume many mutations of small average effect, while the last one ($\lambda = 0.015$, $\bar{s} = 0.2$) assumes few mutations of large average effect. These are the two contrasting models proposed in the literature for spontaneous mutations affecting viability in *Drosophila*. The remaining models represent intermediate situations. The shape parameter of the gamma distribution ($\beta = 0.2, 0.238, 0.263, 0.714, 1.0$, respectively, for the five models) was chosen such that the mutational variance explained by any model is 0.0006, a value that has been experimentally observed (García-Dorado *et al.*, 1999). Note that the range of parameters studied covers the most realistic

			ID of CI†	F of SI‡	SI-CN§	
Experimental results			0.70	0.328	-0.0096	
λ	s	\bar{h}				Test¶
0.5	0.02	0.15	1.47-1.77	0.269-0.310	-0.1492 to -0.0581	*
		0.2	1.44-1.74	0.269-0.313	-0.1442 to -0.0589	*
		0.3	1.34-1.64	0.278-0.324	-0.1247 to -0.0494	*
		0.4	0.82-1.18	0.304-0.372	-0.0811 to -0.0061	*
0.25	0.03	0.15	1.40-1.79	0.266-0.307	-0.1606 to -0.0675	*
		0.2	1.38-1.70	0.269-0.310	-0.1592 to -0.0597	*
		0.3	1.27-1.61	0.278-0.324	-0.1336 to -0.0439	*
		0.4	0.59-0.97	0.317-0.396	-0.0658 to 0.0203	n.s.
0.1	0.05	0.15	1.30-1.60	0.265-0.306	-0.1686 to -0.0603	*
		0.2	1.24-1.54	0.270-0.311	-0.1608 to -0.0586	*
		0.3	0.90-1.27	0.286-0.343	-0.1281 to -0.0197	*
		0.4	0.30-0.71	0.318-0.434	-0.0375 to 0.0656	n.s.
0.05	0.1	0.15	1.08-1.42	0.275-0.324	-0.0369 to 0.0447	*
		0.2	0.86-1.23	0.291-0.356	-0.1072 to 0.0036	*
		0.3	0.34-0.77	0.320-0.436	-0.0347 to 0.0722	n.s.
		0.4	0.16-0.55	0.357-0.456	-0.0033 to 0.0972	*
0.015	0.2	0.15	0.46-0.92	0.321-0.429	-0.0514 to 0.0686	n.s.
		0.2	0.26-0.69	0.352-0.458	-0.0217 to 0.0886	*
		0.3	0.08-0.49	0.375-0.471	0.0014 to 0.0958	*
		0.4	0.05-0.44	0.375-0.469	0.0061 to 0.0983	*

Table 1 Simulated confidence intervals for some parameters studied and their comparison with empirical results.

Mutational parameters: λ , haploid deleterious mutation rate; \bar{s} , average effect of mutations; \bar{h} , average dominance coefficient of mutations. Bold face values in italic denote statistical nonsignificance between simulated and observed results. n.s., overall nonsignificance between simulation and observed results; *, significance at 5%.

†Inbreeding depression rate for the CI lines.

‡Inbreeding coefficient at generation 5 for the SI lines.

§Viability difference at generation 5 between the SI and CN lines.

¶Multivariate test considering the joint probabilities for the three parameters analysed (χ^2 with 6 degrees of freedom).

situations. Other combinations of parameters, for example those involving a large number of mutations of large average effect or, in contrast, a very small number of mutations of small average effect, are incompatible with the results obtained from mutation-accumulation experiments for viability in *Drosophila* (García-Dorado *et al.*, 1999, 2004; Lynch *et al.*, 1999), and are not considered here.

The dominance coefficient of newly arisen mutations, h , was obtained from a uniform distribution between 0 and $\exp(-ks)$, where k is a constant allowing the mean dominance coefficient, \bar{h} , to be the desired one. This model has some experimental support (Caballero & Keightley, 1994). The average dominance coefficients considered were $\bar{h} = 0.15, 0.2, 0.3$ and 0.4 . The value 0.4 is the estimate proposed by Mukai (1969b) for viability in *Drosophila*, while a value of 0.15 has been proposed recently (García-Dorado & Caballero, 2000). Therefore, the set of estimates ($\lambda = 0.25-0.5$, $\bar{s} = 0.02-0.03$, $\bar{h} = 0.4$) corresponds to the classical estimates proposed by Mukai (1964, 1969b), whereas the set ($\lambda = 0.015$, $\bar{s} = 0.2$, $\bar{h} = 0.15$) represents a more recent proposal (García-Dorado *et al.*, 1999).

In all cases, lethal mutations were also included, assumed to occur at a rate 0.015 per generation with dominance coefficient $h = 0.02$ (Simmons & Crow, 1977).

Simulation of the experimental design

An ancestral population of 10 000 individuals was first set up in which gene frequencies for the deleterious alleles (q) were at mutation-selection balance, following equation (6.2.6) of Crow & Kimura (1970):

$$q^2s(1 - 2h) + qsh(1 + u) - u = 0,$$

where u is the per locus deleterious mutation rate and s and h were obtained as explained above. A large number of independent loci (5800) were considered in the simulations so that $u = \lambda/5800$, where λ here includes both lethal and non-lethal mutations.

A design as close as possible to the experimental one was followed, including the three generations previous to the start of the selection and inbreeding experiments (Fig. 1a). Each replicate was initiated from a sample of 40 pairs of individuals taken from the large outbred base population (see generation -3 in Fig. 1a). New deleterious mutations were also allowed to occur during the course of the selection and inbreeding experiments but their impact on the results was small given the few generations considered.

Inbreeding coefficients were calculated from the genealogies. The inbreeding depression rate was calculated as the regression of the inbred mean values on the inbreeding coefficients. Selection response was calculated as the regression of the mean of the selected lines, deviated from the mean of the corresponding unselected controls, on generation number. The expected additive

genetic variance in an infinite population was calculated for each simulation model as $2\lambda sh$ (Charlesworth & Hughes, 1999), where $\bar{s}h$ is the average over 1000 sampled mutational effects for each particular model (including lethal mutations). The simulated additive variance at generation 0 was calculated from the gene frequencies (q) of mutations, as $2\alpha^2q(1-q)$, where $\alpha = s[h(1-2q)+q]$ (Falconer & Mackay, 1996).

Statistical comparison between simulated and empirical results

To contrast different models and observations, we carried out, for each particular combination of mutational parameters, 1000 simulations of the experimental design, each simulation including three replicates of each of the selected/control and inbred/non-inbred lines run for three previous plus five experimental generations, averaging results over the three replicates. This gave a set of 1000 independent simulation results for each of the studied parameters (selection response, inbreeding coefficient, inbreeding depression, etc.) The 1000 simulated results were ordered from the lowest to the largest, and the values corresponding to the 2.5 and 97.5 percentiles of the ordered distribution (values in positions 26th and 975th respectively) were recorded. Thus, a given experimental result was considered incompatible with the particular simulation if it was outside these 95% confidence limits, whereas it was considered compatible if it was inside the bounds. This statistical approach to compare simulation and experimental results is data distribution independent and has been used in other studies (Caballero & Keightley, 1998; Caballero *et al.*, 2002).

In order to combine the analyses made for the different parameters studied, we performed a multifactorial analysis as described by Sokal & Rohlf (1997), pp. 794–797. The correlation between the simulated parameters analysed was shown to be close to zero and nonsignificant. Thus, assuming independence between the variables, the probabilities (P) of the observed results for each of the parameters analysed can be tested jointly, because the sum of the log-probabilities ($-\sum \ln P$) for the k tests is distributed as a χ^2 with $2k$ degrees of freedom.

Results

Experimental results

Figure 2a,b show the average coefficient of inbreeding and the average egg-to-adult viability, respectively, for each experimental line. Replicates behaved in a consistent way in all cases. The corresponding averages over replicates for each treatment are shown in Figs 6a and 7a respectively. In the case of mean viabilities (Fig. 7a), these are shown as deviations from the control non-inbred averages (CN), so this treatment appears as a horizontal line at zero.

The average inbreeding coefficient was kept low (0.09) at generation 5 in the CN and SN lines (Figs 2a and 6a). As expected, the average inbreeding rose in the CI lines up to a value of 0.41 at generation 5. However, in the SI lines a smaller rate of increase of inbreeding was observed from the second generation onwards ($F = 0.33$ at generation 5). The reason is that generation 1 was the first one in which a proportion of inbred (75%) and non-inbred (25%) individuals appeared. Individuals showing higher levels of viability tend to be those with lower levels of inbreeding (not suffering from inbreeding depression). Thus, in the SI lines, the selected group included a higher proportion of non-inbred individuals than the corresponding CI lines, and the average coefficient of inbreeding was correspondingly lower in the former.

The inbreeding depression rate for the trait, estimated from the CI lines, and corrected for environmental effects was $0.70 \pm 0.11\%$ decline in mean viability per 1% increase in inbreeding coefficient, expressed as a percentage of the mean at generation 0. The decline in mean was almost linear on F in real scale, with a small recovery towards the control non-inbred mean in the last generation (Fig. 3).

The observed drop in viability in the CI lines in the initial generations (Fig. 7a) allows deducing the magnitude of the inbreeding depression for the trait ascribed to maternal and offspring components. The mean inbreeding of generations 0, 1 and 2 for the evaluated offspring (Fig. 6a) was about 0, 0.2 and 0.3 respectively. Note that in generation 1 the offspring was inbred but not the mothers, so the mean inbreeding in the mothers was always one generation delayed with respect to that of the offspring. The observed drop in mean viability (deviated from the CN lines; Fig. 7a) was about 0.08 from generation 0 to 1. This depression is exclusively ascribed to the inbred offspring, being $0.08/0.2 = 0.4$. Assuming this rate is maintained linearly from generation 1 to 2,

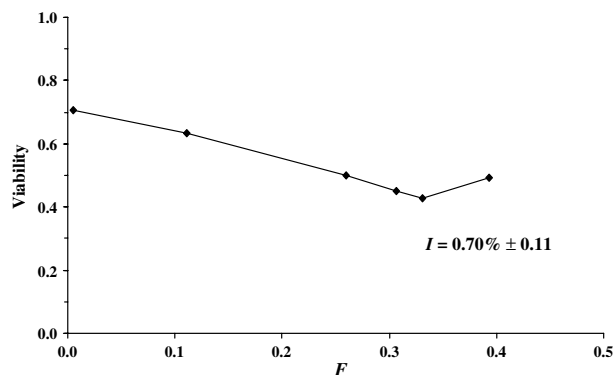


Fig. 3 Average egg-to-adult viability of control inbred (CI) lines corrected for environmental effects plotted against inbreeding coefficient (geometric mean of progeny and maternal average inbreeding coefficients). I : Inbreeding depression estimated by regression slope \pm standard error as a percentage of the initial mean.

the expected drop from generation 0 to 2 is $0.4 \times 0.3 = 0.12$, whereas the observed drop is 0.18. Therefore, the inbreeding depression ascribed to the maternal component of the trait is, at least, $0.06/0.2 = 0.3$. This shows that the maternal and offspring components of the trait are responsible for about one half of the total inbreeding depression, justifying the use of a geometric average of maternal and offspring mean heterozygosities to estimate the overall inbreeding depression rate, as well as a geometric average of maternal and offspring genotypic values for viability in the simulations.

The selected inbred (SI) lines showed a decline in mean similar to that of the CI lines (Fig. 7a) but thereafter there was a substantial recovery towards the control non-inbred value, suggesting purging selection of deleterious mutations.

The response to upward selection was very small for the selected non-inbred (SN) lines (0.011 ± 0.006), giving a nonsignificant additive genetic variance of 0.003 ± 0.002 and a realized heritability of 0.06 ± 0.07 (Fig. 4). This suggests that the wild type alleles (i.e. the nondeleterious ones) should be at very high frequencies in the base population, implying little opportunity for the mean viability to increase by artificial selection. In the selected inbred (SI) lines, a more consistent and significant response was observed (relative to the control inbred, CI, lines), being about twice the response in the SN lines (0.027 ± 0.006 ; see Fig. 4). The nonlinearity of selection response with partial inbreeding (Kelly, 1999) precludes the estimation of unbiased additive genetic variances and heritabilities in this case.

Simulation results

The results from the different simulation models were compared with the empirical observations to investigate what sort of models could explain the observations. A simulation model was considered to be compatible with the observations for a given parameter when the observed value of this parameter (averaged over the three replicates) fell inside the 95% confidence limits

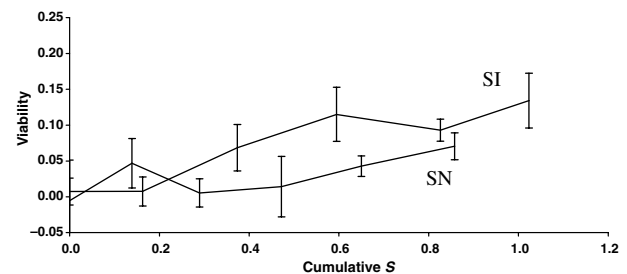


Fig. 4 Average egg-to-adult viability of selected lines deviated from their corresponding controls, plotted against the cumulative selection differential. SI, selected inbred lines; SN, selected non-inbred lines.

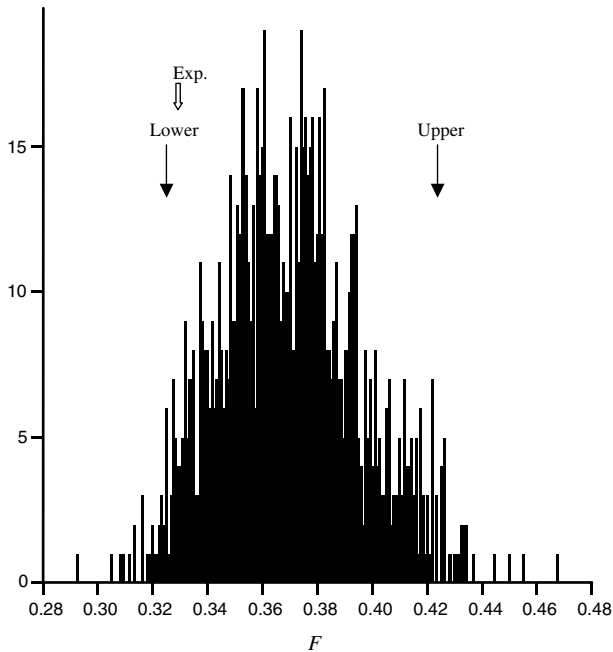


Fig. 5 Illustration of the statistical comparison between simulations and observations. Distribution of 1000 simulation results (each being the average of three replicates) for the mean inbreeding coefficient at generation 5 in selected inbred lines (model $\lambda = 0.015$, $\bar{s} = 0.2$, $\bar{h} = 0.15$). The upper and lower 95% confidence intervals and the observed result are shown by arrows.

from 1000 analogous simulation results. An illustration of this procedure is shown in Fig. 5, which shows 1000 simulation results for the average inbreeding coefficient, the corresponding confidence intervals and the observed result, in this case within the confidence intervals.

All models could satisfactorily explain the average observed levels of inbreeding attained in the CN, SN and CI lines, which were 0.09, 0.09 and 0.41 at generation 5 respectively. The corresponding simulation 2.5 percentiles ranged from 0.077 to 0.078, 0.076 to 0.079 and 0.380 to 0.386, respectively, considering all models studied, and the 97.5 percentiles ranged from 0.099 to 0.101, 0.094 to 0.103 and 0.463 to 0.469 respectively. Some illustrative examples of inbreeding coefficients from simulations are shown in Fig. 6b–e, where the averages over 1000 simulated replicates are represented, and can be compared with the empirical observations (Fig. 6a). Some parameters, such as the inbreeding coefficient in the control lines, are almost independent of the mutational model, and simulated and observed values were practically identical (cf. Fig. 6a,b–e for CI and CN lines), ensuring the reliability of the simulation procedure.

All simulation models were also compatible with the selection responses observed in the SN and SI lines (0.011 and 0.027 respectively). The corresponding simulation

2.5 percentiles ranged from -0.006 to 0.000 and 0.003 to 0.025 , respectively, considering all models studied, and the 97.5 percentiles ranged from 0.019 to 0.021 and 0.030 to 0.052 , respectively. Some illustrative examples of mean simulation values of SN and SI lines are shown in Fig. 7b–e, and these can be compared with the empirical observations (Fig. 7a). From these results it can be concluded that the response of selected lines was not a useful parameter to discriminate among mutational models, as all of them were compatible with the observations. Accordingly, the simulated additive variances at generation 0 ranged from 0.002 to 0.005 considering all simulated models, whereas the experimental observation was 0.003 ± 0.002 in the SN lines.

The only clear discrepancies between simulation and empirical results refer to the magnitudes of the inbreeding depression rates of CI lines, and the inbreeding coefficients and the rates of recovery of the SI lines. Simulated confidence intervals for these parameters and their comparison with the corresponding empirical results are shown in Table 1. In the table, italicized intervals denote cases where the empirical result (at the top of the table) falls within them, indicating compatibility between simulations and observations. For some models, the inbreeding depression rate of the CI lines was too large or too small. Moreover, the inbreeding coefficient of the SI lines increased in excess or in defect, and these lines did not recover the levels of the CN lines at generation 5, or they recovered it too early. Some illustrative examples of simulations agreeing or disagreeing with the empirical observations are also shown in Figs 6 and 7 and discussed below.

A multifactorial test was performed assuming independence between the individual parameters analysed in Table 1. This is justified because these parameters are almost independent of one another, as deduced from their low correlations. For example, for the 1000 replicates of the model $\lambda = 0.5$, $\bar{s} = 0.02$, $\bar{h} = 0.15$, the correlations were: 0.050 (between *ID* of CI and *F* of SI), 0.044 (between *ID* of CI and SI–CN), and 0.018 (between *F* of SI and SI–CN), all of them nonsignificant. Similar low correlations were found for other mutational models. The multifactorial significance at 5% is shown at the right-hand side of each row in Table 1. The only models overly compatible with the observed results were ($\lambda = 0.25$, $\bar{h} = 0.4$), ($\lambda = 0.1$, $\bar{h} = 0.4$), ($\lambda = 0.05$, $\bar{h} = 0.3$) and ($\lambda = 0.015$, $\bar{h} = 0.15$). Thus, only those models for which all separated tests were nonsignificant were still nonsignificant after considering the multivariate analysis.

Models of many mutations of small effect were compatible with the experimental results only for large values of the average dominance coefficient but were inconsistent for lower values (Table 1). For example, for a model of many mutations of small, nearly additive, effects ($\lambda = 0.5$, $\bar{s} = 0.02$, $\bar{h} = 0.4$; the Mukai–Ohnishi classical model), the simulations gave results very similar

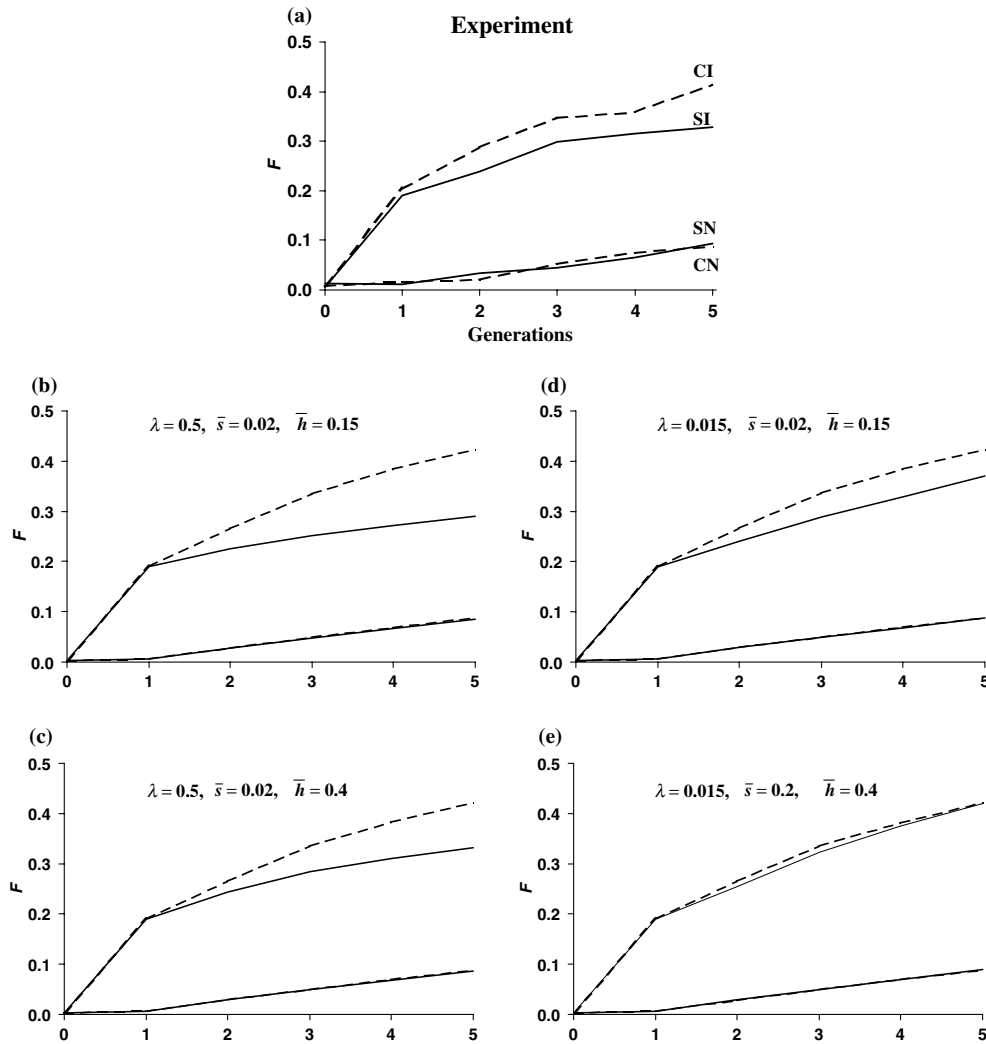


Fig. 6 Average inbreeding coefficient of the different lines. (a) Experimental results. (b–e) Simulation results. CI, control inbred lines; CN, control non-inbred lines; SI, selected inbred lines; SN, selected non-inbred lines. Continuous lines: selected lines; broken lines: control lines.

to the empirical ones (cf. Figs 6c and 7c with Figs 6a and 7a). However, when mutations were nearly recessive in this model ($\lambda = 0.5, \bar{s} = 0.02, \bar{h} = 0.15$; Figs 6b and 7b), the coefficient of inbreeding of SI lines increased less than empirically observed (Fig. 6b), the inbreeding depression of CI lines was too large (Fig. 7b), and the mean viability of the SI lines was not recovered (Fig. 7b). The reason is that with recessive mutations of small effect, purging was very slow, delaying the recovery of SI mean values. In addition, the possible association between selected and non-inbred individuals was maintained for several generations, restraining the increase in inbreeding of SI lines.

On the contrary, models of few mutations of large effect were consistent with observed results for small values of the average dominance coefficient but not for

large values (Table 1). For example, for a model of few mutations of large, nearly recessive, effects ($\lambda = 0.015, \bar{s} = 0.2, \bar{h} = 0.15$; the more recent mutational model), simulations agreed closely with observations (cf. Figs 6d and 7d with Figs 6a and 7a). However, if mutations had nearly additive effects ($\lambda = 0.015, \bar{s} = 0.2, \bar{h} = 0.4$; Figs 6e and 7e), the coefficient of inbreeding of SI lines increased more than empirically observed (Fig. 6e), the inbreeding depression of CI lines was too small (Fig. 7e), and the mean viability of the SI lines was recovered too early (Fig. 7e).

Discussion

We carried out an experiment of artificial selection and inbreeding for viability in a recently captured population

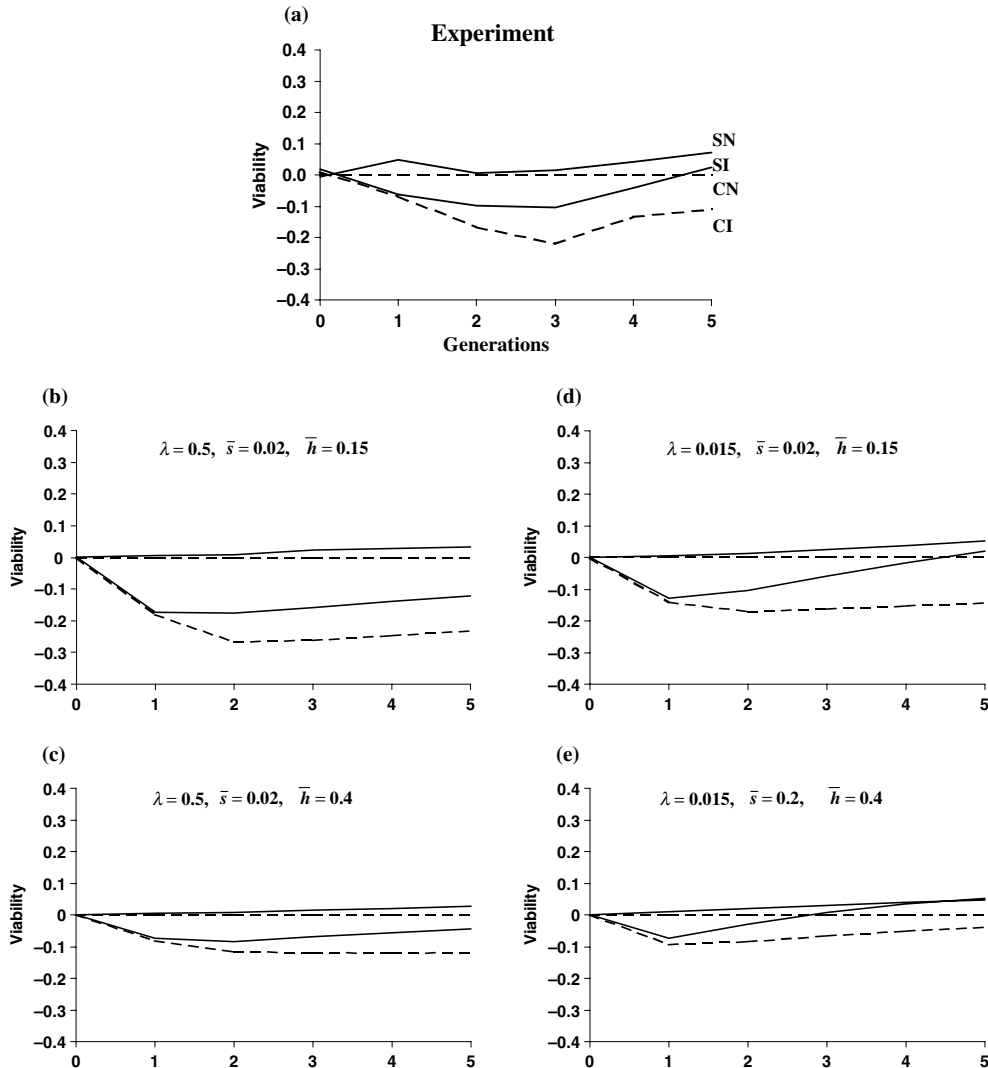


Fig. 7 Average egg-to-adult viability of the different lines, deviated from the viability of the control non-inbred lines. (a) Experimental results. (b–e) Simulation results. CI, control inbred lines; CN, control non-inbred lines; SI, selected inbred lines; SN, selected non-inbred lines. Continuous lines: selected lines; broken lines: control lines.

of *D. melanogaster*, as well as computer simulations of the experimental design. The objective was to ascertain whether the genetic variation for this trait can be explained by the balance between the appearance of deleterious mutations and their elimination by selection, and to investigate the set of mutational parameters that better explain the data. The inbreeding depression rate was about 0.7% of the initial mean per 1% increase in inbreeding, in good agreement with other estimates obtained for the same trait and species (0.82%, López-Fanjul & Villaverde, 1989; 0.54%, García *et al.*, 1994; 0.42–0.96%, Ehiobu *et al.*, 1989). These estimates strictly refer to egg-to-adult viability, but other compound traits that include viability show similar values (Lynch & Walsh, 1998, p. 271).

The estimate of realized heritability (0.06) was also of the same order as others obtained from upward selection of different fitness components in *Drosophila* (Frankham, 1990). The corresponding additive variance (0.003) was of the same order as those obtained by Mukai (1985) in the northernmost populations of *D. melanogaster* analysed by his group (around 0.005, after multiplying by 2.5 to scale for the whole genome), and one order of magnitude smaller than those in the southernmost populations (around 0.05). Mukai (1985) suggested that the genetic variance for viability in the northernmost populations was maintained by mutation-selection balance, whereas the southernmost populations had an excess of additive variance that could be explained by some form of diversifying selection. The results of our work also

indicate that a balance between partially recessive deleterious mutations and their selective elimination can explain the experimental observations without the need for invoking additional factors. This is suggested by the good agreement between the observed and simulated behaviour of selected and control lines for a set of mutational parameters currently assumed in the literature.

To our knowledge, the only previous experiments of artificial selection for viability in *D. melanogaster* are those by López-Fanjul & Villaverde (1989) and García *et al.* (1994). López-Fanjul & Villaverde (1989) carried out one generation of upward selection for egg-to-pupa viability. The realized heritability obtained from a non-inbred base population was 0.10, and the corresponding additive genetic variance (given in the same scale as in the present experiment) was 0.022. García *et al.* (1994) carried out an artificial selection experiment of egg-to-pupa viability for 27 generations on a single population, as well as in subdivided populations with or without previous inbreeding. All lines behaved in a similar manner, showing a clear response (around 20%), which was exhausted after five generations of selection. The realized heritability obtained from a non-inbred base population was 0.29, and the corresponding additive genetic variance was 0.092. The conclusion from the latter experiment was that the base population should carry a number of genes with substantial additive effects besides an indefinite number of partially recessive deleterious alleles. Thus, it is evident that the levels of additive genetic variance estimated in these experiments (0.022–0.092) are one to two orders of magnitude larger than that estimated in the present experiment (0.003). However, it should be borne in mind that the base populations used by López-Fanjul & Villaverde (1989) and García *et al.* (1994) were not recently caught from the wild, but had been maintained in the laboratory for about 2 years. It is highly likely that the genetic architecture of life-history traits in wild populations will change considerably after a period in the laboratory. On the one hand, a population in captivity may suffer from frequent bottlenecks, which may randomly increase the frequency of rare deleterious recessive alleles segregating in nature. On the other, deleterious recessive alleles may also become more common under benign laboratory conditions, as a consequence of adaptation to captivity (Shabalina *et al.*, 1997; Hoffmann *et al.*, 2001). These effects could result in an increased response to upward selection from laboratory populations. Our base population is a very recently caught one and it is therefore not expected that any adaptation to laboratory conditions has as yet occurred.

One of our objectives was to compare the observed results with those of simulations using a mutation-selection balance model of variation and a range of mutational parameters. Classical estimates from mutation-accumulation experiments suggest a large number

of mutations of small average effect and close to additive gene action (Mukai, 1964, 1969b; Mukai *et al.*, 1972; Ohnishi, 1977a,b), whereas other recent estimates and reanalyses of previous ones point to lower rates of mutations of larger effect and highly recessive gene action (García-Dorado *et al.*, 1999). The discrepancy between estimates has been explained in different ways (reviewed by García-Dorado *et al.*, 1999, 2004; Keightley & Eyre-Walker, 1999; Lynch *et al.*, 1999): (i) real differences between populations, perhaps caused by differences in transposition rates, (ii) bias in the estimates from Mukai and Ohnishi experiments because of nonmutational declines in viability or errors in the assignment of marker phenotypes, and (iii) bias in the estimates from some recent and past experiments, because of the difficulty of maintaining valid control populations. Estimates from other traits and species are less conflicting, showing, in general, an overall agreement with a model of few mutations of large effect (García-Dorado *et al.*, 2004). However, all the above estimates may possibly ignore the class of mutations of very small effect, because of the lack of power to detect them experimentally. In any case, although these mutations may be important in an evolutionary long-term context, their contribution to the parameters studied in this work, namely inbreeding depression and short-term selection response, should be negligible and would not affect the conclusions.

A similar approach to ours was followed by Caballero & Keightley (1998), who compared the behaviour of initially isogenic control lines of mice and *Drosophila* with simulations involving accumulation of deleterious mutations. Their results pointed out incompatibilities between the classical model of many mutations of small effect and the observations. In our simulations, some mutational models could be disregarded because (i) they implied too low or too high inbreeding depression, (ii) their average inbreeding increased too much or too little, and (iii) the selected inbred lines did not recover their original mean. It is interesting and, to some extent, unfortunate that the two contrasting models currently debated as alternatives for viability in *Drosophila* were both able to explain all or most observations. A model of many mutations of small effect agreed with all the observations when a high average dominance coefficient was assumed (except for a model of $\lambda = 0.5$, which was incompatible with the observed inbreeding depression; Table 1), but not otherwise. On the contrary, a model of few mutations of large effect was satisfactory when the average degree of dominance was low, but not otherwise (Table 1).

It is remarkable that no differences in selection response were found between the contrasting simulation models despite a substantial difference in the expected additive variances for infinite populations. For example, the additive genetic variance expected in an infinite population for the model $\lambda = 0.5$, $\bar{s} = 0.02$, $\bar{h} = 0.4$ is 0.0031 whereas for the model $\lambda = 0.015$, $\bar{s} = 0.2$,

$\bar{h} = 0.15$ is 0.0004, about eight times smaller. However, the simulated responses with both models were similar (see Figs 7c,d). There are two explanations. First, the sample of 80 individuals to start each replicate implies a bottleneck, and inbreeding increases the additive genetic variance in models involving a large amount of recessive genes at low frequency (Robertson, 1952). Thus, despite the additive variance in the base population being the expected one, the simulated additive variances at generation 0 were 0.0047 and 0.0021 for the above models respectively. The increase in additive variance is proportionately much larger in the second model, with more recessive genes, bringing together the initial variances of both models. Secondly, the above estimates of additive variance assume a between-loci additive model of gene effects, whereas the simulations involved a multiplicative model of gene effects. The actually expressed genetic variance is smaller under a multiplicative model than under an additive model, particularly for the case of many mutations of small effect, and this also contributes to a similar response under both models in real scale. Simulated responses were also evaluated using log-abilities, showing a slight increase in response for the model of many mutations, but still nonsignificantly different from the observed response in log scale.

The two contrasting sets of mutational parameters above have been obtained through mutation-accumulation experiments, where the only assumption is that deleterious mutations are fixed in the mutation-accumulation lines by random drift. Therefore, their use to produce the mutation-selection balance simulated predictions, against which experimental results were contrasted, does not create a problem of circularity. Such a problem could have been argued if the estimates of mutational parameters used in the simulations had been obtained from segregating populations, a procedure that assumes mutation-selection balance. However, no such estimates are available for viability in *Drosophila*. Estimates for other species (some selfing plants and *Daphnia*) obtained in this way have generally been in agreement with a model of many mutations (Lynch *et al.*, 1999 and references therein), but it is precisely the assumption of mutation-selection balance that constitutes the main weakness of these estimates, as balancing selection will produce overestimations of the mutation rate (Lynch *et al.*, 1999).

The trait under study (egg-to-adult viability) has been previously considered as a trait fully dependent on the maternal genotype (López-Fanjul & Villaverde, 1989; García *et al.*, 1994). However, our results indicate that inbreeding depression has maternal and offspring components of similar magnitudes. This justifies the use of a geometric average of maternal and offspring genotypic values for viability in the simulations. If the trait is wholly ascribed to the mothers in the simulations, no inbreeding depression appears in the first generation, in disagreement with the observations. Analogously, if the

trait is wholly ascribed to the progeny, too little inbreeding depression occurs from generation 1 to 2, also in disagreement with the observations. Thus, the geometric mean seems to be justified.

As genes were randomly assigned in the base population, no linkage is considered in the simulations. However, it is unlikely that linkage disequilibrium generates a large amount of genetic variance under mutation-selection balance (Charlesworth & Hughes, 1999). On the contrary, the inclusion of linkage in the simulations appears to be irrelevant. We made simulations assuming a single chromosome of length 1.25 M (the assumed genome length in *D. melanogaster*). Thus, selected and neutral genes were linked in a single chromosome allowing a Poisson distributed random number of crossovers with mean 1.25, occurring in randomly chosen places of the chromosome without interference. The results were almost identical to those given in Table 1 for free recombination.

The simulation model used assumes recurrent mutation. We also considered an alternative model of nonrecurrent mutations. By means of diffusion approximations (Kimura, 1969) we obtained the expected number of segregating sites and the distribution of frequencies in a population of size $N = 10\,000$ individuals, for mutations sampled under each mutational model. From these, we obtained, by binomial sampling (Wang *et al.*, 1998), the corresponding values in a sample of 80 individuals (the starting number for each replicate). To start each replicate and treatment we included the number of sites appropriate for each mutational model. For models of large mutation rate, the number of segregating sites was usually much larger than the 5800 sites involved in the recurrent mutation model. However, the results of this method (not shown) were qualitatively similar to those presented above. For the mutational parameters studied, the larger number of segregating sites under nonrecurrent mutation seems to be approximately compensated for by a larger gene frequency per site in the recurrent mutation model.

As laboratory conditions are likely to be more benign than natural ones, it is possible that selection coefficients are generally smaller in the laboratory (Kondrashov & Houle, 1994). Thus, it may turn out that the frequencies of mutations in natural populations are lower than predicted under mutation-selection balance with the assumed selection coefficients (estimated also in laboratory conditions). If this is the case, our simulation results are likely to give overestimations of the inbreeding depression and selection response. However, the assumption that a harsher environment implies larger selection coefficients is arguable. For example, Chavarrías *et al.* (2001) found similar effects for viability mutations in *D. melanogaster* under competitive and noncompetitive conditions.

Our simulation results would also give overestimations of the inbreeding depression and selection response if

mutations affecting egg-to-adult viability had a pleiotropic-negative effect on other fitness components, such that the overall effect on fitness of such mutations is larger than for viability alone (Mukai & Yamaguchi, 1974; Charlesworth & Hughes, 1999). This argument leads to caution about our conclusion that mutation-selection balance is enough to explain genetic variance for viability in natural populations. In fact, Charlesworth & Hughes (1999) concluded that mutation-selection balance alone is unlikely to account for all the inbreeding depression and additive genetic variance observed for life-history traits using a mutational model analogous to that assumed in this paper for many mutations of small effect. However, the arguments used to deduce that overall fitness effects of viability mutations are larger than for viability alone, are based on indirect methods, with an arguable support. Thus, the calculations of Mukai & Yamaguchi (1974) that imply an overall fitness effect of viability mutations of about twice the viability effect, need accurate estimates of the mean decline in viability due to mutation accumulation, the average mutation effect and dominance, and the homozygous load due to partially dominant and overdominant genes. Several of these estimated values are under discussion (García-Dorado *et al.*, 1999), so the conclusions based on them cannot be taken too seriously. In addition, the calculation of Charlesworth & Hughes (1999), that leads to a 2.7-fold overall fitness effect of viability mutations, is based on the ratio between an estimate of the mutational variance for global fitness (Houle *et al.*, 1992) and the average mutational variance for several life-history traits (Mukai *et al.*, 1972; Ohnishi, 1977a; Houle *et al.*, 1994), after omitting two of them that fall out of the usual range. This calculation implies that the mutation rate for any life-history trait is the same as that for global fitness, a clearly arguable assumption.

Finally, in our simulations we assume a multiplicative model of gene effects among mutations. Synergistic epistatic effects are likely to produce a larger inbreeding depression than expected without epistasis, but little effect on the expected additive variance is predicted (Charlesworth & Hughes, 1999). However, synergistic epistatic effects of deleterious mutations is supported only by very scarce data, in particular, a single point in the experiment of Mukai (1969a) and another one in the experiment of Whitlock & Bourguet (2000).

Although neither of the two sets of mutational parameters proposed in the literature could be discarded, we have shown that a simple model of mutation-selection balance is enough to explain the genetic variation for viability in a natural population, at least under the specific laboratory conditions of the evaluation. Although other sources of variation (such as balancing selection from antagonistic pleiotropy or genotype-environment interaction) cannot be disregarded, we can conclude, at least, that these additional sources of variation are unnecessary to explain the results.

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